



BioCAD[®] 700E Workstation for Perfusion Chromatography[®]

Version 3 Series Software

User Guide

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WARNING

For continued protection against fire hazard, replace fuses with those of the same type and rating.

AVERTISSEMENT

Remplacez les fusibles par ceux de même type et puissance pour éviter les risques d'incendie.

WARNING

Before handling any chemicals, refer to the Material Safety Data Sheet provided by the manufacturer, and observe all relevant precautions.

AVERTISSEMENT

Avant de manipuler des produits chimiques, veuillez consulter la fiche de sécurité du matériel fournie par le fabricant, et observer les mesures de précaution qui s'imposent.

WARNING

Always wear eye protection when working with solvents or any pressurized liquids.

AVERTISSEMENT

Porter toujours des protections pour les yeux lorsque vous manipulez des solvants ou tout autre liquide sous pression.

WARNING

PEEK tubing is a polymeric material. Use caution when working with any polymer tubing under pressure.

- 1. Always wear eye protection when in close proximity to pressurized polymer tubing.*
 - 2. Extinguish all nearby flames if using flammable solvents.*
 - 3. Do not use PEEK tubing that has been severely stressed or kinked.*
 - 4. Do not use PEEK tubing with tetrahydrofuran or concentrated nitric and sulfuric acids.*
 - 5. Methylene chloride and dimethyl sulfoxide cause PEEK tubing to swell and greatly reduce the rupture pressure of the tubing.*
 - 6. With high solvent flow rates (40 ml/min), a static charge may build up on the surface of the tubing. Electrical sparks may result.*
-
-

AVERTISSEMENT

Le tubage PEEK est un matériau polymère. Prenez des précautions lorsque vous travaillez avec un tubage polymère sous pression.

- 1. Porter toujours des protections pour les yeux lorsque vous vous trouvez à proximité d'un tubage polymère sous pression.*
 - 2. Éteindre toutes les flammes avoisinantes si vous utilisez des solvants inflammables.*
 - 3. Ne pas utiliser un tubage PEEK qui a déjà été sérieusement travaillé ou plié.*
 - 4. Ne pas utiliser un tubage PEEK avec du tétrahydrofurane ou des acides nitriques et sulfuriques concentrés.*
 - 5. Le chlorure de méthylène et le sulfoxyde de diméthyle entraînent un gonflement du tubage PEEK et réduisent fortement la résistance à la rupture du tubage.*
 - 6. Avec des débits d'écoulement de solvants élevés (>à 40 ml/min), une charge statique peut se développer sur la surface du tubage. Des étincelles électriques peuvent en résulter.*
-
-

WARNING

Use this product only as specified in this document. Using this system in a manner not specified may result in injury or damage to the system.

AVERTISSEMENT

Veillez suivre avec attention les indications figurant dans ce document lorsque vous utilisez ce produit. Un usage différent le système causer un accident ou endommager le système.

CAUTION

Failure to leave the pH and conductivity monitors in a 1 M salt solution and to run the SYSCLEAN method after daily use may result in system downtime. See Section 2.6, Starting Up and Shutting Down, for the recommended procedure.

CAUTION

Do not use methylene chloride, tetrahydrofuran, dimethyl sulfoxide, or concentrated nitric or sulfuric acids. These materials are not compatible with the PEEK components in the BioCAD 700E Workstation.

CAUTION

Certain organic solvents, such as acetonitrile and isopropanol used over long time periods or in high concentrations, can weaken PEEK tubing on the BioCAD 700E Workstation. Change the PEEK tubing on the workstation periodically if you use organic solvents frequently or in high concentrations. For more information, contact Applied Biosystems Technical Support.

CAUTION

*If you are using organic solvents, bypass the pH and conductivity monitors to prevent damage. You can bypass the pH and conductivity monitors by clicking the **Bypass** option button in the pH/Cond section of the Control Panel or in the General Settings of a method.*

US Safety and EMC (Electromagnetic Compatibility) Standards

Safety

This instrument has been tested to and complies with standard ANSI/UL 3101-1, "Electrical Equipment for Laboratory Use; Part 1: General Requirements", 1st Edition. It is an ETL Testing Laboratories listed product.

EMC

This device complies with Part 15 of the FCC Rules. Operation is subject to the following two conditions: (1) This device may not cause harmful interference, and (2) this device must accept any interference received, including interference that may cause undesired operation.

Warning: *Changes or modifications to this unit not expressly approved by the party responsible for compliance could void the user's authority to operate the equipment.*

Note: *This equipment has been tested and found to comply with the limits for a Class A digital device, pursuant to Part 15 of the FCC Rules. These limits are designed to provide reasonable protection against harmful interference when the equipment is operated in a commercial environment. This equipment generates, uses, and can radiate radio frequency energy and, if not installed and used in accordance with the instruction manual, may cause harmful interference to radio communications. Operation of this equipment in a residential area is likely to cause harmful interference in which case the user will be required to correct the interference at his own expense.*

Note: *Shielded cables must be used with this unit to ensure compliance with the Class A FCC limits.*

Canadian Safety and EMC (Electromagnetic Compatibility) Standards

Safety

This instrument has been tested to and complies with standard CSA 1010, "Safety Requirements for Electrical Equipment for Measurement, Control, and Laboratory Use; Part 1: General Requirements". It is an ETL Testing Laboratories listed product.

Sécurité

Cet instrument a été vérifié avec la norme CSA 1010, «Spécifications de sécurité du matériel électrique utilisé pour les mesures, les contrôles et dans les laboratoires ; Partie 1 : Spécifications générales», et il est conforme à cette norme. C'est un produit homologué par les ETL Testing Laboratories.

EMC

This Class A digital apparatus meets all requirements of the Canadian Interference-Causing Equipment Regulations.

Cet appareil numérique de la classe A respecte toutes les exigences du Règlement sur le matériel brouilleur du Canada.

European Safety and EMC (Electromagnetic Compatibility) Standards

Declaration of Conformity

Application of Council Directive(s):	73/23/EEC "Low Voltage" 89/336/EEC "Electromagnetic Compatibility"
Standard(s) to which conformity is declared:	EN61010-1:1993, "Safety Requirements for Electrical Equipment for Measurement, Control and Laboratory Use" EN55011:1991, Group 1, Class B "Radiated Emissions" EN50082-1:1991 "Generic Immunity"
Manufacturer's Name:	Applied Biosystems
Manufacturer's Address:	500 Old Connecticut Path Framingham, Massachusetts 01701 USA
Type of Equipment:	Laboratory Instrumentation
Model Name:	BioCAD 700E Workstation
Model Number:	5-1004-75
Serial Number:	1068 and later
Year of Manufacture:	1997 and later

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Glossary

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How to Use This Guide

Purpose Applied Biosystems' *BioCAD® 700E Workstation User Guide* details the procedures for installing, running, maintaining, and troubleshooting the BioCAD® 700E Workstation.

Audience This guide is intended for novice and experienced BioCAD 700E Workstation users who are developing and executing analytical and preparative separation protocols of biomolecules based on POROS® Perfusion Chromatography® media and conventional chromatography media.

Structure Applied Biosystems' *BioCAD 700E Workstation User Guide* is divided into ten chapters. Each chapter page is marked with a tab and a header to help you locate information within the chapter.

The table below describes the material covered in each chapter.

<i>Chapter 1,</i> Introducing the BioCAD 700E Workstation	Describes the system hardware and software. Includes a quick introduction to Microsoft® Windows®.
<i>Chapter 2,</i> Installing the BioCAD 700E Workstation	Provides procedures for installing the system, connecting components (such as tubing and external devices), installing the software, starting up, and shutting down.
<i>Chapter 3,</i> Preparing the Workstation and External Devices	Describes how to prepare the hardware components of the system for operation, including columns, plumbing, buffers/solvents, samples, and external devices. Explains how to prime and flush the system.

<i>Chapter 4,</i> Configuring and Preparing the BioCAD Software	Describes how to configure and prepare the BioCAD software for operation. Includes instructions for configuring and preparing the pump, buffers/solvents, samples, columns, detectors, autosampler, and fraction collector.
<i>Chapter 5,</i> Controlling the Workstation from the Control Panel	Describes how to control the system manually from the Control Panel and Sample Table.
<i>Chapter 6,</i> Creating and Running Methods	Describes how to use the Method Editor to create and run methods.
<i>Chapter 7,</i> Creating and Running Templates and Multi-Methods	Describes how to use templates and multi-methods to systematically run and develop methods.
<i>Chapter 8,</i> Viewing Results and Checking Status	Describes how to use the Method Queue, Status Window, Strip Chart Recorder, Timer, and Event Log to view the results and status of an injection.
<i>Chapter 9,</i> Maintenance	Describes maintenance procedures. Includes a checklist of preventative maintenance tasks and schedule.
<i>Chapter 10,</i> Troubleshooting and Error Codes	Includes troubleshooting information and error codes.

<i>Appendix A,</i> Specifications	Includes system specifications.
<i>Appendix B,</i> Spare Parts	Provides a list of spare parts.
<i>Appendix C,</i> Warranty/ Service Information	Includes warranty information.
<i>Appendix D,</i> BioCAD Sample Methods	Describes the sample methods supplied with the system.
<i>Appendix E,</i> External Instrument Connections	Lists the pin assignments for the connectors on the left panel of the workstation.
<i>Appendix F,</i> Autosampler Injection Types	Describes the processes that occur when making each type of autosampler injection.
<i>Appendix G,</i> Maintenance Log	Contains log sheets that you can use to keep track of scheduled maintenance.
<i>Appendix H,</i> Autoloader Flow Path	Includes diagrams of the autoloader flow path during various operations.

Conventions This guide uses the following conventions to make the text easier to understand.

General conventions The following general conventions are used:

- **Bold** text indicates user action:
“Type **0** and press **Enter** for the remaining fields.”
- *Italic* text denotes new or important words, and is also used for emphasis:
“Prior to operation, *always* check for eluent miscibility.”

Notes, Hints, Cautions, and Warnings Notes, Hints, Cautions, and Warnings are used as follows:

- A note calls out important information to the user:

NOTE: *Record your result before proceeding with the next step.*

- A hint provides helpful suggestions not essential to the use of the product:

Hint: *Use Heat/Mix segments after Dilute segments to produce a uniform mixture.*

- A caution calls out information to avoid damage to the system or equipment:

CAUTION

Do not touch the flow cell window. This may damage the flow cell.

- A warning calls out information essential to the safety of the user:

WARNING

Always observe safe laboratory practices when operating your system.

*Remarques,
recommandations
et avertissements*

Une remarque fournit une information importante à l'opérateur et se présente ainsi:

REMARQUE: *Si on vous demande d'insérer la disquette de démarrage dans le lecteur, insérez-la puis appuyez sur n'importe quelle touche.*

Une recommandation fournit une information destinée à éviter des détériorations du système et de la synthèse ou la perte de données:

RECOMMANDATION

La lampe peut être endommagée. N'y touchez pas.

Un avertissement fournit une information indispensable à la sécurité de l'opérateur et se présente ainsi:

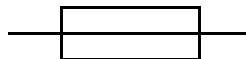
AVERTISSEMENT

Conformez-vous toujours aux règlements du laboratoire quand vous utilisez votre système.

***Symbols used
on the system***

The following symbol appears next to the values of the fuses required by the system.

Le symbole ci-contre figure à côté de la valeur des fusibles nécessaires avec ce système.



**Related
documentation**

These additional documents are shipped with your system:

- **Applied Biosystems' BioCAD 700E Workstation Getting Started Guide**—Use this guide to learn the basics of operating your BioCAD 700E Workstation. It provides step-by-step information for running your first experiment.
- **Applied Biosystems' Data Analysis Getting Started Guide and User's Guide**—Use these guides to learn to how to use the Data Analysis software provided with your BioCAD 700E Workstation.
- **Applied Biosystems' UV/Vis-201/205 Absorbance Detectors Operating Manual**—Use this manual if you are setting up an external or auxiliary UV/Vis detector. If you are using the internal UV/Vis detector in the BioCAD 700E Workstation, you do not need this manual.
- **Applied Biosystems' 304/305 Fluorescence Detectors Operating Manual**—If your system includes a fluorescence detector, use this manual to obtain detailed operating instructions, advanced programming information, and maintenance information. The *BioCAD 700E Workstation User Guide* includes information for connecting and configuring a fluorescence detector as an external or auxiliary detector.
- **Applied Biosystems' SCOUT® Column Selector User's Guide**—If your system includes a SCOUT Column Selector, use this guide to obtain procedures for installing, running, maintaining, and troubleshooting the SCOUT Column Selector.
- **Printer documentation** (depends on the printer you purchase)—Use this documentation to set up and service your printer.

- **Microsoft® Windows® User Guide** and related documents—Use this guide to learn detailed information about the Microsoft Windows user interface. The *BioCAD 700E Workstation User Guide* includes a quick introduction to Windows.
- **Microsoft® Excel User's Guide** and related documents—If your workstation includes the Microsoft Office option, use these documents to learn how to use the Microsoft Excel software after you export data from the BioCAD software.
- **SpectraSYSTEM® Autosamplers Reference Manual**—If your system includes an optional autosampler, use this manual to obtain detailed operating instructions, advanced programming information, and maintenance information for the autosampler. The *BioCAD 700E Workstation User Guide* contains basic programming information.
- **Advantec® Super Fraction Collector SF-2120-Operation Instruction Manual** or **Gilson® FC 205 Fraction Collector User Guide**—If your system includes an optional fraction collector, use this manual to obtain detailed operating instructions, advanced programming information, and maintenance information for the fraction collector. The *BioCAD 700E Workstation User Guide* contains basic programming information.

**Send us your
comments**

We welcome your comments and suggestions for improving our manuals. You can send us your comments in two ways:

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How to Use This Guide

1 Introducing the BioCAD® 700E Workstation



This chapter includes the following sections:

1.1	System Features.....	1-2
1.2	Systematic Method Development with Perfusion Chromatography	1-4
1.3	Parts of the System.....	1-8
1.4	BioCAD Software Overview	1-21
1.5	BioCAD Software File Structure	1-30
1.6	A Quick Introduction to Microsoft Windows	1-36

1.1 System Features

Overview Applied Biosystems' BioCAD® 700E Workstation for Perfusion Chromatography® is a computer-aided chromatography workstation that integrates all aspects of biomolecule purification with Perfusion Chromatography technology.

The BioCAD 700E Workstation is designed for:

- **Chromatographic method execution**—Routine execution of chromatographic methods for the purification and analysis of biomolecules.
- **Systematic method development**—The goal of method development is to optimize conditions for your application. Systematic method development makes it easy to identify variables that affect your application, systematically change the variables, observe the effects, and use the information to optimize chromatographic conditions.

Features The BioCAD 700E Workstation provides the following features:

- **Simplicity**—Microsoft® Windows®-based software that is intuitive and easy to use. You can efficiently develop and execute separations for analytical and preparative applications.
- **Full Process Capability**—Full capability from sample injection through fraction collection, including automated column washing and regeneration.
- **Speed**—Chromatographic separations in as little as one to three minutes, when using POROS® media and columns.
- **Versatility**—Multiple column configurations and injection mode options.
- **Gradient Capability**—Six-buffer/solvent capability, with simultaneous blending between any four buffers/solvents.
- **Detection**—Monitoring of pH, conductivity, dual-wavelength UV/Vis absorbance, fluorescence (optional), and system pressure.
- **Templates**—Predefined templates for systematic method development of key variables such as pH, gradient slope, and sample load.
- **Data Analysis**—Data analysis of single data files or groups of data files.
- **SCOUT® Column Selector**—Automated multi-column switching device that allows flow to be diverted to any one of six attached columns (optional).

1.2 Systematic Method Development with Perfusion Chromatography

Overview You can manipulate many variables in the separation of biological molecules. Ideally, you identify the variables most likely to affect your separation and systematically optimize the variables.

The development of each new method proceeds differently, but follows a typical method development cycle (Figure 1-1). Often, the cycle does not proceed linearly. Instead, results from each step can require that you reoptimize a variable from a previous step.

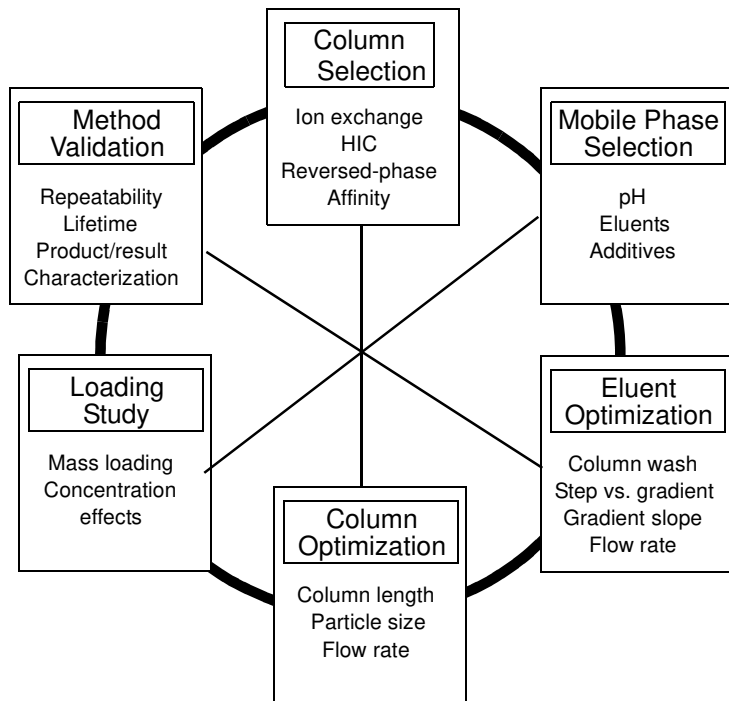


Figure 1-1 Method Development Cycle

Conventional chromatography

With conventional liquid chromatography columns and media, run times range from 30 minutes to several hours. To develop a method in a timely manner, you must often make guesses and change more than one variable at a time.

Perfusion Chromatography and BioCAD

With POROS Perfusion Chromatography media and columns and the BioCAD 700E Workstation, separations can often be completed in less than five minutes. Short run times, the unique templating and multi-method features, and the automated analysis features of the BioCAD software allow you to systematically investigate and optimize each variable, one at a time.

Variables affecting a separation

Table 1-1, “Variables Affecting Separations,” on page 1-6 lists variables that affect separations and the BioCAD 700E template you can use to optimize the variable. The table also rates the effect (Strong, Moderate, Weak) each variable has on four different modes of chromatography.

NOTE: Variables listed as Strong have a major effect on a separation and should be examined. Moderate variables can have a significant effect. Weak variables can often be ignored.

Table 1-1 Variables Affecting Separations

Variable	BioCAD 700E Template	Mode			
		Ion Exchange	Hydrophobic Interaction	Reversed-Phase	Affinity
Surface chemistry	Column Switching	Strong	Strong	Moderate	Weak
pH	pH Map	Strong	Moderate	Moderate	Moderate
Eluting agent	Combinatorial	Moderate	Moderate	Weak	Strong
Buffers/ additives	Combinatorial	Moderate	Weak	Strong	Weak
Elution steps	Step Optimization	Strong	Strong	Moderate	Moderate
Gradient profile	Linear Gradient	Strong	Strong	Strong	Weak
Column length	Column Switching	Weak	Weak	Moderate	Weak
Flow rate ¹	Flow Rate	Weak	Weak	Weak	Weak
Sample load	Loading Study	Strong	Strong	Strong	Weak
Cleanup/ regeneration ²	Auto Cycling	Moderate	Moderate	Moderate	Moderate

1. Weak variable for Perfusion Chromatography media only. Flow rate can have a strong effect on conventional chromatography media.

2. Importance varies highly with sample.

***Example of
systematic
method
development***

The following example is a typical procedure that you might use for systematic method development of an ion exchange method:

1. Identify the key variables that affect the separation: pH, solvent blending, and sample load.
2. Create the method.
3. Run a pH Map template to determine the optimum pH for the separation.
4. Using the method that generated the data file with optimized pH, run a Linear Gradient template to determine the optimum gradient for the separation.
5. Using the method that generated the data file with optimized gradient, run a Loading Study template to determine the optimum sample load for the separation.

You now have a method that is optimized for pH, gradient, and sample load.

1.3 Parts of the System

Overview The BioCAD 700E Workstation is shown in Figure 1-2.

Opening front doors The BioCAD 700E Workstation has two front doors that open from the center. To open the front doors, run your hand along the bottom of the door (not the bottom of the workstation) until you locate the notch. Pull out on the notch.

Parts of the system The BioCAD 700E Workstation includes:

- Front panel indicators and controls
- Buffer/solvent and pump system
- Sample loader
- Columns and plumbing
- Detector and monitors
- Fraction collection
- Computer components

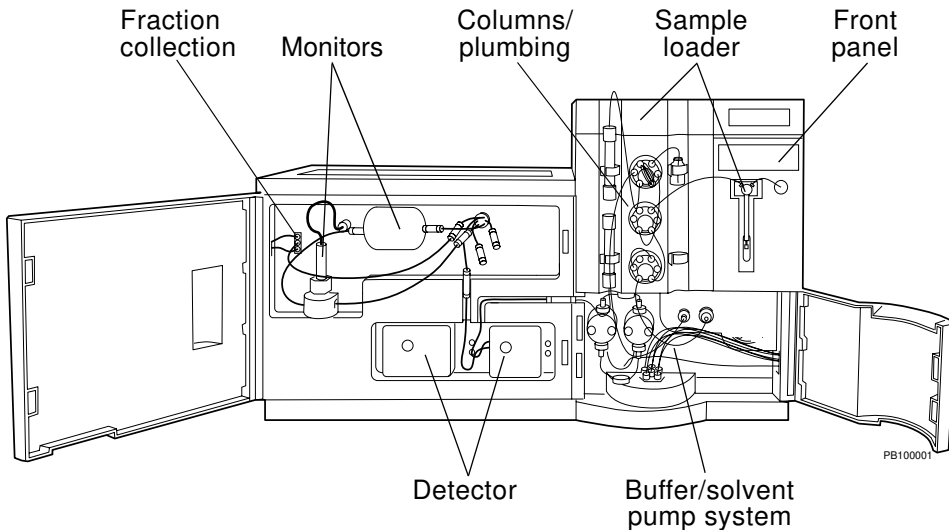


Figure 1-2 BioCAD 700E Workstation

1.3.1 Front Panel Indicators and Controls

The front panel is located at the top-right side of the workstation (Figure 1-3).

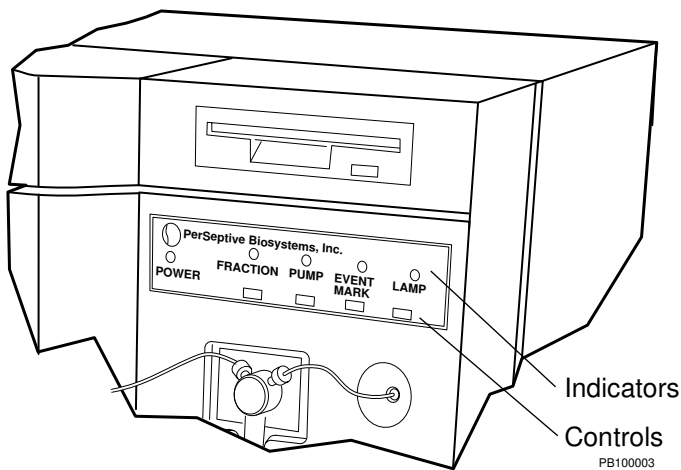


Figure 1-3 Front Panel Indicators and Controls

Indicators The front panel includes LED (light emitting diode) indicators:

- **Power On/Off**—Indicates status of system power
- **Fraction**—Indicates valve position of the built-in fraction/waste valve
- **Pump On/Off**—Indicates status of pump
- **Event Mark**—Indicates status of Event Mark control
- **Lamp On/Off**—Indicates status of UV/Vis detector lamp

Controls The front panel includes push-button controls:

- **Fraction**—Selects fraction/waste valve position (fraction or waste)
- **Pump On/Off**—Turns pump on and off
- **Event Mark**—Marks the electronic strip chart recorder manually with an MRK notation
- **Lamp On/Off**—Turns UV/Vis detector lamp on and off

1.3.2 Buffer/Solvent and Pump System

The buffer/solvent and pump system is located behind the door at the bottom-right side of the workstation (Figure 1-4). It includes:

- Tubing
- Proportioning valve
- Pump
- Pump seal wash system
- Prime/purge valve
- Filter/Mixer
- Sparge panel (located on the right side panel)

NOTE: Do not add inline or endline solvent filters before the pumps. Filters before the pumps can cause pump cavitation (vacuum formation).

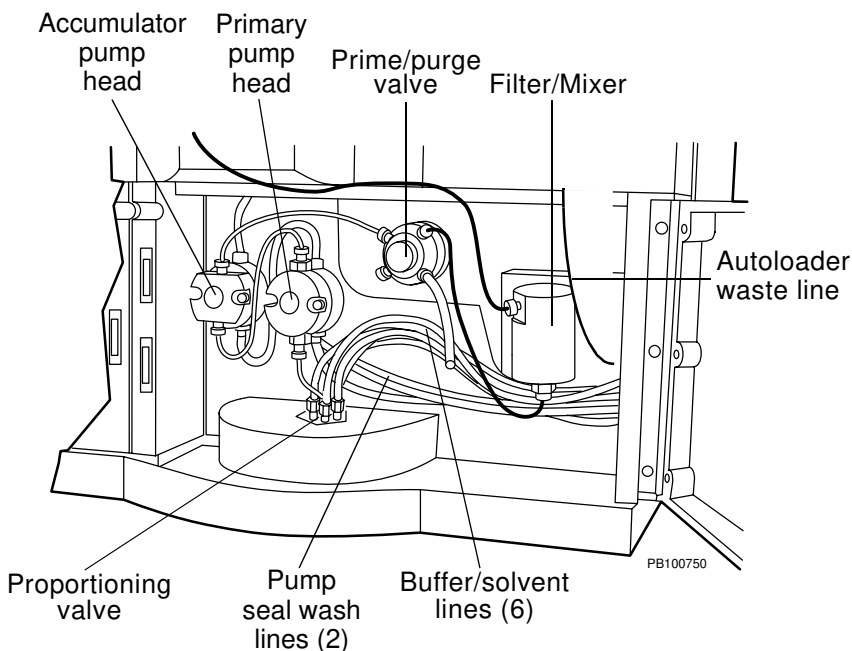


Figure 1-4 BioCAD 700E Buffer/Solvent and Pump System

Tubing The lines that extend out of the right side of the workstation include:

- Six buffer/solvent lines, labeled A through F
- Two urethane pump seal wash lines
- One autoloader waste line

Proportioning valve The proportioning valve is located behind the door at the bottom of the BioCAD 700E Workstation (see Figure 1-4). It can simultaneously blend up to four solvents.

Pump The pump is located behind the door at the bottom of the workstation (see Figure 1-4). It includes:

- **Primary pump**—Located on the right, this larger pump receives the blend from the six buffer/solvent lines and delivers to the Accumulator pump.
- **Accumulator pump**—Located on the left, this smaller pump delivers the blend to the system.

The pump heads protrude through the plastic panel to allow easy removal for service.

Pump seal wash system The pump seal wash system consists of the pump seal wash lines and pump seal wash solution. The pump seal wash system minimizes pump seal deterioration by flushing the back side of the seals continuously for maximum cleaning. This method is superior to gravity flow seal-flushing systems or scheduled manual cleaning. Each time the piston cycles, wash solution is drawn over the back side of the piston, keeping the piston clean and prolonging the lifetime of the pump seals.

Prime/purge valve The prime/purge valve is located to the right of the pump heads (see Figure 1-4). You can control this valve to remove air from the buffer/solvent flow path.

Filter/mixer The mixer is located to the right of the prime/purge valve (see Figure 1-4). It contains a frit that filters mobile phase as it leaves the pump to remove particulate matter that may occur as pump seals wear.

Sparge panel The sparge panel is located on the right side panel of the workstation (Figure 2-3).

There are six sparge valves, labeled A through F. Six sparge lines connect from the sparge valves to the buffer/solvents. Each sparge valve has an individual gas flow control knob.

Table 1-2 BioCAD 700E Workstation Injection Modes

Injection Mode	Description	When to Use	Volume Range
Autoloader	Automatically injects from one of four sample vessels.	For routine injections. To switch between various samples.	25 ml maximum
Direct Autoloader	Bypasses the sample lines and automatically injects directly from one sample vial mounted on the column clamp adjacent to the valve.	To minimize sample loss by reducing prime volume.	25 ml maximum
Manual	Bypasses the autoloader and allows you to inject directly into the injection valve and sample loop using a syringe and needle port adapter.	To further minimize sample loss by eliminating prime volume.	Limited by syringe size
Pump	Bypasses the injection valve and injects large volume samples through the pump using the buffer/solvent lines.	To inject large volume samples.	Greater than 5 ml
Direct Wire Autosampler	Injects from an optional, external autosampler. You control the autosampler functions from the autosampler controls.	To make multiple injections of single or multiple samples for analytical applications.	Limited by vial size
Integrated Autosampler	Injects from an optional, external autosampler (AS 3500). You control the autosampler functions from the BioCAD software.	To make multiple injections of single or multiple samples for analytical applications.	Limited by vial size

For information on making injections, see Section 5.3, Making Injections From the Control Panel, or Section 6.3, Creating Typical Methods.

1.3.4 Columns and Plumbing

Columns and plumbing Columns and plumbing are located on the front-center panel of the workstation (Figure 1-6).

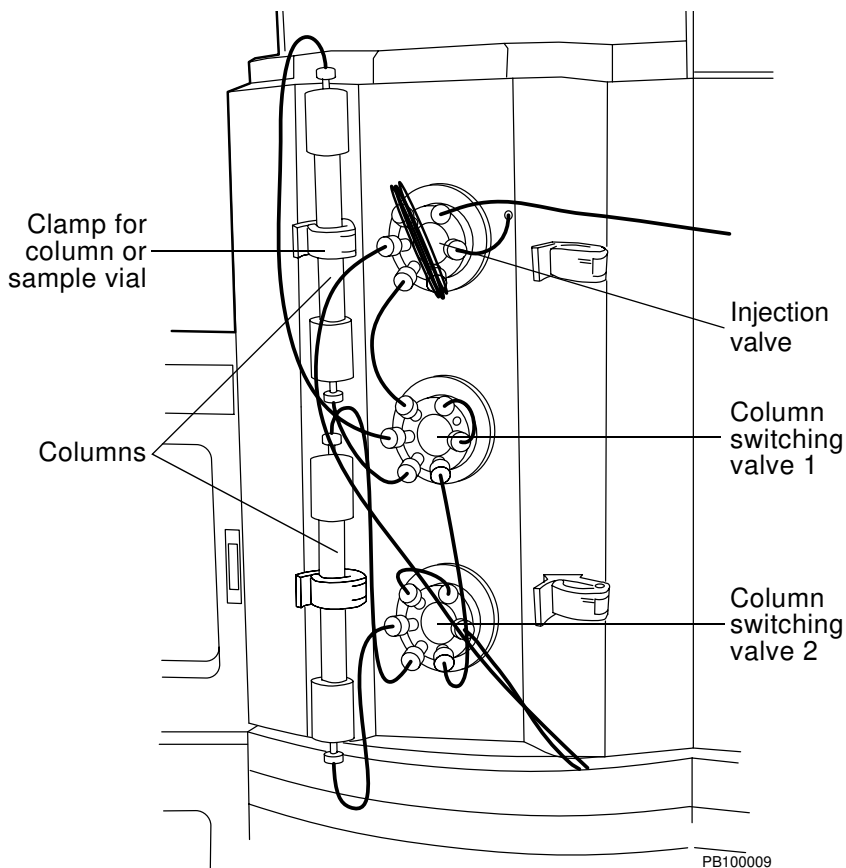


Figure 1-6 BioCAD 700E Columns and Plumbing

Columns and plumbing include:

- Four clamps for columns or sample vials
- Two 6-port, high-pressure column switching valves
- One 6-port, high-pressure injection valve

Column plumbing configurations

Five column plumbing configurations are available on the BioCAD 700E Workstation:

- Single column
- Tandem column
- Three column
- Direct control
- Low dispersion

Plumbing configurations are illustrated in Section 3.1.3, Plumbing Tubing and Columns.

Single column

Single-column configuration allows you to:

- Operate Column 1 alone
- Bypass Column 1 directly to the detectors
- Backflush Column 1 for washing, regeneration, or reversed elution

Tandem column

Tandem-column configuration allows you to:

- Operate Column 1 alone
- Operate Column 2 alone
- Operate Column 1 and Column 2 in series
- Bypass both columns directly to the detectors

Three column

Three-column configuration allows you to operate any one of the three columns individually. You cannot operate more than one column at a time. You cannot bypass all of the columns. One column is inline at all times.

Direct control

Direct control configuration allows you to customize plumbing in configurations other than those directly supported by the software. You plumb columns and column switching valves any way you desire. You control flow direction by specifying column switching valve positions (clockwise or counterclockwise).

Low dispersion

Low-dispersion configuration is a single-column configuration. It shortens the flow path and minimizes delay volume by bypassing the two column switching valves. However, only forward flow is available, and the column is inline at all times.

SCOUT

The optional SCOUT® Column Selector is an automated multi-column switching device that allows the flow to be diverted to up to six columns. For more information, see the *SCOUT® Column Selector User's Guide*.

1.3.5 Detectors and Monitors

Detectors and monitors are located on the left side of the workstation, behind the front door (Figure 1-7). The BioCAD 700E Workstation includes three detectors and monitors:

- pH monitor
- Conductivity monitor
- UV/Vis absorbance detector

You can connect two optional detectors:

- External detector (not shown)
- Auxiliary detector (not shown)

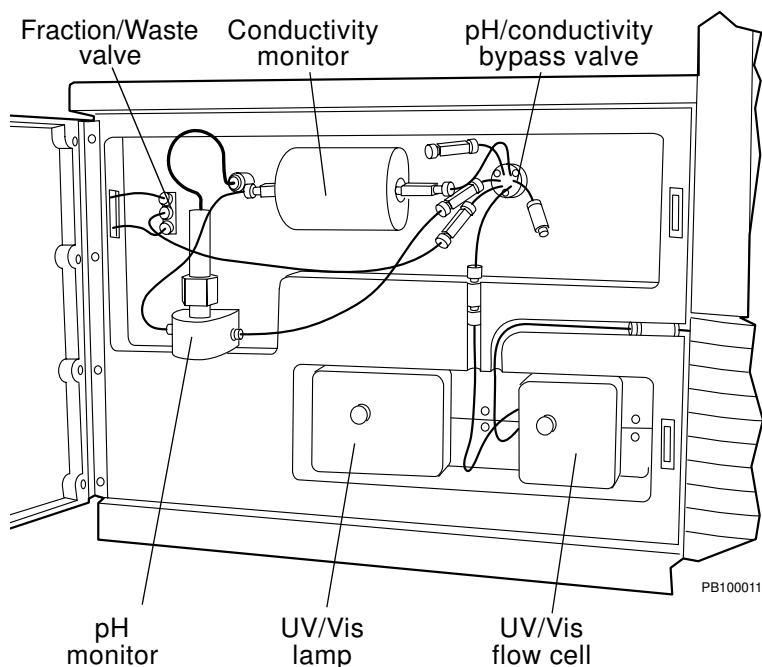


Figure 1-7 BioCAD 700E Detectors and Monitors

pH and conductivity monitors

The BioCAD 700E Workstation includes pH and conductivity monitors to monitor the buffer/solvent composition.

A 6-port selection valve allows you to bypass the pH and conductivity monitors. When you take the pH and conductivity monitors out of line, the output from the UV/Vis absorbance detector flows directly to the fraction collection valve.

NOTE: *Detector bypass affects only pH and conductivity monitors. You cannot bypass the UV/Vis detector.*

UV/Vis detectors

The BioCAD 700E Workstation has two UV/Vis detector options:

- **Internal dual-wavelength**—A Model 205 detector is installed in the workstation. This detector can function in single- or dual-wavelength mode. It includes deuterium and tungsten lamps.

A motorized grating allows data sampling at two wavelengths. However, only one lamp can be used for dual wavelength operation. The lamp used depends on the wavelength setting. The wavelength range depends on whether you select single- or dual-wavelength mode when you control detectors. See Section 5.2.6, Controlling Detectors.

Single Wavelength Range 190 nm to 800 nm	Dual wavelength Range 190 nm to 700 nm
Deuterium lamp (UV)— 190 to 365 nm	Deuterium lamp (UV)— 190 to 450 nm
Tungsten lamp (Vis)— 366 to 800 nm	Tungsten lamp (Vis)— 366 to 700 nm

- **External dual-wavelength**—An external Model 201, 204, or 205 detector is connected to and controlled from the BioCAD 700E Workstation. Dual-wavelength mode is always available, because the internal detector is used for one wavelength and the external detector is used for the second wavelength. See “External detectors” on page 1-18 for more information.

NOTE: The maximum number of UV/Vis wavelengths you can monitor is two. When you connect an external detector, only dual-wavelength mode is available, because the internal detector is used for one wavelength and the external detector is used for the second wavelength. The internal UV/Vis detector is limited to single-wavelength monitoring when you install an external UV/Vis detector.

External detectors

You can optionally connect an external UV/Vis or external fluorescence detector to the BioCAD 700E Workstation.

Use UV/Vis or fluorescence detectors provided by Applied Biosystems only (see below). The BioCAD software has complete control over the external detector functions. You program the external detector using the BioCAD software.

See Section 2.4.1, Connecting an External Detector (UV/Vis or Fluorescence), for installation instructions.

UV/Vis

You can optionally install one of the following as an external UV/Vis detector:

- Model 201 UV/Vis Detector (single-wavelength)
- Model 204 UV/Vis Detector (single-wavelength)
- Model 205 UV/Vis Detector (dual-wavelength)

See “UV/Vis detectors” on page 1-17 for more information on external UV/Vis detectors.

Fluorescence

You can optionally install a Model 304/305 Fluorescence Detector as an external detector.

Auxiliary detectors

You can optionally connect an auxiliary detector to the BioCAD 700E Workstation and collect the analog signal. Auxiliary detectors can be UV/Vis, fluorescence, refractive index, electrochemical, or any detector with an analog output.

The BioCAD software has *no* control over the auxiliary detector functions. You program the auxiliary detector using the detector controls.

See Section 2.4.2, Connecting an Auxiliary Detector, for installation instructions.

1.3.6 Fraction Collection

The fraction/waste valve (Figure 1-8) is located on the top-left side of the workstation, behind the front door.

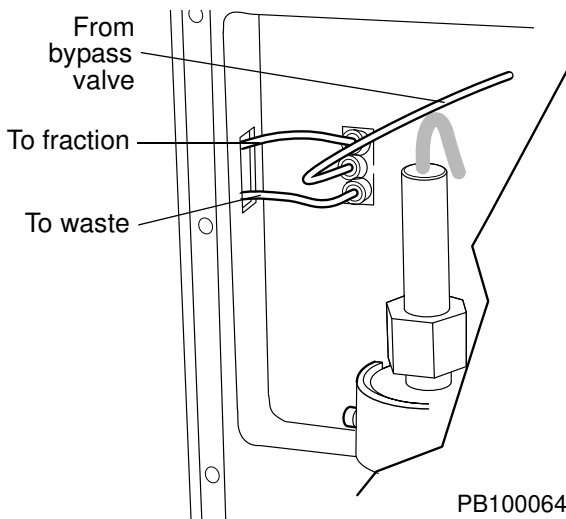


Figure 1-8 BioCAD 700E Fraction/Waste Valve

The 3-port fraction/waste valve allows you to divert the flow to waste or to collect fractions. By controlling the fraction/waste valve from the Control Panel or in a method, you have direct control over the fractions you collect.

Optional fraction collector

You can also install an optional fraction collector and control it from the Control Panel or in a method. For more information, see the following sections:

- Section 2.4.3, Connecting the Fraction Collector
- Section 3.5, Preparing the Fraction Collector
- Section 4.6, Configuring and Preparing the BioCAD Software for a Fraction Collector
- Section 5.4.1, Controlling the Fraction Collector
- Section 6.2.6, Adding Events

1.3.7 Computer Components

Hardware The BioCAD 700E Workstation includes the following minimum computer hardware components:

- Internal Pentium® 133 MHz computer with a 3.2 gigabyte hard disk drive and 64 MB RAM (Random Access Memory)
- Internal 3.5-inch HD (high density) 1.44 MB floppy disk drive
- 14-inch color monitor (optional 17-inch or 21-inch monitors available)
- Windows 95 104-key Keyboard
- Microsoft-compatible mouse (PS/2 or bus mouse)
- Network card
- SCSI (Small Computer System Interface) card
- Printer (optional)

Software The BioCAD 700E Workstation includes the following software:

- Microsoft Windows 95 Version OSR 2.5 (minimum)
- BioCAD Software
- Microsoft Office 97 (optional)
- Supervisor™ Software (optional)

NOTE: *Hardware and software components may change without notice. Contact Technical Support for more information.*

1.4 BioCAD Software Overview

BioCAD software consists of the following:

- System Control
- Configuration dialog box
- Method Editor
- Data Analysis Software

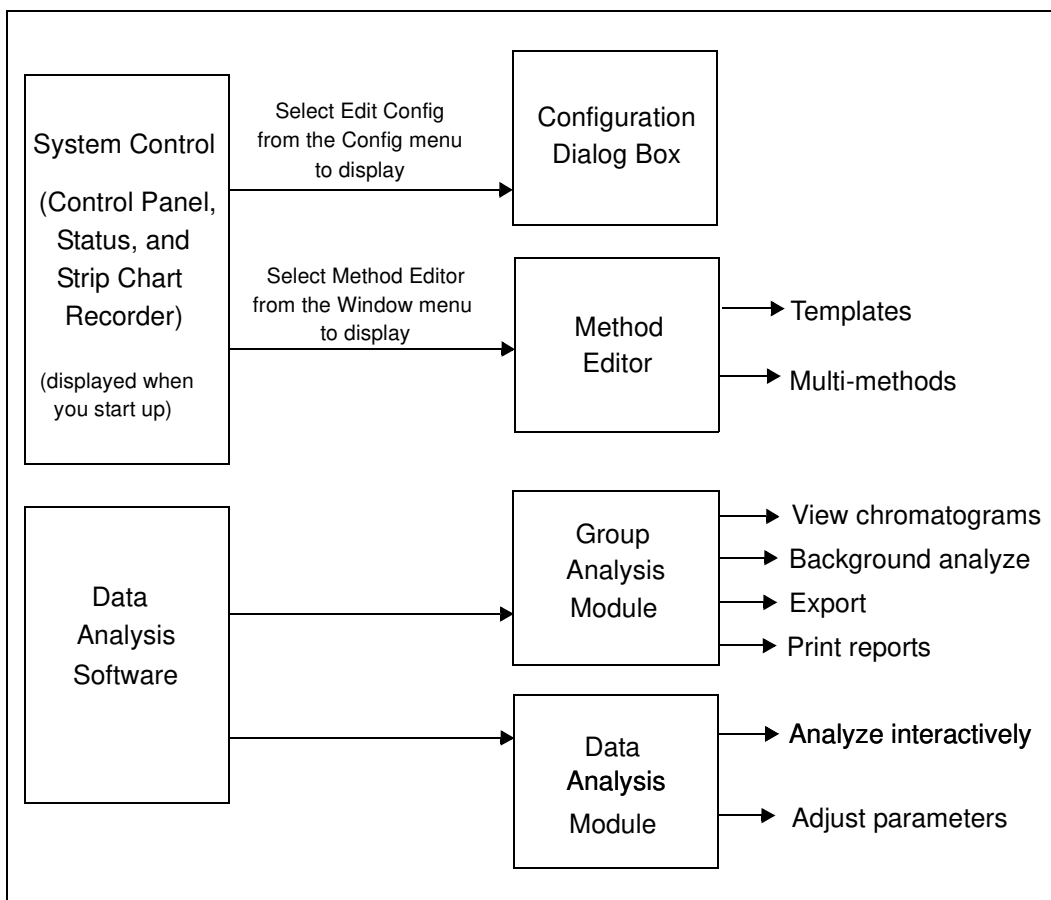


Figure 1-9 Overview of BioCAD Software

**Supervisor™
software option**

The Supervisor software option is a stand-alone version of the BioCAD software, designed to run on a computer without a BioCAD 700E Workstation. The Supervisor software option contains all software modules listed above except the Control Panel. It allows you to create and edit methods and configurations, and analyze data.

**Displaying
software version**

To display the version number of BioCAD software loaded on your workstation, select **About BioCAD** from the Window menu.

1.4.1 System Control

The System Control module allows you to:

- Manually control the workstation
- Observe run status
- Observe data collection

The System Control module includes:

- Control Panel window
- Status window
- Timer window
- Strip Chart Recorder window

The Control Panel, Status, and Strip Chart Recorder windows are displayed when you start the BioCAD software.

Control Panel The Control Panel window (Figure 1-10) allows you to manually control the workstation. You can control all parts of the system, including the flow rate, gradients, injections, detectors, and fraction collection, without creating a method.

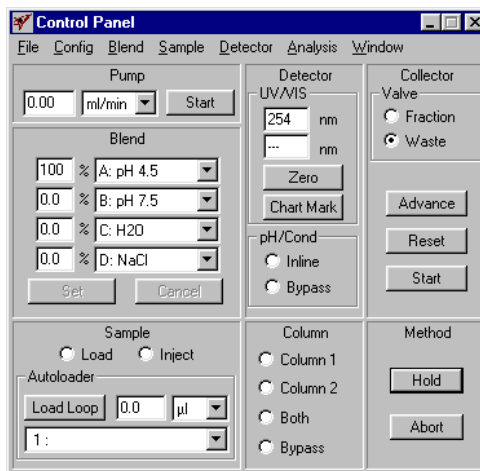


Figure 1-10 BioCAD 700E Control Panel

NOTE: Control Panel actions override a currently running method and are recorded in the method file.

The Control Panel is described in Chapter 5, Controlling the Workstation From the Control Panel.

Status The Status window (Figure 1-11) displays data about the workstation status, including pump flow rate, system pressure, monitor and detector signals (pH, conductivity, absorbance, temperature), fraction collection valve position, and information about the method that is running.

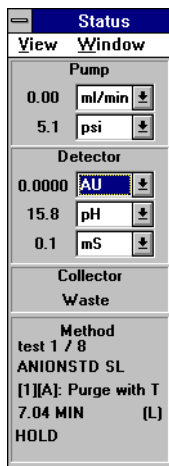


Figure 1-11 Status Window

The Status window is described in Section 8.2, Using the Status Window.

Timer The Timer window (Figure 1-12) is a utility which allows you to record elapsed volume as well as time. Use it as you would use a stop watch.

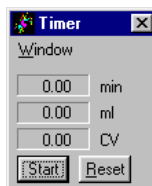


Figure 1-12 Timer Window

The Timer is described in Section 8.4, Using the Timer.

Strip Chart Recorder

The Strip Chart Recorder window (Figure 1-13) is an electronic recorder that displays and temporarily stores data as it is acquired during a method run. You determine the amount of data displayed and stored by the Strip Chart Recorder.

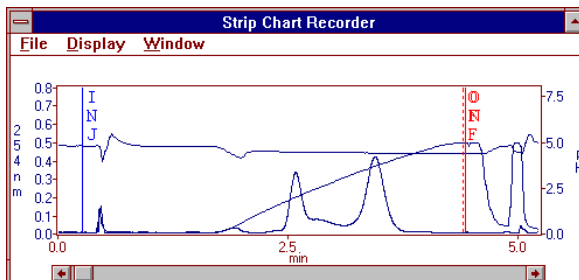


Figure 1-13 Strip Chart Recorder Window

Strip Chart Recorder data:

- Is collected in the Strip Chart Recorder whenever the pump is running, independent of the running of methods
- Is independent of data stored in data files
- Can include information from different runs
- Can be recalled and saved as data files for analysis and reporting

The Strip Chart Recorder is described in Section 8.3, Using the Strip Chart Recorder.

1.4.2 Configuration

You access the Configuration dialog box (Figure 1-14) by selecting Edit Config from the Config menu in the Control Panel.

You can create different configurations for different applications. Each configuration includes information identifying system pressure, samples, buffers/solvents, plumbing, columns, injection mode, detection, fraction collection, and data collection.

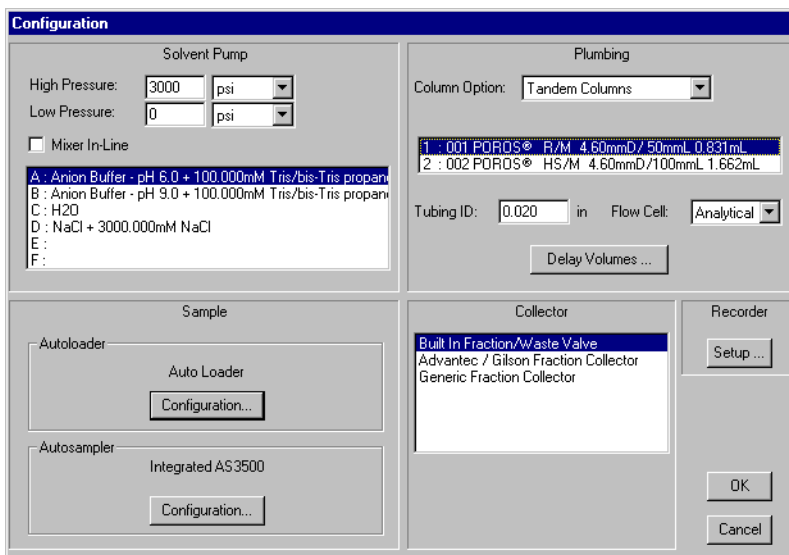


Figure 1-14 Configuration Dialog Box

The Configuration dialog box is described in Section 4.2, Creating a New Configuration.

1.4.3 Method Editor

The Method Editor window (Figure 1-15) allows you to construct and edit methods (instructions) that the BioCAD 700E Workstation uses to perform a separation and generate a data file (chromatogram).

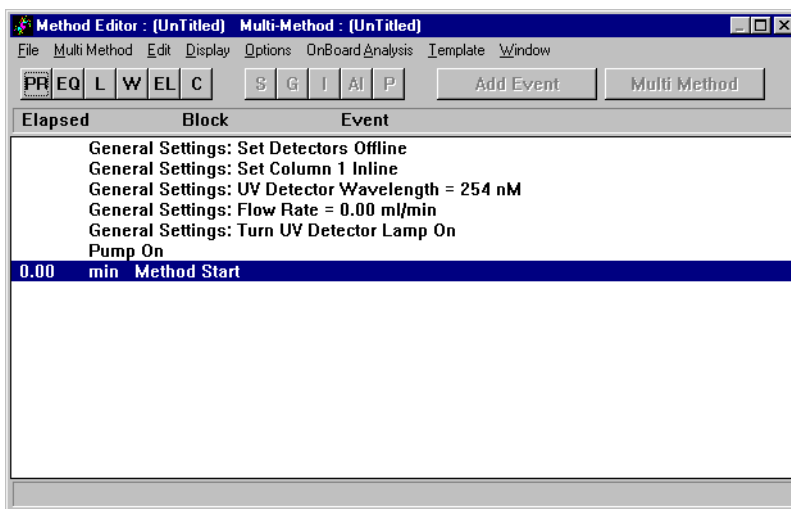


Figure 1-15 Method Editor Window

The Method Editor is described in Chapter 6, Creating and Running Methods.

Blocks, segments, and events

The Method Editor window uses an intuitive approach to building and editing methods. You build a chromatographic run using:

- **Blocks**—Six types of operations that you compile to create a method
- **Segments**—Actions you can add to a block
- **Events**—Additional customized actions you can add to a segment

For more information on blocks, segments, and events, see Chapter 6, Creating and Running Methods.

Templates

The Template feature in the Method Editor allows you to set up experimental sequences in which one or more variables changes with each method run.

For more information on templates, see Section 7.1, Creating and Running Templates.

Multi-methods

The Multi-method feature in the Method Editor allows you to:

- Set up experimental sequences in which one or more variables change
- Create your own experimental sequences for which templates do not exist
- Link methods together

For more information on Multi-methods, see Section 7.2, Creating and Running Multi-Methods.

1.4.4 Data Analysis Software

The Data Analysis Software is described in detail in the *Data Analysis User's Guide*.

The Data Analysis window allows you to:

- View chromatograms
- Background analyze single data files or groups of data files
- Export data files or chromatograms
- Print reports and chromatograms
- Analyze data files interactively
- Adjust analysis parameters
- Adjust report format parameters

1.5 BioCAD Software File Structure

This section describes:

- File types
- File contents
- Directories

1.5.1 File Types

The file types that you can create using the BioCAD software include:

File Type	File Extension
Data	.BIO or.B##, where ## are sequential numbers incremented for each data file
Method	.MET .G##, when you create methods using a multi-method
Configuration	.CFG
Group	.GRO
Multi-method	.GMM
Strip chart recorder	.DRC
Event log	.EVL

1.5.2 File Contents

Figure 1-16 illustrates the file contents for each file type.

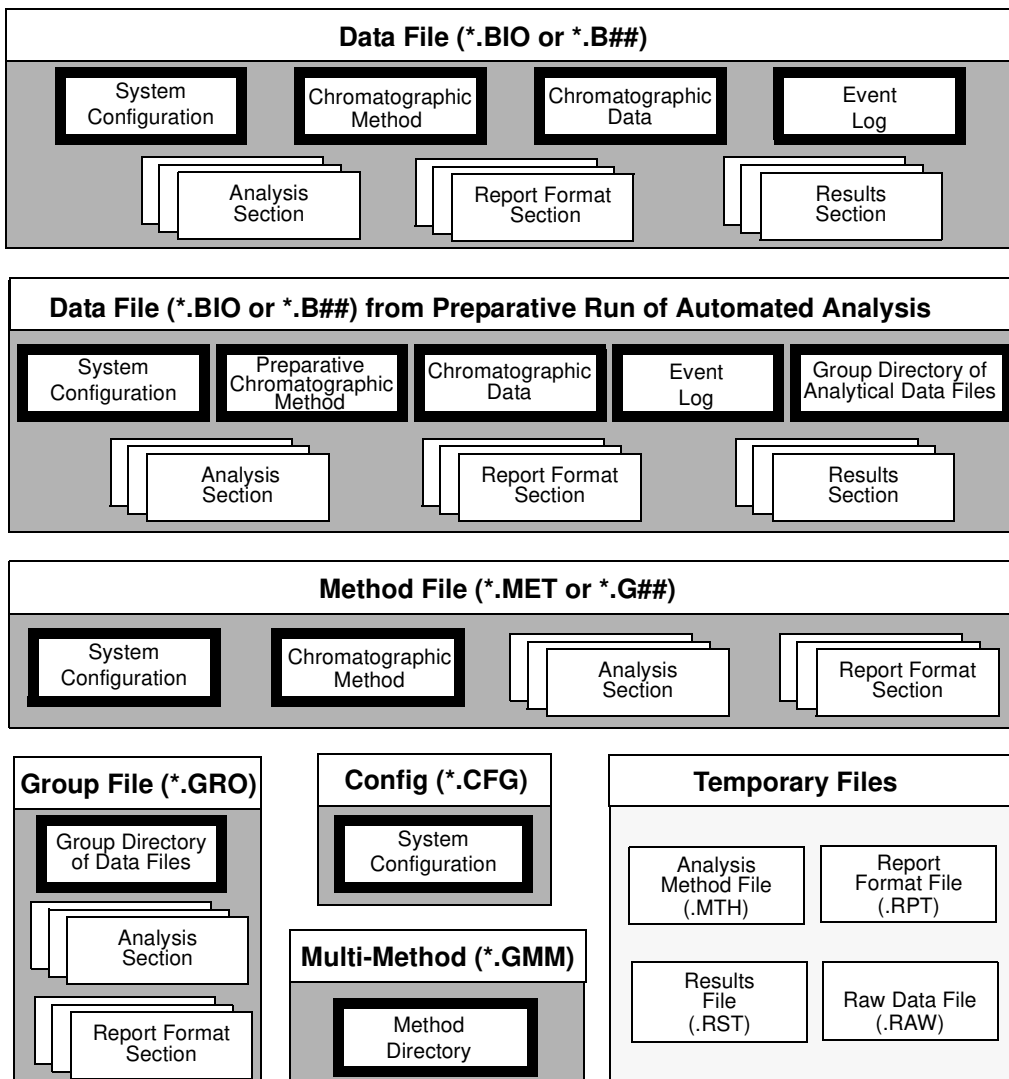


Figure 1-16 BioCAD File Contents for Each File Type

NOTE: If you save data files (.BIO or .B##) from the Strip Chart Recorder, the chromatographic method and configuration information are not automatically saved and the data cannot be exported to Microsoft Excel.

The following table describes the file contents for each file type:

File Content	Description
System Configuration	Instrument setup information
Chromatographic Method	Information that controls method run and data collection
Chromatographic Data	Chromatogram
Group Directory	List of data files in a group file
Method Directory	List of method files in a multi-method
Event Log	List of all events and errors that occur
Analysis Section	Processing (peak detection, integration, report/replot printing), and calibration parameters
Report Format Section	Parameters included in a printed report
Results Section	Analyzed data

1.5.3 Directories

Default directories

The BioCAD software is set up to store files in default directories (Figure 1-17). The default directories are:

- Biocad
- Data
- GettingStarted
- Group
- Methods
- Setup
- Temp
- Penexe (not shown)

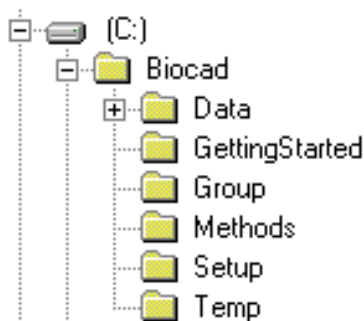


Figure 1-17 BioCAD Default Directories

You can save your files in the default directories, or create new subdirectories to save your files. To store a file in a directory other its corresponding default directory, select **Save As** instead of Save.

NOTE: It is strongly recommended that you create different subdirectories for different users and different projects. This practice helps you easily locate files of interest. See “Creating subdirectories for data files” on page 1-35.

CAUTION

The PENEXE directory contains the Data Analysis software files. Do not delete this directory.

The following table lists the file types stored in each BioCAD default directory:

Default Directory	File Type
biocad	Configuration (.CFG)—SYSCONF.CFG Event log (.EVL) Calibration (.CAL): <ul style="list-style-type: none"> • pH.CAL • COND.CAL • BLND.CAL Dictionary (.DCT library files): <ul style="list-style-type: none"> • SOLVENT.DCT • SAMPLE.DCT • SYRINGE.DCT • COLUMN.DCT <p>NOTE: Do not delete any .DCT files or the BLND.CAL file.</p> Other files stored in this directory are internal files used by the BioCAD software.
data	Data files (.BIO and .B##)
group	Group files (.GRO)
methods	Method files (.MET) Multi-method files (.GMM) Method files created in a multi-method (.G##)
setup	Setup files used to update hardware setup
temp	Strip chart recorder (.DRC and DATAREC.C##) <p>NOTE: Other files stored in this directory are internal files used by the BioCAD software.</p>

Creating subdirectories for data files

To easily store and find data files related to specific users and projects, you can create subdirectories in the C:\BIOCAD\DATA directory (Figure 1-18).

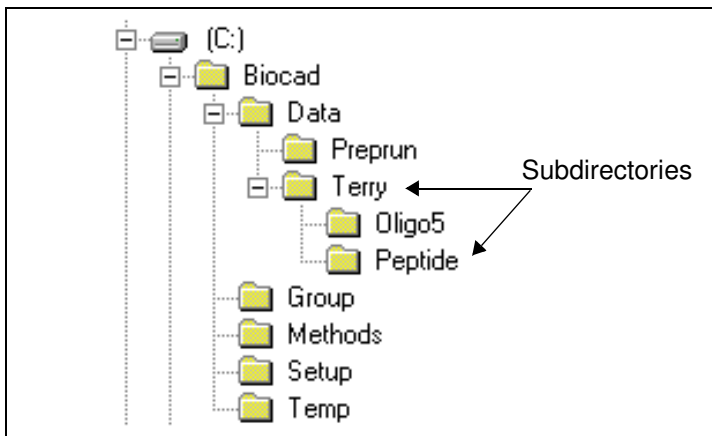


Figure 1-18 Creating Subdirectories for Data Files

You can create new subdirectories:

- When you use the Method Editor. See Section 6.2.10, Specifying Data Storage Options, for more information on creating subdirectories.
- From Windows Explorer. Refer to the *Microsoft Windows User's Guide* for more information on creating subdirectories.

1.6 A Quick Introduction to Microsoft Windows

This section introduces you to Microsoft® Windows® features that you need to know to use the BioCAD software. Refer to the *Microsoft Windows User's Guide* for more information.

This section describes:

- Using the desktop and windows
- Using the mouse and menus
- Using the Open File dialog box

1.6.1 Using the Desktop and Windows

Overview All work you perform using Windows software occurs in the *desktop*. The desktop is the screen space that Windows occupies (Figure 1-19).

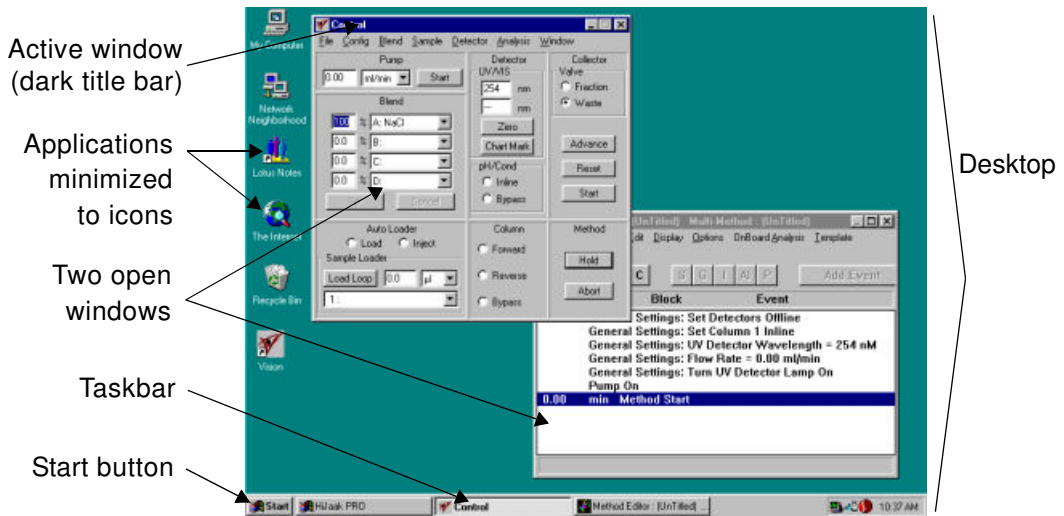


Figure 1-19 Desktop and Windows

Starting BioCAD

When you start up the BioCAD software, a *window* is displayed. A window is an area on the desktop on which a software application (for example, the BioCAD software) runs.

You can open more than one window at a time on the desktop.

Hint: When you have more than one window open, it is sometimes hard to find a particular window. To display a list of all open windows, press **Control+Escape** to display the Windows Task List. Double-click the window you want to view.

Parts of a window

A sample window is shown in Figure 1-20.

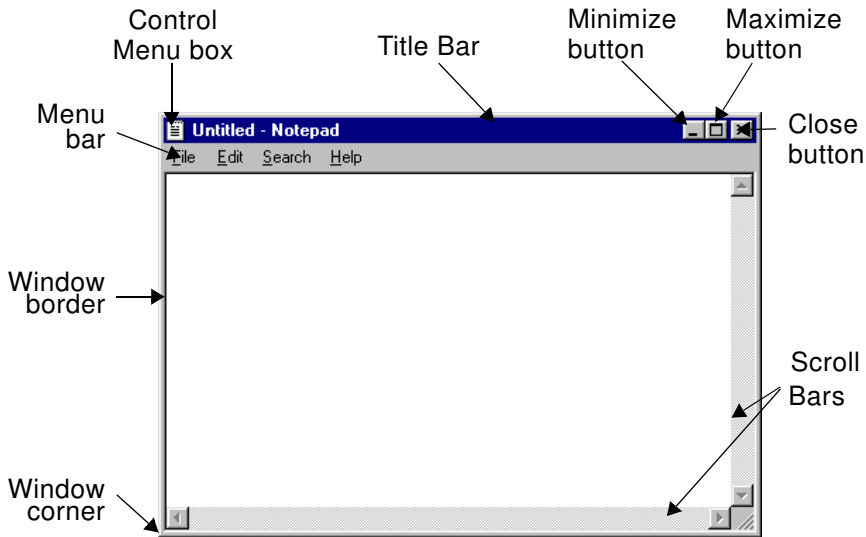


Figure 1-20 Parts of a Window

Parts of a window Parts of a window that you need to know about include:

- **Control Menu Box**—Allows you to close a window. Double-click on the Control Menu box to close a window.
- **Title Bar**—Displays the name of the window. In some BioCAD windows, the title bar also displays instructions.

If more than one window is open, only one window is the *active* window. The active window is the window affected when you type or click. The title bar of the active window is a different color than the non-active windows (see Figure 1-19).

To move a window, click-drag the title bar.

- **Close Button**—Closes the active window. You will be prompted to save any changes made before closing the file.
- **Minimize Button**—Allows you to shrink a window. Click on the Minimize button to shrink a window to a button on the taskbar.
- **Maximize Button**—Allows you to enlarge a window. Click on the Maximize button to enlarge a window to full screen size.
- **Scroll Bar**—Allows you to view different parts of a window. Click the arrows at the ends of the scroll bar to move different parts of the window into view.
- **Window Borders and Corners**—Allow you to resize a window. Move the cursor over window edges until the cursor changes to a two-headed arrow. Click-drag the border or corner to resize the window.

Click-dragging a window *border* resizes the width or length of the window. Click-dragging a window *corner* resizes the window proportionately.

- **Menu Bar**—Lists available menus. Each menu contains a list of commands.

Parts of a dialog box

A dialog box is displayed when you select a menu command followed by an ellipses (...) (Figure 1-21). See Section 1.6.2, Using the Mouse and Menus. A dialog box requests additional information.

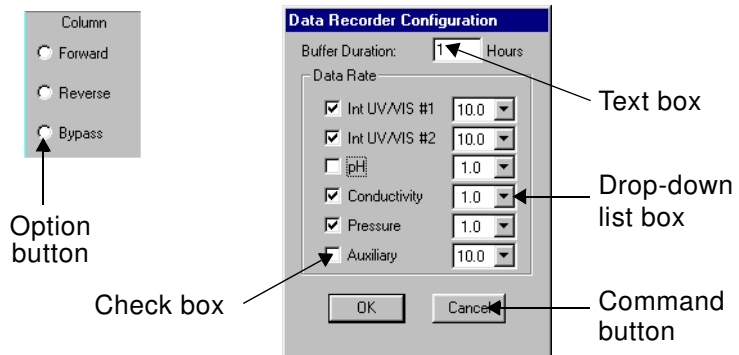


Figure 1-21 Dialog Box

Parts of a dialog box that you need to know about include:

- **Command buttons**—Click a command button to activate a command. For example, click **OK**. Command buttons that contain an ellipses (...) display a second dialog box.
- **Option buttons**—Click to enable or disable an option. Option buttons are mutually exclusive. You can select only one option at a time. The selected option contains a black dot.
- **Check boxes**—Click to enable or disable an option. You can select more than one check box at a time. A selected check box contains an "X".
- **Text box**—Type text in the box.
- **Drop-down list box**—Click the down arrow on the right to display a list of selections. To select an item, click on the item.
- **Combination box**—Functions as both a text box and a drop-down list box. You can type text or select an item.

1.6.2 Using the Mouse and Menus

Using the mouse There are several actions you perform using the mouse:

NOTE: All mouse actions listed below require that you click the left mouse button. For information on using the keyboard instead of the mouse, refer to the *Microsoft Windows User's Guide*.

- **Click**—Position the mouse pointer on the item you want to click and press the left mouse button one time to select an item.

For example, click the **OK** button or click the **Events** box.

- **Double-click**—Position the mouse pointer on the item you want to double-click and press the left mouse button two times to activate an item.

For example, double-click the **Control menu box** to close a window, or double-click an **icon** to maximize it.

- **Click-drag**—Position the mouse pointer on the item you want to drag. Press and hold the left mouse button while dragging the mouse.

For example, click-drag a window title bar to move the window or click-drag a window border or corner to resize the window.

Using menus Most menus on the menu bar have a list of associated commands. To display menu commands, click the menu. To select a command, click the command.

Note the following when using menus:

Menu Convention	Meaning
Command is dimmed or missing	<p>Command not active at this time. Either you have not selected the information needed to activate this command, or this command does not apply to the window at this time.</p> <p>For example, the Edit Multi-method command is dimmed until you open a multi-method.</p>
Ellipsis (...) follows command	<p>A dialog box is displayed when you select the command. You must enter additional information in the dialog box before the command is activated.</p> <p>For example, selecting Edit Config... from the Edit menu displays the Configuration dialog box.</p>
Check mark next to command	<p>Command is enabled.</p> <p>For example, selecting the Block View command from the Display menu in the Method Editor enables or disables this view. When checked, the Method Editor displays methods in block view. When not checked, the Method Editor displays methods in sequence view.</p>
Triangle follows command	<p>When you select this command, a cascade menu listing additional commands is displayed.</p> <p>For example, selecting the CV Units command from the Display menu in the Method Editor displays a list of columns.</p>

1.6.3 Using the Open File Dialog Box

Overview You use the Open File dialog box (Figure 1-22) to open a file, such as a configuration (.CFG), method (.MET), or data (.BIO) file.

Understanding how the Open File dialog box works will help you easily find directories and files. You access the Open File dialog box by selecting **Open Configuration** from the Control Panel File menu or **Open** from the Method Editor File menu.

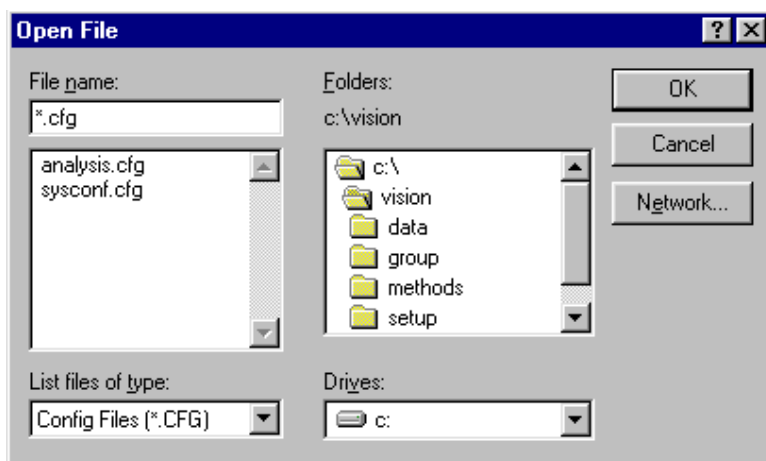


Figure 1-22 Open File Dialog Box

Parts of the Open File dialog box

The Open File dialog box includes:

- **File Name**—Allows you to type or select a file name. When opening a file, you can select from the list of file names instead of typing a file name. If the desired file names are not displayed, you probably are not viewing the correct directory or are not displaying the correct file type.
- **List Files of Type**—Allows you to display different file types (designated by extensions). To display all file types, select **All Files** [*.*].
- **Directories**—Allows you to display files from different directories and subdirectories. A directory is an area on a disk drive in which you store files. The active directory is the directory with the open file folder next to it. The File Name list box displays all files stored in the active directory.
- **Drives**—Allows you to display directories and files from different drives. Typically, your hard drive is C:, your floppy drive is A:, and network drives are designated with other letters.

File naming conventions

Maximum file name length is 8 characters (not including the extension). File names must start with a letter or number.

Do not include spaces or the following special characters in file names:

- Period (.)
- Quotation mark (")
- Slash (/)
- Backslash (\)
- Brackets ([])
- Colon (:)
- Semicolon (;)
- Vertical bar (|)
- Equal sign (=)
- Comma (,)

It is not necessary to type the extension. The BioCAD software automatically appends the correct extension for you.

Selecting a directory

You select a directory or subdirectory to display all files contained in that directory.

To select a directory, click the directory folder in the Directories list box (Figure 1-23). The directory file folder opens. The subdirectories that the directory contains are displayed below it in the Directories list box. The files that the directory contains are displayed in the File Name list box.

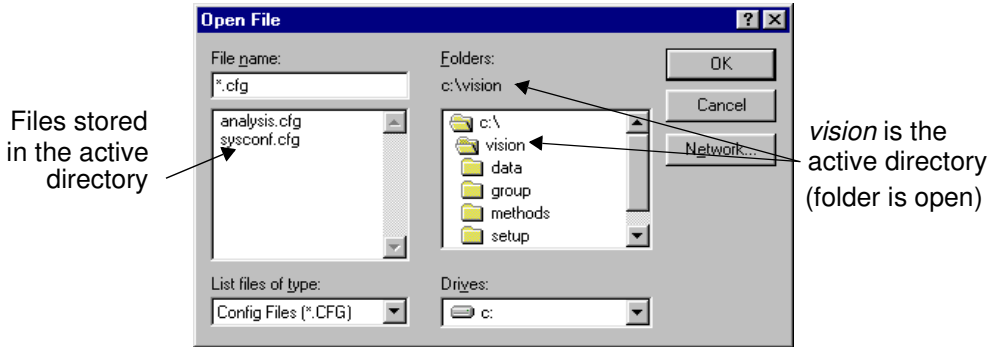


Figure 1-23 Selecting a Directory

Selecting a subdirectory

To select a subdirectory contained within the active directory, click the subdirectory folder below the open folder (Figure 1-24).

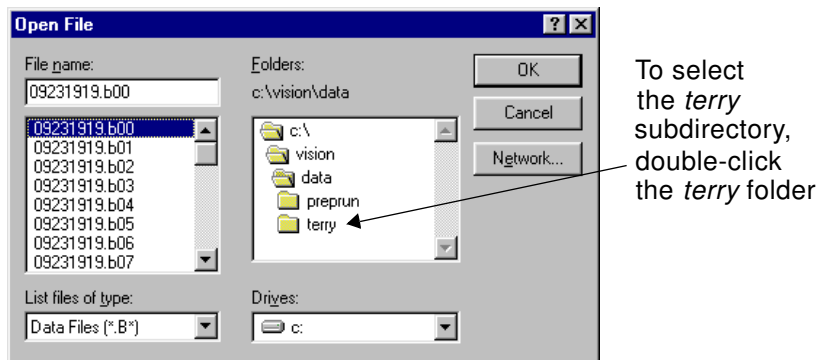


Figure 1-24 Selecting a Subdirectory

Selecting an equal or higher level directory

To select a directory at the same or higher level than the active directory, close the active directory (Figure 1-25).

For example, if you are in the C:\BIOCAD\DATA directory, but want to view method files (C:\BIOCAD\METHODS directory), double-click the **biocad** folder to close the data directory. All folders in the biocad directory are listed. Double-click the **methods** folder. You must also select **Method Files (*.MET)** from the List Files of Type drop-down list box.

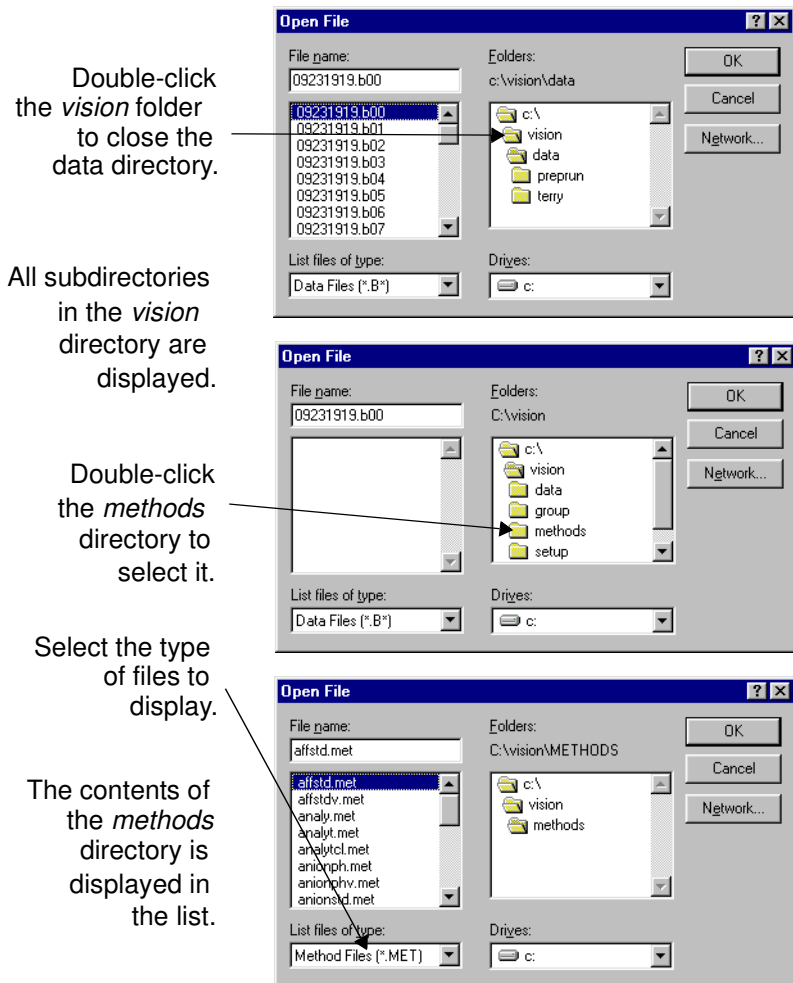
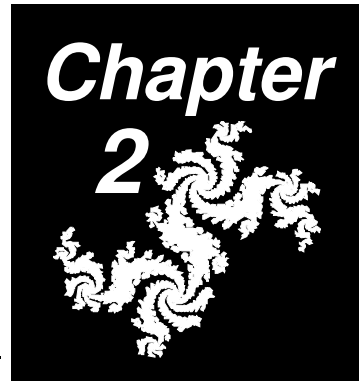


Figure 1-25 Selecting an Equal or Higher Level Directory

1

2 Installing the BioCAD 700E Workstation



This chapter includes the following sections:

2.1	Initial Installation	2-2
2.2	Site Requirements	2-3
2.3	Connecting Tubing	2-7
2.4	Connecting External Devices	2-13
2.5	Installing the BioCAD Software	2-27
2.6	Starting Up and Shutting Down	2-37

2.1 Initial Installation

Your BioCAD 700E Workstation is initially installed by a Applied Biosystems technical representative. All internal plumbing and electronic connections have been made. Do not run the workstation before it is properly installed.

Use the information in this chapter if you:

- Move the BioCAD 700E Workstation
- Install optional external components

CAUTION

If the workstation will be exposed to freezing temperatures or left unused for an extended period of time, remove the pH probe to prevent damage. Store the probe in the storage solution (saturated KCl) and protective container provided with the probe. For information on removing the probe, see Section 9.5, pH and Conductivity Monitor Maintenance.

2.2 Site Requirements

This section describes:

- General requirements
- Changing the voltage setting
- Cold room operation

2.2.1 General Requirements

Required space The BioCAD 700E Workstation requires a space measuring:

- 36 inches (91 cm) deep
- 72 inches (183 cm) wide, which includes 18 inches (46 cm) on either side of the BioCAD 700E Workstation for computer monitor, keyboard, and buffers
- 36 inches (91 cm) high

Additional space If your system includes an autosampler, fraction collector, external or auxiliary detector, or optional large monitor, allow additional space:

- **Autosampler**—18 inches (46 cm) above.
- **Fraction Collector**—18 inches (46 cm) to the left.
- **External or Auxiliary Detector**—18 inches (46 cm) above. If you are also installing an autosampler, place the detector to the left of the workstation.
- **Large Monitor**—10 additional inches (25 cm) to the left.

Weight The BioCAD 700E Workstation weighs approximately 275 pounds (125 kg).

2

Gas supplies

The BioCAD 700E Workstation requires:

- Helium source for sparge panel, with regulated maximum pressure of 5 psi (0.3 bar)
- Compressed dry air or nitrogen source for valve operation, regulated between 80 and 100 psi (5.4 to 6.8 bar)

Power and voltage requirements

The BioCAD 700E Workstation requires a grounded outlet for its three-pronged plug. Check that the BioCAD 700E Workstation is configured for the voltage of your power source (Figure 2-1). The voltage setting is displayed on the rear panel near the power plug. For instructions on changing the voltage setting, see Section 2.2.2, Changing the Voltage Setting.

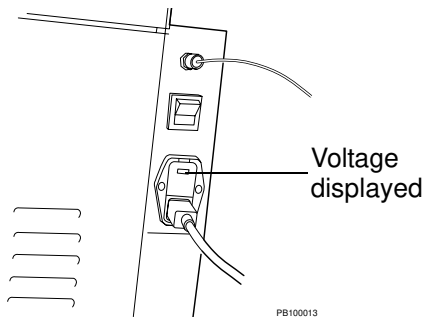


Figure 2-1 Checking Voltage

The power and voltage requirements are:

Electrical Ratings	
Volts/Amps	Fuse
100V~6A	T6A 250V
120V~5A	T5A 250V
220V~3A	T3A 250V
240V~2.5A	T2.5A 250V
50/60 Hz AC	

2.2.2 Changing the Voltage Setting

To change the voltage setting on the BioCAD 700E Workstation:

1. Power down the workstation.
2. Remove the power cord from the workstation.
3. Pry out the voltage selector cover using a small flat-blade screwdriver (Figure 2-2).

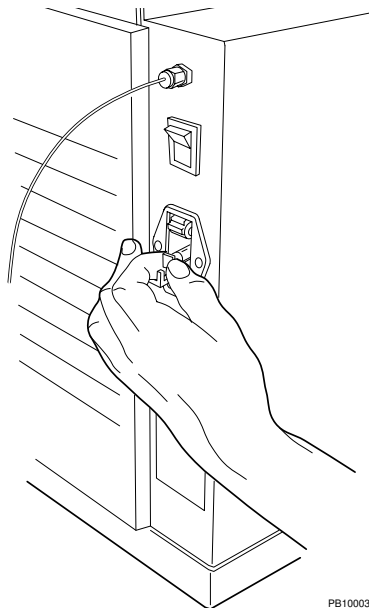
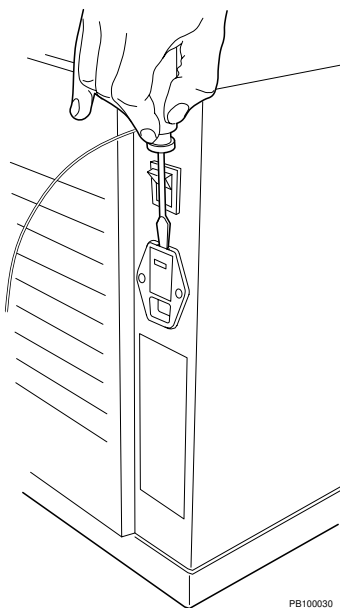


Figure 2-2 Removing the Voltage Selector Cover and Removing the Fuse Block

4. Rotate the voltage selection wheel until the proper voltage is displayed.
5. Pull out on the white tab to remove the fuse block (Figure 2-2).



- Remove the fuse and replace with a fuse of the proper rating.

Voltage	Fuse Rating
100V~6A	T6A 250V
120V~5A	T5A 250 V
220V~3A	T3A 250V
240V~2.5A	T2.5A 250V

- Insert the fuse block into the receptacle.
- Replace the voltage selector cover.
- Plug in and power up the workstation.

2.2.3 Cold Room Operation

You can install and operate the BioCAD 700E Workstation in a cold room (39° F/4° C).

CAUTION

Any instrument that you operate in the cold runs the risk of damage due to corrosion or electrical shorts caused by condensation.

Take the following precautions when installing and operating the workstation in a cold room:

- As soon as the instrument is placed in the cold room, connect the power cord and power up the workstation.
- Leave workstation powered up at all times while the workstation is in the cold room. When not in use, turn off the UV/Vis detector lamp.
- When you remove the workstation from the cold room, connect the power cord and power up the workstation as soon as possible. Leave the system power on until the workstation reaches room temperature.

2.3 Connecting Tubing

This section describes connecting:

- Compressed gas supply line
- Helium supply line
- Buffer/solvent and sparge lines
- Pump seal wash lines
- Waste lines

2.3.1 Connecting the Compressed Gas Supply Line

The BioCAD 700E Workstation requires compressed dry gas (air or nitrogen) to operate the valves.

To connect the compressed gas supply line:

1. Using Teflon® tubing (1/16-inch ID, 1/8-inch OD), connect the gas source to the fitting labeled Air on the right side of the workstation, near the top rear corner.
2. Regulate the gas between **80** and **100** psi.

2.3.2 Connecting the Helium Supply Line

The BioCAD 700E Workstation requires helium to sparge the buffers/solvents.

To connect the helium supply line:

1. Using Teflon tubing (1/16-inch ID, 1/8-inch OD), connect the helium source to the fitting labeled Helium on the right side of the system. The fitting is also labeled with the appropriate pressure, 5 psi max (Figure 2-3).
2. Regulate the helium source to **5 psi**.

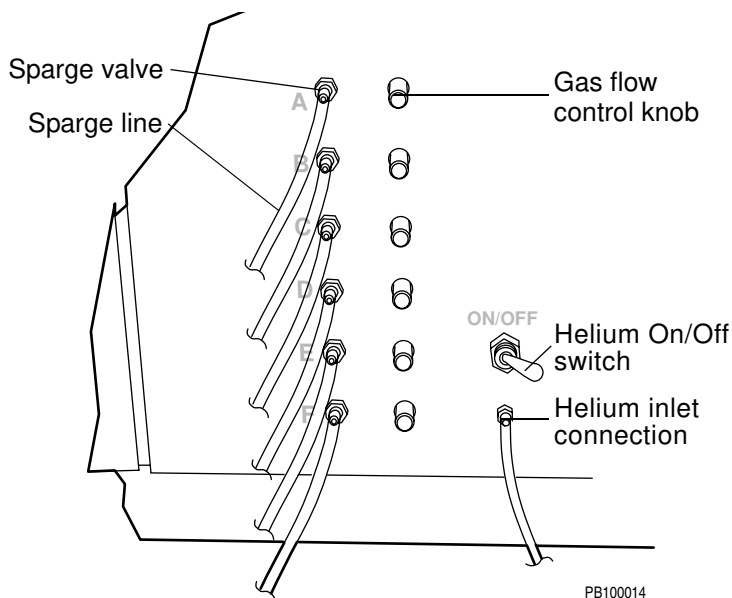


Figure 2-3 Sparge Panel Connections

CAUTION

Do not close the helium tank without turning off the helium switch. Helium diffuses out of the line, forming a vacuum, which pulls liquid into the sparging system.

2.3.3 Connecting the Buffer/Solvent and Sparge Lines

Connect the buffer/solvent bottles to the buffer/solvent pump system and the sparge panel (see Figure 2-3).

To connect the buffer/solvent and sparge lines:

- Prepare the lines and bottle caps
- Connect the sparge element
- Prepare the buffer/solvent reservoirs
- Wet the sparge element

CAUTION

Do not use methylene chloride, tetrahydrofuran, dimethyl sulfoxide, or concentrated nitric or sulfuric acids. These materials are not compatible with the PEEK components in the BioCAD 700E Workstation.

CAUTION

Certain organic solvents, such as acetonitrile and isopropanol, used over long time periods or in high concentrations can weaken PEEK tubing on the BioCAD 700E Workstation. Change the PEEK tubing on the workstation periodically if you use organic solvents frequently or in high concentrations. For more information, contact Applied Biosystems Technical Support.

Sparge kit The BioCAD 700E Workstation is provided with a sparge kit, which contains the following:

- Teflon tubing (0.085-inch ID, 1/8-inch OD, 4 feet long)
- Bottle caps (45 mm screw thread, for 1 liter bottles)
- Bottle cap inserts
- Vent plugs
- Sparge elements
- Urethane tubing (1/4-inch OD, 2 inches long)

Unused lines Place unused buffer/solvent lines in filtered deionized water. Do not leave these lines dry.

Preparing lines and bottle caps

To prepare the lines and bottle caps:

1. Slide one of the buffer/solvent lines (labeled A through F) through a bottle cap, with the top of the cap toward the system.
2. Slide a sparge line through the bottle cap. If the sparge line is connected to a sparge valve, make sure it is the sparge line that corresponds to the buffer/solvent line above (A through F).
3. Hold a bottle cap insert as shown in Figure 2-4.

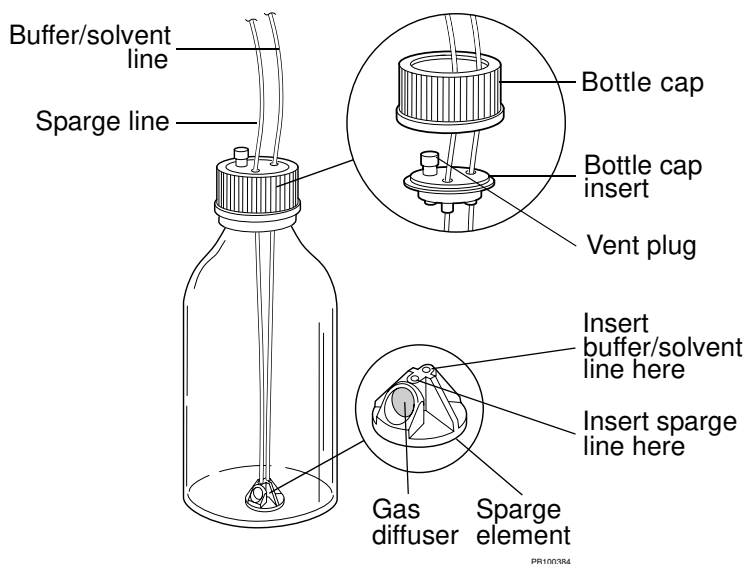


Figure 2-4 Buffer/Solvent Connections

4. Push approximately 8 inches of the sparge line through the 1/8-inch port in the bottle cap insert.
5. Push approximately 8 inches of the buffer/solvent line through the 3/16-inch port in the bottle cap insert.
6. Insert a vent plug in the largest port in the bottle cap insert.

Connecting the sparge element

To connect the sparge element to the lines:

1. Insert the reservoir-end of the sparge line snugly into the port next to the gas diffuser on the sparge element (see Figure 2-4).
2. Insert the reservoir-end of the buffer/solvent line through the open port on the sparge element. The tubing should extend just beyond the opening on the underside of the sparge element. *Do not* push the tubing beyond the bottom plane of the sparge element.

NOTE: *Do not insert the buffer/solvent line into the port next to the gas diffuser on the sparge element. This may restrict flow and cause pump cavitation.*

3. Submerge the sparge element and connected lines in the appropriate buffer/solvent or filtered deionized water.
4. Screw the bottle cap onto the bottle.

Preparing the buffer/solvent reservoirs

To prepare the buffer/solvent reservoirs:

1. Place buffer/solvent reservoirs to the right of the workstation. Place reservoirs at the same level as or slightly higher than the workstation. Place all reservoirs at the same approximate height.

CAUTION

Do not place the buffer/solvent reservoirs below the level of the workstation. Doing so could cause loss of system prime, pump cavitation, and faulty flow rate readings.

2. On the right side of the workstation, set the Helium On/Off switch to the **On** position (see Figure 2-3).
3. Open the sparge valves for the reservoirs in use by turning the gas flow control knobs on the right side of the workstation (see Figure 2-3). Open the valves until you see a steady stream of bubbles in the reservoir.

CAUTION

Do not close the helium tank without turning off the helium switch. Helium diffuses out of the line, forming a vacuum, which pulls liquid into the sparging system.

2.3.4 Connecting the Pump Seal Wash Lines

Insert the two pump seal wash lines (see Figure 1-4 on page 1-10) in a wetting agent for the pump seal wash solution. A 30 percent isopropanol/water mixture is recommended.

Place the reservoir containing the pump seal wash solution at the same level as or slightly higher than the workstation.

Filter the wash solution through a 0.22 or 0.45 micron filter before using. Change the wash solution weekly or more often if you run the system very frequently. Prime the pump seal wash lines if they become drained. See Section 3.4.1, Priming the Pump Seal Wash Lines.

2.3.5 Connecting the Waste Lines

Insert the following lines in waste containers before operating the BioCAD 700E Workstation:

- **Autoloader waste line**—1/16-inch OD Teflon tubing on the right side of the workstation (see Figure 1-4 on page 1-10).
- **Fraction/waste valve line**—1/8-inch or 1/16-inch OD Teflon tubing attached to the waste (bottom) port of the fraction/waste valve at the top-left of the workstation (see Figure 1-8 on page 1-19).

NOTE: You can also connect the fraction/waste valve line to an optional fraction collector. For more information, see Section 2.4.3, Connecting the Fraction Collector.

2.4 Connecting External Devices

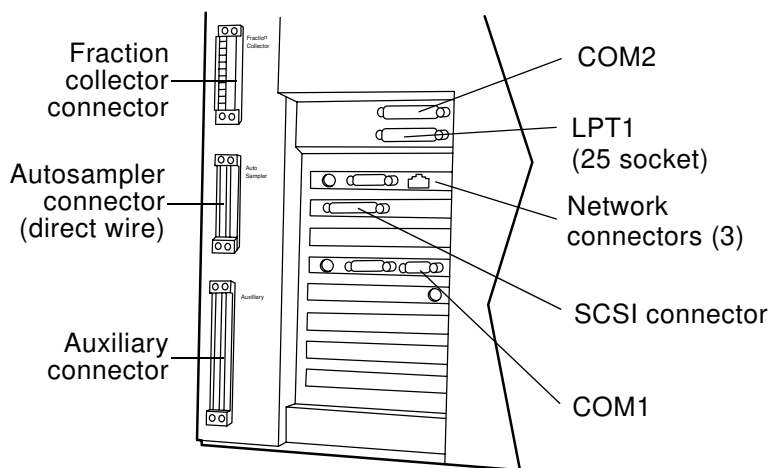
This section describes connecting:

- External detector (UV/Vis or fluorescence)
- Auxiliary detector
- Fraction collector
- Autosampler (integrated and direct wire)
- SCOUT Column Selector
- Strip chart recorder or integrator
- Keyboard, monitor, and mouse
- Printer
- Network
- Peripheral devices (SCSI)

Side panel connections

The left side panel of the BioCAD 700E Workstation contains the connectors to which you connect external devices (Figure 2-5).

NOTE: The arrangement of boards in your computer may not match Figure 2-5. To locate COM2, look for the 25-pin connector. To locate LPT1, look for the 25-socket connector.



PB100686

Figure 2-5 Connections for External Devices

2.4.1 Connecting an External Detector (UV/Vis or Fluorescence)

2

CAUTION

If you install a second detector in the system, it must have a semi-prep flow cell installed. If the second detector has an analytical flow cell, high system pressure can damage the flow cell in the first detector.

Overview

If you have one of the following external detectors provided by Applied Biosystems, you can control it from the BioCAD 700E Workstation:

- Model 201 UV/Vis Detector (single-wavelength)
- Model 204 UV/Vis Detector (single-wavelength)
- Model 205 UV/Vis Detector (dual-wavelength)
- Model 304/305 Fluorescence Detector

To use the external detector, you must:

- Connect the external detector
- Update the hardware setup
- Enable UV/Vis Channel #2 in configuration

Connecting an external detector

NOTE: *The BioCAD 700E Workstation has two COM ports. You can connect only two of the following external devices to these COM ports: external detector, integrated autosampler, or SCOUT Column Selector.*

To connect an external detector:

1. Power down the BioCAD 700E Workstation.
2. Place the external detector on top of the workstation.
If your system includes an autosampler, place the external detector to the left of the workstation.
3. Connect the detector cable to COM1 or COM2 on the left side of the workstation (see Figure 2-5).

Two cables are provided with the external detector kit. Use the cable that is compatible with the COM port. COM1 is the 9-pin connector, and COM2 is the 25-pin connector.

NOTE: When you update the hardware setup, select the same COM port you connected to above. See Section 2.5.2, Updating BioCAD Hardware Setup Options.

4. Plumb the external detector inline after the internal UV/Vis detector and before the detector bypass valve (see Figure 1-7 on page 1-16):
 - Connect the outlet line (top) from the internal UV/Vis flow cell to the inlet (bottom) of the external detector flow cell. Do not overtighten.
 - Connect the outlet line (top) from the external detector flow cell to the green connector (port 5) on the Detector Bypass valve.

CAUTION

Plumb the UV flow cell so that fluid flows into the cell at the bottom and out of the cell at the top. If plumbing connections are reversed, air bubbles can be trapped in the flow cell and cause excessively noisy baselines.

**Updating
hardware setup**

After connecting the external detector, update the hardware setup to recognize the external detector. When you update the hardware setup, select the COM port you connected to above. For directions, see Section 2.5.2, Updating BioCAD Hardware Setup Options.

**Enabling UV/Vis
channel #2**

Before using the external detector, enable the UV/VIS #2 channel in the Data Recorder Configuration. For directions, see Section 4.2.5, Configuring Detectors.

NOTE: When you connect an external detector (UV/Vis or fluorescence), the signal is displayed in the UV/VIS #2 channel in the Strip Chart Recorder and in data files.

2.4.2 Connecting an Auxiliary Detector

2

Overview

You can connect an auxiliary detector (UV/Vis, fluorescence, refractive index, electrochemical, or any detector with an analog output) to the BioCAD 700E Workstation to collect another channel of data.

You cannot control an auxiliary detector from the BioCAD 700E Workstation.

Connecting an auxiliary detector

To connect an auxiliary detector:

1. Place the auxiliary detector on top of the workstation. If your system includes an autosampler, place the auxiliary detector to the left of the workstation.

NOTE: When connecting a device to the Auxiliary connector, use a shielded cable with a ferrite bead to maintain CE compliance. Recommended source and part number for the ferrite bead is:
Fair-Rite Products Corporation, No. 2643164151.

2. Connect a cable between the 1 V output signal on the auxiliary detector and the Auxiliary connector on the workstation (see Figure 2-5 on page 2-13). Connect to the following pins on the Auxiliary connector on the left side of the workstation:
 - Pin 1 Analog Input (+) 0–1 Volt Full Scale
 - Pin 2 Analog Input (-) 0–1 Volt Full Scale

3. Plumb the auxiliary detector inline after the internal UV/Vis detector and before the detector bypass valve (see Figure 1-7 on page 1-16):
 - Connect the outlet line (top) from the internal UV/Vis flow cell to the inlet (bottom) of the auxiliary detector flow cell. Do not overtighten.
 - Connect the outlet line (top) from the auxiliary detector flow cell to the green connector (port 5) on the Detector Bypass valve.

CAUTION

Plumb the UV flow cell so that fluid flows into the cell at the bottom and out of the cell at the top. If plumbing connections are reversed, air bubbles can be trapped in the flow cell and cause excessively noisy baselines.

***Enabling
auxiliary
channel***

Before using the auxiliary detector, enable the Auxiliary channel in the Data Recorder Configuration. For directions, see Section 4.2.5, Configuring Detectors.

***Programming
event outputs***

You may need to program event outputs from the workstation to the auxiliary device. For directions, see the reference manual for the device and Section 5.2.10, Programming Event Outputs.

2.4.3 Connecting the Fraction Collector

Connecting fraction collector

To connect the fraction collector provided by Applied Biosystems to the BioCAD 700E Workstation:

1. Place the fraction collector to the left of the workstation.
2. Connect the thick cable supplied with the fraction collector:
 - Attach the orange connector to the fraction collector.
 - Attach the green connector to the left side of the workstation, to the connector labeled Fraction Collector (see Figure 2-5 on page 2-13).
3. Connect the thin valve control cable supplied with the fraction collector:
 - Attach one end to the white connector protruding from the thick cable connected to the fraction collector
 - Attach the other end to the fraction collector diverter valve
4. Plumb from the waste port (bottom port) of the BioCAD 700E fraction/waste valve (see Figure 1-8 on page 1-19) to the IN connector on the fraction collector diverter valve. Use the tubing provided with the fraction collector.
5. Plumb from the NO (normally open) line on the fraction collector to a waste container. Use the tubing provided with the fraction collector.

Configuring fraction collector

Before using the fraction collector, configure the fraction collector in the Configuration dialog box. For directions, see Section 4.6, Configuring and Preparing the BioCAD Software for a Fraction Collector.

Other fraction collectors

You can connect fraction collectors other than those supplied by Applied Biosystems to the BioCAD 700E Workstation in two ways:

- Connect to the BioCAD Fraction Collector outputs
- Connect to the BioCAD Auxiliary outputs

NOTE: You can connect the fraction collector diverter valve to the BioCAD 700E Workstation. For assistance, contact Applied Biosystems Technical Support.

Connecting to Fraction Collector outputs

You can connect any fraction collector to the Fraction Collector outputs on the workstation. You can control the diverter valve on the fraction collector from the BioCAD software if you make the connections described in the following table. Connect only to the pins that transmit the signals you need.

Fraction Collector Connection	BioCAD 700E Connection	Type of Signal
Start/Run	Fraction Collector pin 4	External Start Out
Stop	Fraction Collector pin 5	External End Out
Advance (if desired)	Fraction Collector pin 6	External Count (Advance) Out
Ground	Fraction Collector pin 3	Ground
Tube change	Fraction Collector pin 7	Mark
Valve -	Fraction Collector pin 9	Valve switch
Valve + (12 V valve)	Fraction Collector pin 10	Valve power
Valve + (24 V valve)	Auxiliary pin 10*	+24 Vdc
<p>*NOTE: When connecting a device to the Auxiliary connector, use a shielded cable with a ferrite bead to maintain CE compliance. Recommended source and part number for the ferrite bead is: Fair-Rite Products Corporation, No. 2643164151.</p>		

Connecting to Auxiliary outputs

NOTE: After connecting a fraction collector to the Fraction Collector outputs, configure the fraction collector in the Configuration dialog box. See Section 4.6, Configuring and Preparing the BioCAD Software for a Fraction Collector. Set up the fraction collector to accept inputs from the BioCAD 700E Workstation. See the fraction collector manual.

You can connect any fraction collector to the BioCAD Auxiliary outputs. However, you cannot control the fraction collector from the BioCAD software. You must program all information on the fraction collector.

Fraction Collector Connection	BioCAD 700E Connection	Type of Signal
Start/Run	Auxiliary pin 10*	Aux Out 1
Stop	Auxiliary pin 11	Aux Out 2
Advance (if desired)	Auxiliary pin 6	Spare out LC
Ground	Auxiliary pin 6	Ground
Detector +	Chart Recorder Red	Chart Rec +
Detector -/ground	Chart Recorder Black	Chart Rec -
Tube change	Fraction Collector pin 7	Mark
<p>*NOTE: When connecting a device to the Auxiliary connector, use a shielded cable with a ferrite bead to maintain CE compliance. Recommended source and part number for the ferrite bead is: Fair-Rite Products Corporation, No. 2643164151.</p>		

NOTE: When you connect a fraction collector to Auxiliary outputs as described above, do not configure the fraction collector in the Configuration dialog box.

2.4.4 Connecting the Autosampler

There are two ways to connect the autosampler:

- **Integrated**—The BioCAD software has complete control over autosampler functions. You program sample information using the BioCAD software. You must use an AS 3500 autosampler provided by Applied Biosystems.
- **Direct Wire**—The BioCAD software triggers the autosampler to make injections. You program sample information using the autosampler controls. You can use any autosampler.

Connecting integrated autosampler

NOTE: *The BioCAD 700E Workstation has two COM ports. You can connect only two of the following external devices that connect to COM ports: external detector, integrated autosampler, or SCOUT Column Selector.*

To connect the AS 3500 autosampler provided by Applied Biosystems as an integrated autosampler:

1. Power down the BioCAD 700E Workstation.
2. Place autosampler on top of or beside the workstation.
3. Connect the ribbon-style cable supplied with the autosampler:
 - Connect the 8-pin telephone-style connector to the connector labeled COMM on the autosampler.
 - Connect the 9- or 25-pin D-connector to COM1 or COM2 on the left side of the workstation (Figure 2-5 on page 2-13).

NOTE: *When you update the hardware setup, specify an integrated autosampler and select the same COM port you connected to above. See Section 2.5.2, Updating BioCAD Hardware Setup Options.*

4. Power up the autosampler.



Connecting direct wire autosampler

To connect the AS 3500 autosampler provided by Applied Biosystems as a direct wire autosampler:

1. Place the autosampler on top of or beside the workstation.
2. Connect the thin gray cable supplied with the autosampler:
 - Connect the 12-pin black connector to the autosampler.
 - Connect the green connector to the left side of the workstation, to the connector labeled Autosampler (see Figure 2-5 on page 2-13).

NOTE: When you update hardware setup, specify a direct wire autosampler. See Section 2.5.2, Updating BioCAD Hardware Setup Options.

3. Do not power up a direct wire autosampler until you configure the BioCAD software. For more information, see Section 4.8, Configuring and Preparing the BioCAD Software for a Direct Wire Autosampler.

Other autosamplers

You can connect autosamplers other than those available from Applied Biosystems to the workstation, but only as a direct wire autosampler. Connect the autosampler to the pins in the Autosampler connector (see Figure 2-5 on page 2-13) on the left side of the workstation. Connect only to the pins that transmit the signals you need. Pin assignments are listed below:

BioCAD 700E Connection	Type of Signal
Autosampler pin 1	Logic Input Ground
Autosampler pin 2	Input Injection Trigger—TTL
Autosampler pin 3	Input Stop—TTL
Autosampler pin 4	Busy Output—TTL
Autosampler pin 5	Start Output—TTL
Autosampler pin 6	Stop Output—TTL
Autosampler pin 7	Logic Output Ground

2.4.5 Connecting a SCOUT Column Selector

If your system includes a SCOUT Column Selector, refer to the *SCOUT® Column Selector User's Guide*, Chapter 2, Installing the SCOUT Column Selector.

NOTE: *The BioCAD 700E Workstation has two COM ports. You can connect only two of the following external devices that connect to COM ports: external detector, integrated autosampler, or SCOUT Column Selector.*

2.4.6 Connecting a Strip Chart Recorder or Integrator

To connect a strip chart recorder or integrator to the BioCAD 700E Workstation, connect the cables to the Chart Recorder or Integrator connectors on the left side panel of the workstation (see Figure 2-5 on page 2-13). Connect the red cable to the red connector and the black cable to the black connector.

Signals for these devices are:

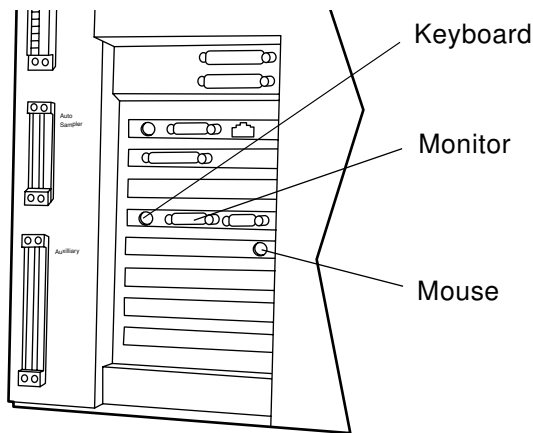
Device	Signal
Strip chart recorder	0–10 mV
Integrator	0–1 V

2.4.7 Connecting the Keyboard, Monitor, and Mouse

Connect the keyboard, monitor, and mouse to the following connectors on the left side panel of the BioCAD 700E Workstation:

Component	Connector
Keyboard	Small round connector next to monitor connector
Monitor	15-pin high-density connector (3 rows of pins), between keyboard and COM1
Mouse	Small round connector

NOTE: The arrangement of boards in your computer may not exactly match Figure 2-6. To identify the correct connectors, look for the connectors described in the above table.



PB100686

Figure 2-6 Connecting Keyboard, Monitor, and Mouse

2.4.8 Connecting a Printer

Supported printers You can connect one printer to the BioCAD 700E Workstation. Printers must be supported by Microsoft Windows 95.

Connecting Connect the printer to LPT1 connector (25-socket) on the left side panel of the workstation (see Figure 2-5 on page 2-13).

Installing Install the printer by clicking the **Start** button, pointing to **Settings**, and clicking **Printers**. Refer to the *Microsoft Windows User's Guide* for more information. If there is no print driver listed for the printer you are installing, contact Applied Biosystems Technical Support for a recommendation.

2

2.4.9 Connecting to a Network

Connecting to local area network Your BioCAD 700E Workstation is provided with a pre-installed, pre-configured Ethernet network card (see Figure 2-5 on page 2-13). You can connect the workstation to a local area network (LAN) using one of the following cables:

- 10BASE-T
- 10BASE2
- 10BASE5

Your network manager is responsible for connecting your workstation to the network, installing the appropriate client software, and maintaining your network. For more information, see the *Microsoft Windows User's Guide* and the documentation provided for the network card.

CAUTION

Do not change the configuration settings for the network card. Doing so will disrupt the operation of your workstation and can invalidate your warranty.

Diagnostic software for the network card is installed on your workstation. If you need assistance, contact Applied Biosystems Technical Support.

2.4.10 Connecting Peripheral Devices

Your BioCAD 700E Workstation is provided with a pre-installed, pre-configured SCSI (Small Computer System Interface) card (see Figure 2-5 on page 2-13). The SCSI card allows you to connect peripheral devices such as:

- CD ROM drive
- Hard drive
- Iomega® Zip™ drive

To connect a peripheral device to the SCSI card, connect the device to the SCSI connector shown in Figure 2-5 on page 2-13. For more information, see the documentation provided with the SCSI card.

CAUTION

Do not change the configuration settings for the SCSI card. Doing so will disrupt the operation of your workstation and can invalidate your warranty.

Diagnostic software for the SCSI card is installed on your workstation. If you need assistance, contact Applied Biosystems Technical Support.

2.5 Installing the BioCAD Software

This section describes:

- Installing the software
- Updating the hardware setup options
- Installing the hardware key
- Starting the software
- Exiting the software

2.5.1 Installing the Software

When to install

The BioCAD 700E Workstation is provided with the software pre-installed. If you need to re-install the software or install a new version of software, follow the procedures in this section.

Installation requirements

The instrument version of BioCAD software requires:

- A BioCAD 700E Workstation
- 100 MB of free disk space
- CD ROM drive
- Microsoft Windows 95 Version OSR 2.5 or later (provided with the system)

The Supervisor option (stand-alone version) of BioCAD software requires:

- Pentium 100 MHz or greater computer
- VGA (600 x 800) or greater resolution display
- Mouse or trackball
- 32 MB of RAM required (64 MB suggested)
- 100 MB of free disk space
- Hardware key
- 1 parallel port (for the hardware key)
- CD ROM drive access
- Microsoft Windows 95 (minimum)

Before installing

Before installing the BioCAD software, make sure all display, network, and printer driver software is installed on your computer.

Install procedure

The following procedure assumes you have an external CD ROM drive attached to your workstation. For more information on connecting an external CD ROM drive, see Section 2.4.10, Connecting Peripheral Devices.

To install the BioCAD software, you must install the Data Analysis software first, then the instrument software:

Installing Data Analysis software

1. Insert the BioCAD CD in the CD drive attached to the BioCAD 700E Workstation.
2. From the Windows desktop, click **Start**, then click **Run**.
3. In the Run dialog box, click **Browse** and navigate to find **D:\TURBOCHROM\DISK1\SETUP.EXE**. Click **Open**. Click **OK**.
4. Click **Next** to bypass the Welcome dialog box.
5. In the Choose Destination Directory dialog box, click **Next** to accept the default directory.

Temporary setup files are copied to your hard drive.

6. Click **Next** to bypass the Welcome dialog box.
7. In the Choose Destination Location dialog box, click **Next** to accept the default directory of C:\PENEXE.
8. If the Installation Options dialog box appears, select **Complete Installation** and click **Next**.
9. In the Setup Options dialog box, select only the **Help Files** check box and click **Next**.
10. In the Setup Options dialog box, select **60 Hz** and click **Next**.
11. In the Installation Options Summary dialog box, click **Next** to copy the Data Analysis software files.
12. In the Restart Computer dialog box, select **Yes** and click **Finish**.

Your computer restarts automatically.

Installing instrument software

1. From the Windows desktop, click **Start**, then click **Run**.
2. In the Run dialog box, click **Browse** and navigate to find **D:\PERSEPTIVE\DISK1\SETUP.EXE**. Click **Open**. Click **OK**.

3. Click **Next** to bypass the Welcome dialog box.
4. In the Select Instrument dialog box, select **BioCAD 700E** and click **Next**.
5. In the Setup Type dialog box, select one of the following:
 - **Instrument Full Installation**—Prompts you to enter hardware setup options
 - **Instrument Upgrade Installation**—Retains the existing hardware setup options
 - **Stand Alone**—For Supervisor (stand alone) software

Click **Next**.

6. In the Select Components dialog box, select **BioCAD Program Files** and **BioCAD Example Files** and click **Next**.
7. In the Select Program Folder dialog box, select **PerSeptive** and click **Next**.
8. If you selected Full Installation in step 5, continue to step 9. If you selected Upgrade Installation or Stand Alone in step 5, skip to step 11.
9. In the Enter Information dialog box, type the **serial number** (located on the right side panel of your workstation).
10. Enter **hardware setup options**. For more information, see Section 2.5.2, Updating BioCAD Hardware Setup Options.
11. In the Start Copying Files dialog box, click **Next** to copy the instrument software files.
12. When the Setup Complete dialog box appears, click **Finish**.

The BioCAD icon appears on the Windows desktop.

NOTE: If you delete the BIOCAD software or need to replace your hard drive, contact Applied Biosystems Technical Support for assistance in re-installing the software.

**Other
installation
options**

If you do not have an external CD ROM drive connected to your workstation, you can do one of the following:

- Install the BIOCAD software over a local area network from a computer with a CD ROM drive
- Use a computer with a CD ROM drive to create floppy disks of the BIOCAD software
- Obtain the BIOCAD software on floppy disks from Applied Biosystems

For more information, contact Applied Biosystems Technical Support.

2.5.2 Updating BioCAD Hardware Setup Options

- When to update** You must update the BioCAD Hardware Setup options if:
- You change the hardware connected to your workstation
 - No previous software version was installed on your hard drive (for example, if the software was deleted or the hard drive was replaced)

Update procedure To update the BioCAD Hardware Setup options:

1. Click **Start**, point to **Programs**, point to **PerSeptive**, and then click **BioCAD Setup**.

The Welcome dialog box is displayed.

2. Click **Next**.

NOTE: *The BioCAD 700E Workstation has two COM ports. You can connect only two of the following external devices that connect to COM ports: external detector, SCOUT Column Selector, or integrated autosampler.*

External detector 3. Select an external detector option:

- None
- UV 205—Dual-Wavelength
- UV 201—Single-Wavelength
- UV 204—Single-Wavelength
- FL 304/305—Fluorescence Detector

Click **Next**.

4. If you selected a detector in step 3, select the COM port to which the external detector is connected: COM1 or COM2 (see Figure 2-5 on page 2-13). Click **Next**.

Autosampler 5. Select an autosampler option:

- None
- Direct Wire Autosampler
- AS3500 (basic version)
- AS3500 + Cooling option
- AS3500 + Prep option
- AS3500 + Prep and Cooling options
- AFC 2000

NOTE: To determine if your AS 3500 autosampler supports the Cooling option, look for a wick below the left-hand tray.

To determine if your AS 3500 autosampler supports the Preparation option, look for two syringes behind the tray area or look for a heat/mix chamber to the left of the tray area.

6. Click **Next**.

7. If you selected an autosampler in step 5, select the COM port to which the autosampler is connected: COM1 or COM2 (see Figure 2-5 on page 2-13). Click **Next**.

SCOUT 8. If you have a SCOUT Column Selector installed, select **Yes**. If you do not have a SCOUT Column Selector installed, select **No**. Click **Next**.

9. If you selected Yes in step 8, select the COM port to which the SCOUT Column Selector is connected: COM1 or COM2 (see Figure 2-5 on page 2-13). Click **Next**.

10. Click **Next** to bypass the Setup Summary dialog box. Click **Finish** to exit the BioCAD Hardware Setup program.

NOTE: If you configure an AS 3500 autosampler, you must also set the autosampler options using the front panel of the autosampler. Refer to “Setting autosampler options” on page 3-45.

2.5.3 Installing the Hardware Key

To run the Supervisor option (stand-alone software version), you must install a hardware key before you can start the software.

Connect the hardware key to the LPT1 connector (25 socket) on your stand-alone computer. The computer side of the key is labeled. Make sure the key is firmly attached to the connector. Screw in place.

If you have a printer connected to LPT1, connect the hardware key to the connector, then connect the printer cable to the hardware key.

2

2.5.4 Starting the Software

Starting the software

To start the BioCAD software:

1. Power up the workstation and computer.

The Windows desktop screen is displayed.

2. Double-click the BioCAD icon .

The Control Panel (Figure 2-7) is displayed.

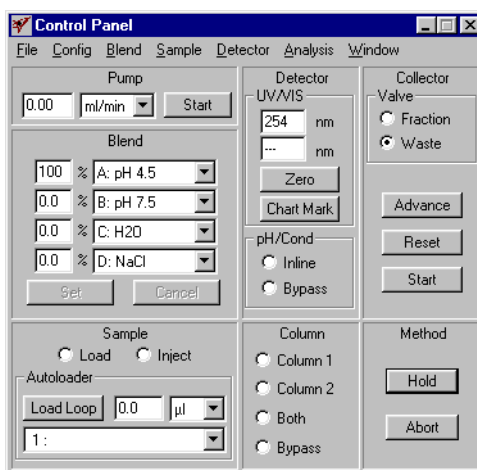


Figure 2-7 BioCAD Control Panel

NOTE: The BioCAD software automatically checks to make sure at least 20 percent of your disk is free. If the space is inadequate, a warning appears. You can exit the BioCAD software and delete files from the disk to make more room.

Supervisor option When you start the Supervisor option (stand-alone software), the Supervisor option main screen is displayed (Figure 2-8). The Supervisor option includes the Method Editor, but not the Control Panel.

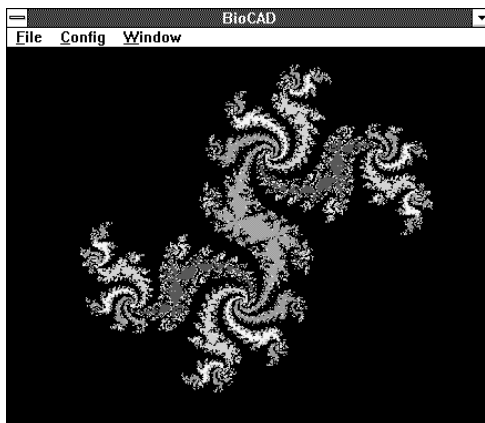


Figure 2-8 Supervisor Option Main Screen

CAUTION

You can use the Supervisor option to open data files acquired on a workstation with software earlier than version 3.0. However, when you analyze these data files in version 3.0 software, the files are updated using version 3.0 Data Analysis and cannot be opened again in the previous version of software. The data files inherit the default analysis (.MTH) and report format (.RPT) parameters of the version 3.0 software. You can adjust these parameters to make them consistent with the original .ANP file used in the earlier software version.

Hint: You may want to backup data files that you acquired using an earlier software version. You can use the backup data files with the earlier software version.

CAUTION

If you use the Supervisor option to write methods, you can run the methods on workstations with version 3.0 software or later. You cannot run these methods on workstations with earlier software versions.

2.5.5 Exiting the Software

CAUTION

If you are running the BioCAD 700E Workstation and you exit the BioCAD software, you can no longer control the workstation. Do not exit the BioCAD software while the workstation is running.

To exit the BioCAD software:

1. Select **Exit BioCAD** from the File menu in the Control Panel.

The BioCAD software closes.

2. To exit Microsoft Windows, click **Start**, then click **Shut Down**.

2.6 Starting Up and Shutting Down

This section describes:

- Starting up
- Shutting down
- Long term shutdown

Starting up

Power up the BioCAD 700E Workstation and external components in the following order:

1. Turn on the compressed dry gas source (air or nitrogen) and regulate to 80 to 100 psi.
2. Turn on the helium source and regulate to 5 psi.
3. Power up the following external components before powering up the workstation:
 - Auxiliary detector
 - External detector
 - Fraction collector
 - Integrated autosampler
 - Monitor and printer
 - SCOUT Column Selector
4. Power up the workstation by turning on the power switch, located on the right side of the workstation, near the top of the panel.
5. When Windows Desktop is displayed, double-click the **BioCAD icon** (see Figure 2-7) to start the BioCAD software.

When the Control Panel or the Supervisor Option window is displayed, the workstation is ready to configure.

NOTE: *If nothing appears on the monitor screen, check that the monitor is powered on, that the cable is connected between the monitor and the workstation, and adjust the contrast of the monitor display.*

6. After configuring the workstation for a direct wire autosampler, power up the direct wire autosampler.

Shutting down To power down the BioCAD 700E Workstation:

CAUTION

Failure to leave the pH and conductivity monitors in a 1 M salt solution and to run the SYSCLEAN method after daily use may result in system downtime.

1. Click the **Bypass** option button in the Column section of the Control Panel to take the column out of line.

NOTE: *If you configure Low Dispersion, Three Columns, or Direct Control column plumbing configurations, the column cannot be bypassed. Physically remove the column and replace with a union before priming.*

2. Purge the system with a 1 M salt solution with the pH and conductivity monitors inline. For more information, see Section 5.2.4, Purging the System.
3. Run the SYSCLEAN method supplied with the system:

NOTE: *As an alternative to running the SYSCLEAN method, you can purge the system with water from the Control Panel. Take the pH monitor offline by clicking the **Bypass** option button in the pH/Cond section of the Control Panel. Then purge the system with filtered deionized water. For more information, see Section 5.2.4, Purging the System.*

- Place all buffer/solvent lines and sample lines in filtered deionized water.
- Select **Open** from the File menu in the Method Editor to display the Open File dialog box.

- Select **SYSCLEAN.MET** from the C:\BIOCAD\METHODS directory and click **OK**.
You may see some configuration mismatch errors, which are usually due to differences in buffer/solvent and column configuration. Click **Update Method with System Configuration** to accept changes.
- Select **Run Method** from the File menu. This method takes the column, pH monitor, and conductivity monitor out of line.

4. Close the BioCAD software by selecting **Exit BioCAD** from the Control Panel File menu.

The BioCAD software closes.

5. To exit Microsoft Windows, click **Start**, then click **Shut Down**.
6. Power down the components of the workstation in any order.
7. Close the sparge valves by turning the gas flow control knobs on the right side of the workstation.
8. Set the Helium On/Off switch to the **Off** position.
9. Power down the BioCAD 700E Workstation.
10. Turn off the gas and helium sources.

Long term shutdown

If you are shutting down the BioCAD 700E Workstation for more than one week:

1. Click the **Bypass** option button in the Column section of the Control Panel, to take the column out of line.

NOTE: *If you configure Low Dispersion, Three Columns, or Direct Control column plumbing configurations, the column cannot be bypassed. Physically remove the column and replace with a union before priming.*

2. Purge the system with a 1 M salt solution with the pH and conductivity monitors inline. For more information, see Section 5.2.4, Purging the System.
3. Run the **SYSCLEAN** method as described on page 2-38.

NOTE: *As an alternative to running the SYSCLEAN method, you can purge the system with water from the Control Panel. Take the pH monitor offline by clicking the **Bypass** option button in the pH/Cond section of the Control Panel. Then purge the system with filtered deionized water. For more information, see Section 5.2.4, Purging the System.*

4. Purge the system with a solution of water and isopropanol (80:20). For more information, see Section 5.2.4, Purging the System.
5. Remove the pH probe and store as described in Section 9.5, pH and Conductivity Monitor Maintenance.
6. Close the BioCAD software by selecting **Exit BioCAD** from the File menu in the Control Panel.
7. Close Microsoft Windows by clicking **Start**, then clicking **Shut Down**.
8. Power down the components of the workstation in any order.
9. Set the Helium On/Off switch to the **Off** position.

10. Power down the BioCAD 700E Workstation.
11. Turn off the gas and helium sources.

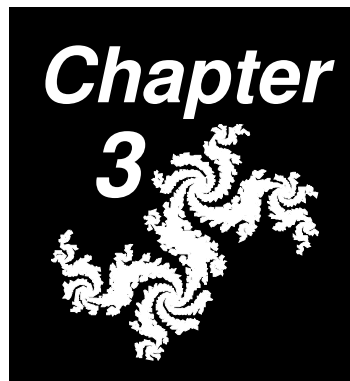
CAUTION

Do not close the helium tank without turning off the helium switch. Helium diffuses out of the line, forming a vacuum, which pulls liquid into the sparging system.

NOTE: *If you shutdown the system for an extended time period, you may want to remove the pH probe. Store the probe in the storage solution (saturated KCl) and protective container provided with the probe.*

2

3 Preparing the Workstation and External Devices



This chapter includes the following sections:

3.1	Plumbing the Workstation	3-2
3.2	Preparing Buffers, Solvents, and Solutions	3-17
3.3	Preparing Samples	3-23
3.4	Priming and Flushing the System	3-28
3.5	Preparing the Fraction Collector	3-39
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3.1 Plumbing the Workstation

This section describes:

- Selecting tubing diameter and length
- Connecting fittings to tubing
- Plumbing tubing and columns
- Changing the syringe and sample loop

NOTE: For information on plumbing a fraction collector, see Section 2.4.3, Connecting the Fraction Collector.

For information on plumbing an autosampler, see Section 3.6, Preparing an Autosampler.

3.1.1 Selecting Tubing Diameter and Length

Tubing diameter Appropriate tubing diameter for plumbing the injection valve and column switching valves is determined by flow rate. See Table 3-1 to determine the appropriate tubing diameter for each flow rate range.

Small Smaller diameter tubing adds less additional delay volume in the system and results in higher efficiency.

For maximum separation efficiency, use the smaller tubing.

Large Larger diameter tubing allows operation at higher flow rates with minimal pressure increase.

If you will be running at significantly different flow rates, use the larger tubing for maximum versatility.

NOTE: Use the appropriate size tubing for your flow rate. If you use smaller tubing and run at higher flow rates, the pump shuts down when the pressure maximum you set in the Configuration dialog box is reached.

Flow rate and tubing size

Use Table 3-1 to determine the appropriate tubing diameter for your application flow rate.

Table 3-1 Flow Rate and Tubing Size

Flow Rates	Tubing ID	Tubing Color	Tubing Volume	
			µl/ft	µl/cm
0 to 20 ml/min	0.010"	Blue	15	0.51
0 to 60 ml/min	0.020"	Orange	62	2.03
0 to 100 ml/min	0.030"	Green	139	4.56
0 to 100 ml/min, low pressure columns	0.030"	Green	139	4.56
0 to 100 ml/min, glass columns	0.030"	Green	139	4.56

NOTE: All tubing has a 1/16-inch OD.

NOTE: When using the blue 0.010-inch ID tubing, it is recommended that you run at flow rates under 20 ml/min. However, you can take the column out of line and increase the flow rate to quickly purge the system when changing to a new buffer/solvent blend.

NOTE: To minimize the back pressure of the system when running low-pressure columns, use the variable length flow cell (part number P5-1085-53-0000).

Tubing length

To determine the lengths of tubing you need for plumbing the injection valve and column switching valves for each column plumbing configuration, refer to Figure 3-6 through Figure 3-10.

Cutting tubing If you cannot find an appropriate length of tubing for the connection that you are making, you can cut your own.

Use the tubing cutter and coils of PEEK tubing provided in the startup kit. When cutting tubing:

- Make sure you cut PEEK tubing ends squarely
- Minimize the length of tubing you use

3.1.2 Connecting Fittings to Tubing

The BioCAD 700E Workstation uses four types of fittings:

- Rheodyne RheFlex® fittings (Figure 3-2)
- Compression ring fittings (Figure 3-3)
- Wing nut fittings (Figure 3-4)
- Low-pressure plastic fittings (Figure 3-5)

Figure 3-1 shows the location of the four fitting types.

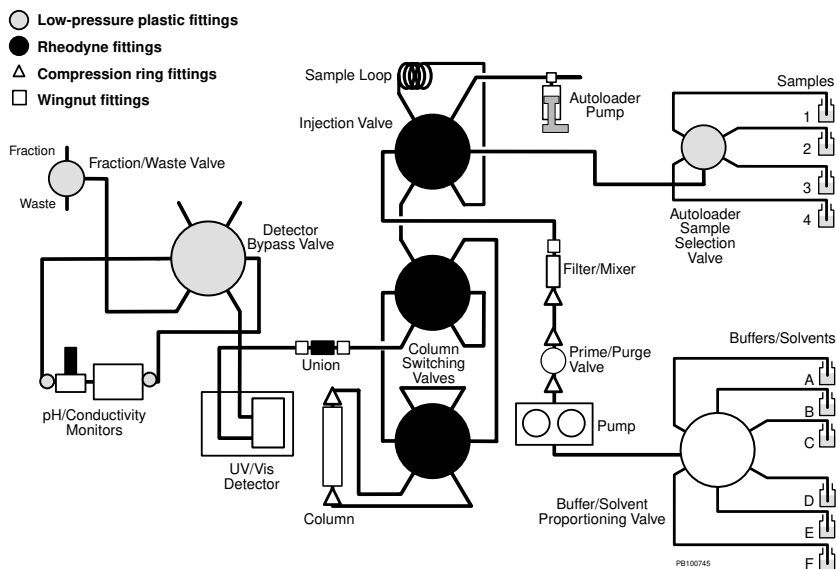


Figure 3-1 BioCAD 700E Workstation Fitting Diagram

CAUTION

Plumb the UV/Vis flow cell so that fluid flows into the cell at the bottom and out of the cell at the top. If plumbing connections are reversed, air bubbles can be trapped in the flow cell and cause excessively noisy baselines.

NOTE: Fittings on the buffer/solvent proportioning valve are plastic barbed slip-on fittings, not low-pressure plastic screw-on fittings.

Rheodyne RheFlex fittings

Use Rheodyne RheFlex fittings to connect tubing to:

- 6-port injection valve
- 6-port column switching valves

To connect Rheodyne RheFlex fittings to tubing:

1. Slip a compression fitting over the end of the tubing.
2. Slip a split ferrule over the end of the tubing, with the tapered end of the ferrule pointing away from the compression fitting. The split end of the ferrule should point toward the compression fitting. Make sure at least 1/16-inch of tubing extends beyond the ferrule (Figure 3-2).

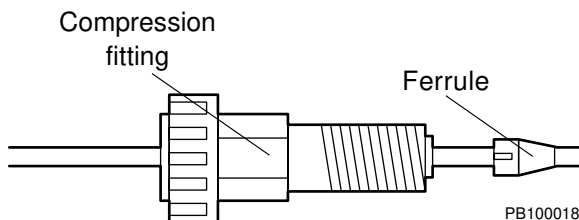


Figure 3-2 Connecting Rheodyne RheFlex Fittings to Tubing

3. Bottom the tubing in a port. Finger-tighten the compression fitting.

Compression ring and wing nut fittings

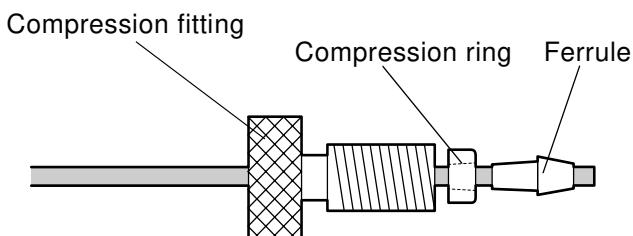
Use compression ring fittings or wing nut fittings to connect tubing to:

- Columns
- Prime/purge valve
- Filter/Mixer
- Union to the UV/Vis detector flow cell

NOTE: To connect PEEK columns, do not use standard stainless steel fittings that require tightening with a wrench. Overtightening can strip the threads of the column.

To connect compression ring fittings to tubing:

1. Slip a stainless steel compression fitting over the end of the tubing.
2. Slip a stainless steel ring over the end of the tubing with the flat end of the ring toward the fitting.
3. Slip a PEEK ferrule over the end of the tubing with the narrow end of the ferrule toward the ring. Make sure at least 1/16-inch of tubing extends beyond the ferrule (Figure 3-3).



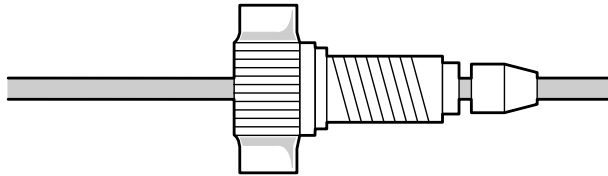
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Figure 3-3 Connecting Compression Ring Fittings to Tubing

4. Bottom the tubing in a port. Finger-tighten the compression fitting. Tighten 1/4 turn with needle-nose pliers.

To connect wing nut fittings to tubing:

1. Slip a wing nut fitting over the end of the tubing.
2. Slip a ferrule over the end of the tubing, with the tapered end of the ferrule pointing away from the compression fitting. Make sure at least 1/16-inch of tubing extends beyond the ferrule (Figure 3-4).



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Figure 3-4 Connecting Wing Nut Fittings to Tubing

3. Bottom the tubing in a port. Finger-tighten the compression fitting.

Low-pressure plastic fittings

Use 1/4-28-inch low-pressure plastic fittings to connect low-pressure tubing to:

- Autoloader sample selection valve
- Detector bypass valve
- Fraction/Waste valve
- pH and conductivity monitors

To connect plastic fittings to tubing:

1. Slip the plastic compression fitting over the end of the tubing.
2. Slip a plastic ferrule over the end of the tubing with the tapered end toward the compression fitting. Use a yellow ferrule with 1/8-inch tubing. Use a blue ferrule with 1/16-inch tubing. Make sure tubing is flush with the ferrule (Figure 3-5).

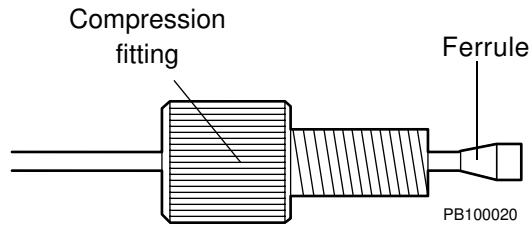


Figure 3-5 *Connecting Low-Pressure Plastic Fittings to Tubing*

3. Bottom the tubing in a port. Finger-tighten the compression fitting.

3.1.3 Plumbing Tubing and Columns

Plumbing configurations

The BioCAD 700E Workstation supports four standard plumbing configurations and one customized configuration:

- Single Column
- Tandem Columns
- Three Columns
- Low Dispersion
- Direct Control

Configurations and tubing lengths for each configuration are shown in Figure 3-6 through Figure 3-10.

Hint: You can display plumbing configurations on the workstation monitor by selecting **Show Column Plumbing** or **Show System Plumbing** from the Config menu in the Control Panel. The system plumbing diagram shows the Single Column plumbing configuration.

Plumbing tubing and columns

Connect tubing and columns to a BioCAD 700E Workstation following one of the plumbing configurations shown in Figure 3-6 through Figure 3-10.

See “Column plumbing configurations” on page 1-15 for more information on each plumbing configuration.

NOTE: After connecting the columns and tubing to the workstation, you must edit the system configuration to match the physical plumbing. See Section 4.2.4, Configuring Columns and Plumbing.

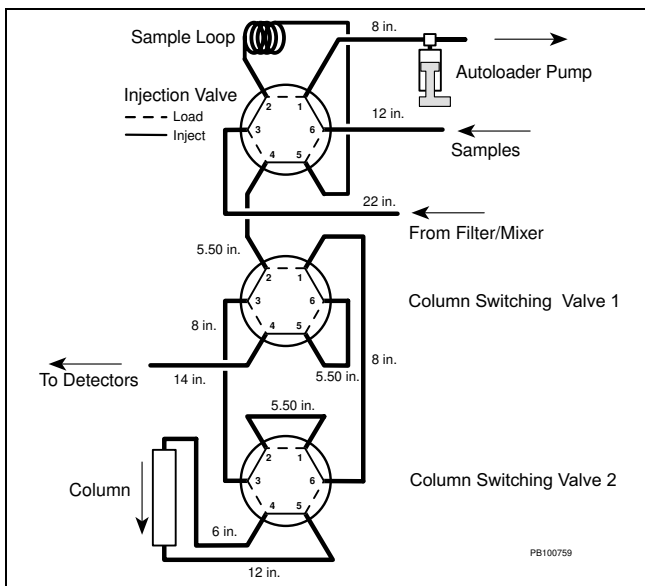


Figure 3-6 BioCAD 700E Single Column Configuration

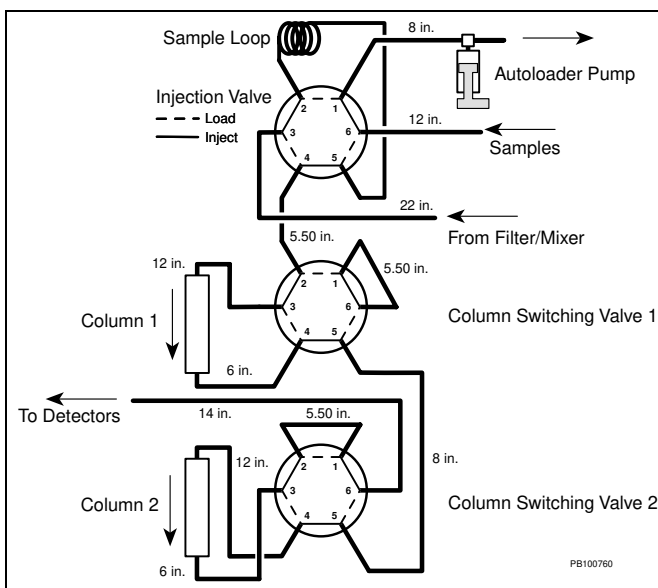


Figure 3-7 BioCAD 700E Tandem Column Configuration

3

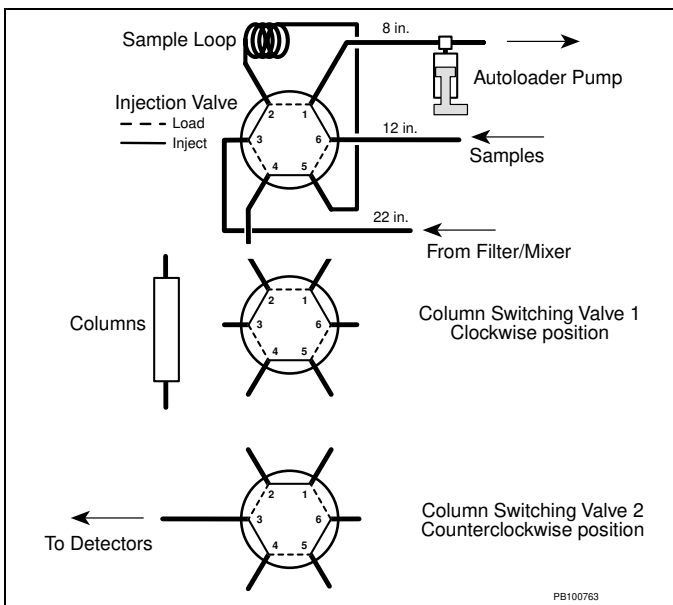


Figure 3-10 BioCAD 700E Direct Control Configuration

3

3.1.4 Changing the Syringe and Sample Loop

You may need to change the syringe and sample loop to accommodate your injection volume when making autoloader or manual injections.

NOTE: Pump injections and autosampler injections do not use the syringe and sample loop on the BioCAD 700E.

Selecting syringe and sample loop size

Injection volume determines the syringe and sample loop size. To achieve maximum injection volume accuracy and precision, follow these guidelines when specifying injection volumes and selecting sample loop sizes:

- For injection volumes less than 50 percent of the sample loop volume, enter the injection volume in the Control Panel or method.
- For injection volumes equal to the sample loop volume, enter a volume of three times the sample loop volume in the Control Panel or method. For example, you can accurately inject a 100 μ l sample using the 100 μ l sample loop if you use an injection volume of 300 μ l.
- Do not inject volumes between 50 percent and 100 percent of the sample loop volume.

NOTE: The actual volume injected onto the column never exceeds the sample loop size.

Changing syringe

Change the syringe to accommodate different injection volumes. Syringes provided with the BioCAD 700E Workstation include:

- 500 μ l—Injection volume range 0.001 to 0.5ml
- 5 ml—Injection volume range 0.01 to 5 ml

To change the syringe, you must:

- Remove the existing syringe
- Attach a new syringe

Removing To remove the syringe:

1. Select **Change Syringe** from the Sample menu on the Control Panel.

The syringe plunger retracts and the Set Syringe Size dialog box is displayed (Figure 3-11).

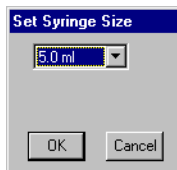


Figure 3-11 Set Syringe Size Dialog Box

CAUTION

*Do not remove the syringe before retracting the plunger.
The syringe will break.*

2. Unscrew and remove the knurled knob at the base of the plunger.
3. Unscrew the body of the syringe (turn to the left) and remove.

Attaching To attach a new syringe:

1. Place the hole in the base of the plunger over the pin on the syringe piston.

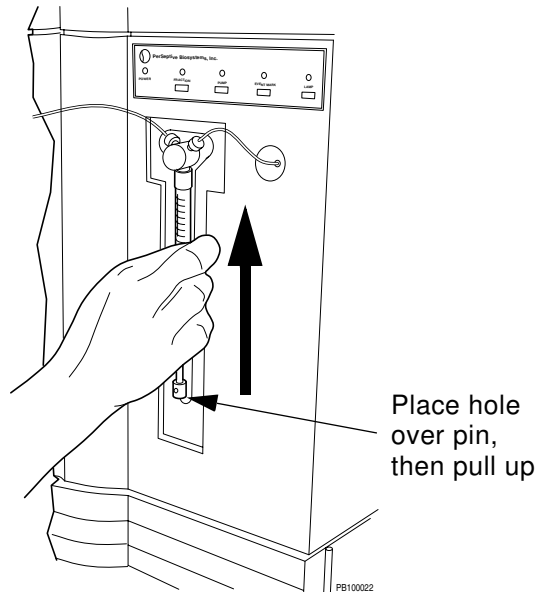


Figure 3-12 Replacing the Syringe

2. Pull up on the syringe body until it reaches the fitting.
3. While pushing up gently, turn the syringe body to the right and screw into place.
4. Replace the knurled knob in the plunger base.
5. Select the **size** of the syringe you installed from the drop-down list box in the Set Syringe Size dialog box. Click **OK**.

The syringe plunger returns to the home position.

NOTE: The home position varies with different syringe sizes. The syringe size you select updates the syringe size in the Configuration dialog box.

Changing sample loop

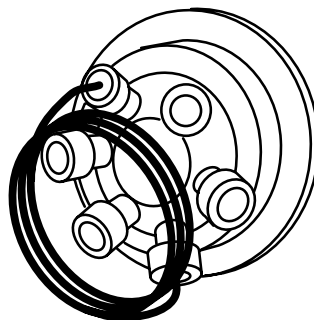
Change the sample loop to accommodate different injection volumes. Sample loops provided with the BioCAD 700E Workstation include:

- 100 μ l
- 2 ml
- 5 ml

To change the sample loop:

1. Unscrew the installed loop from positions 2 and 5 on the sample injection valve.
2. Replace with the appropriate sample loop.

Connect loop to positions 2 and 5 on injection valve



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Figure 3-13 *Installing a Sample Loop*

3. Select **Change Loop Size** from the Sample menu on the Control Panel.

The Set Sample Loop Size dialog box is displayed.

4. Type the **size** of the loop you installed and click **OK**.

NOTE: *The sample loop size you type updates the sample loop size in the Configuration dialog box.*

3.2 Preparing Buffers, Solvents, and Solutions

This section describes preparing:

- Buffers/solvents
- pH blend mode buffers
- Pump seal wash solution

NOTE: For information on preparing autosampler solvents, see Section 3.6, Preparing an Autosampler.

Incompatible solvents

CAUTION

Do not use methylene chloride, tetrahydrofuran, dimethyl sulfoxide, or concentrated nitric or sulfuric acids. These materials are not compatible with the PEEK components in the BioCAD 700E Workstation.

CAUTION

Certain organic solvents, such as acetonitrile and isopropanol, used over long time periods or in high concentrations can weaken PEEK tubing on the BioCAD 700E Workstation. Change the PEEK tubing on the workstation periodically if you use organic solvents frequently or in high concentrations. For more information, contact Technical Support.

Organic solvents

CAUTION

*If you use organic solvents, bypass the pH and conductivity monitors to prevent damage. You can bypass the pH and conductivity monitors by clicking the **Bypass** option button in the pH/Cond section of the Control Panel or in the General Settings of a method.*

3.2.1 Preparing Buffers/Solvents

You can use up to six buffers/solvents on the BioCAD 700E Workstation. You can blend up to four buffers/solvents.

CAUTION

If you use salt solutions, be aware that lower grade salts can have high acid contamination, which can decrease the pH of the buffer/solvent.

Filtering Filter all buffers/solvents through a 0.22 or 0.45 micron filter before using them in the workstation.

You do not need to filter HPLC-grade liquid solvents, but you should sparge them.

Location Place buffer/solvent reservoirs to the right of the workstation. Place reservoirs at the same level as or slightly higher than the workstation. Place all reservoirs at the same approximate height.

CAUTION

Do not place the buffer/solvent reservoirs below the level of the workstation. Doing so could cause loss of system prime, pump cavitation, and faulty flow rate readings.

Unused lines Place unused buffer/solvent lines in filtered deionized water. Do not leave these lines dry.

Sparging Sparge all buffers/solvents used on the BioCAD 700E Workstation. Sparging prevents outgassing and helps ensure pump flow rate precision and gradient accuracy.

***Sparging the
buffer/solvent***

Make sure the buffer/solvent bottles are connected according to Section 2.3.3, Connecting the Buffer/Solvent and Sparge Lines. Adjust the following controls on the right side panel of the system:

1. Set the Helium On/Off switch to the **On** position.
2. Open the sparge valves for the reservoirs in use by turning the gas flow control knobs on the right side panel (see Figure 2-3 on page 2-8). Open the valves until you see a steady stream of bubbles.
3. Allow 15 minutes for the helium sparging to equilibrate.

CAUTION

Do not close the helium tank without turning off the helium switch. Helium diffuses out of the line, causing a vacuum to form, pulling liquid into the sparge system.

***Before changing
buffer/solvent***

Before changing to a new buffer/solvent, make sure the sparge line is clear. If there is any liquid in the sparge line:

1. With the sparge element in the original buffer/solvent, blow helium through the sparge line by opening the gas flow control knob for a few seconds.
2. Remove the sparge element and connected lines from the original buffer/solvent and submerge them in the new buffer/solvent.

NOTE: *This step prevents contamination of the new buffer/solvent.*

3.2.2 Preparing pH Blend Mode Buffers

pH blend mode The BioCAD 700E Workstation allows you to run in three blend modes: Percentage, Eluent, and pH. To run in pH blend mode, you need to prepare four buffers/solvents:

- Two pH blend buffers (anion or cation)
- A salt solution
- Water

NOTE: Configure the two pH buffers as type *Buffer*, the salt solution as type *Eluent*, and the water as type *H2O*. For more information, see Section 4.2.2, Configuring Buffers/Solvents, and Section 4.5.1, Customizing the Buffer/Solvent Library.

The three types of pH blend mode buffers you can prepare are:

- **pH Blend Buffers**—Special anion or cation pH buffers for which the workstation is already calibrated. These buffers exist in the Buffer/Solvent Library. Recipes for these buffers appear on page 3-21. When you blend in pH mode, you specify a *pH* value in the Control Panel, method segment, or pH Map template. The BioCAD software then calculates the percent of each buffer needed to achieve the desired pH.
- **Custom pH Blend Buffers (Calibrated)**—Any pair of buffers can be used for pH blending. You calibrate custom buffers using the Calibrate pH Mode Buffers feature. (For more information, see Section 5.2.9, Calibrating Custom pH Blend Buffers.) You will need to add these buffers to the Buffer/Solvent Library. When you blend in pH mode, you specify a *pH* value in the Control Panel, method segment, or pH Map template. The BioCAD software then calculates the percent of each buffer needed to achieve the desired pH.
- **Custom pH Blend Buffers (Uncalibrated)**—Any pair of buffers can be used for pH blending. You will need to add these buffers to the Buffer/Solvent Library. When you blend in pH mode, you specify a *percentage* value in the Control Panel, method segment, or pH Map template.

pH blend buffers The Buffer/Solvent Library in the BioCAD software contains the following pH blend mode buffers:

- Anion Buffer - pH 6.0 + 100.0 mM Tris/bis-Tris propane
- Anion Buffer - pH 9.0 + 100.0 mM Tris/bis-Tris propane
- Cation Buffer - pH 4.5 + 100.0 mM MES/HEPES/Acetate
- Cation Buffer - pH 7.5 + 100.0 mM MES/HEPES/Acetate
- H₂O
- NaCl + 3000.0 mM NaCl

Recipes for special anion and cation buffers appear below. Buffer strength of the buffers should be two to five times more concentrated than the buffer strength of the blend needed for the separation. For example, 100 mM pH buffers are appropriate for pH separations that require a 20 to 50 mM blend concentration.

Anion buffers Use anion buffers with anion exchange columns. Anion buffers cover the pH range of 6 to 9. Anion buffers for pH Map templates are composed of:

- 50 mM Tris
- 50 mM bis-Tris propane

Make two bottles of buffer with a total buffer concentration of 100 mM. Adjust one buffer to pH 6.0 (with HCl) and the other to pH 9.0 (with NaOH).

Cation buffers Use cation buffers with cation exchange columns. Cation buffers cover the pH range 4.5 to 7.5. Cation buffers for pH Map templates are composed of:

- 33.3 mM HEPES—(N-[2-Hydroxyethyl]piperazine-N'-[2-ethanesulfonic acid])
- 33.3 mM MES—(2-[N-Morpholino]ethane-sulfonic acid)
- 33.3 mM sodium acetate

Make two bottles of buffer each with a total buffer concentration of 100 mM. Adjust one buffer to pH 4.5 (with HCl) and the other to pH 7.5 (with NaOH).

NaCl and water Prepare 3 M NaCl using HPLC-grade water.

3.2.3 Preparing Pump Seal Wash Solution

Choose a wetting agent for the pump seal wash solution. A 30 percent isopropanol/water solution is recommended.

Filtering Filter the wash solution through a 0.22 or 0.45 micron filter before using.

Location Place the reservoir containing the pump seal wash solution at the same level as or slightly higher than the workstation.

Replacement Change the wash solution weekly or more often if you run the system very frequently.

NOTE: For information on connecting the pump seal wash lines, see Section 2.3.4, Connecting the Pump Seal Wash Lines.

For information on priming the pump seal wash lines, see Section 3.4.1, Priming the Pump Seal Wash Lines.

3.3 Preparing Samples

This section describes how to prepare samples for:

- Autoloader injections (small vessels)
- Autoloader injections (large vessels)
- Direct autoloader injections
- Manual injections
- Pump injections

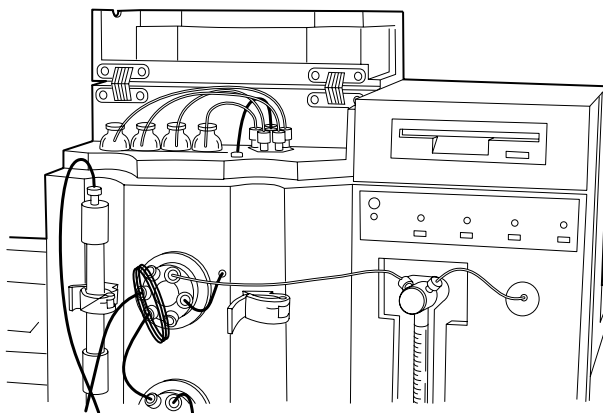
For information on preparing samples for autosampler injections, see Section 3.6, Preparing an Autosampler.

NOTE: To determine which injection mode to use, see “Selecting an injection mode” on page 1-12.

3.3.1 Preparing Samples for Autoloader Injections (Small Vessels)

To prepare samples for autoloader injections using small vessels that fit in the sample compartment:

1. Filter samples through a 0.22 or 0.45 micron filter before injection.
2. Transfer samples to small sample vessels.
3. Place up to four small sample vessels in the sample compartment at the top of the BioCAD 700E Workstation (see Figure 3-14).
4. Insert the four sample lines in the appropriate vessels.
5. Prime or flush the sample lines as described in Section 3.4.3, Priming and Flushing the Autoloader Sample Lines.



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Figure 3-14 Preparing Samples for Autoloader Injection

3.3.2 Preparing Samples for Autoloader Injections (Large Vessels)

To prepare samples for autoloader injections using large vessels that do not fit in the sample compartment:

1. Filter samples through a 0.22 or 0.45 micron filter before injection.
2. Transfer samples to large sample vessels.
3. Place up to four large sample vessels on top of the BioCAD 700E Workstation.
4. Replumb the sample lines with green 0.030-inch ID tubing and insert the tubing into the sample vessels.
5. Prime or flush the sample lines as described in Section 3.4.3, Priming and Flushing the Autoloader Sample Lines.

NOTE: Change the prime and flush volumes in the Configuration dialog box to account for the extra volume added by the sample lines you replumbed. Green 0.030-inch ID tubing volume is 4.56 $\mu\text{l}/\text{cm}$.

3.3.3 Preparing Samples for Direct Autoloader Injections

To prepare samples for direct autoloader injections:

1. Filter samples through a 0.22 or 0.45 micron filter before injection.
2. Transfer the sample to a sample vial.
3. Place the sample vial in the top right column clamp on the workstation, next to the injection valve (Figure 3-15).

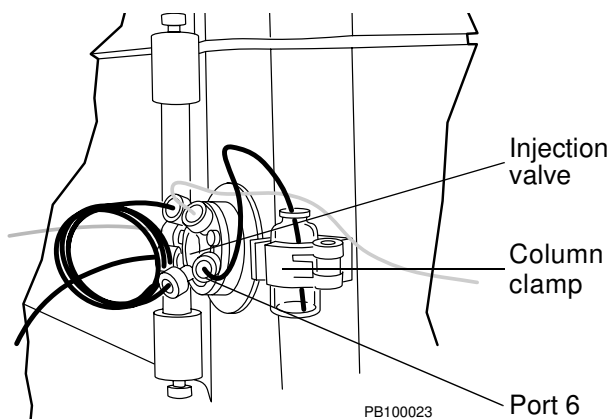


Figure 3-15 Preparing Sample for Direct Autoloader Injection

4. Disconnect the autoloader line from port 6 on the injection valve (top valve).
5. Connect a short length of orange 0.020-inch ID tubing from the sample vial to port 6 on the injection valve.
6. Prime or flush the sample lines as described in Section 3.4.3, Priming and Flushing the Autoloader Sample Lines.

NOTE: Reduce the prime and flush volumes in the Configuration dialog box to account for the shorter length of sample tubing used for the direct autoloader injection. Orange 0.020-inch ID tubing volume is 2.03 $\mu\text{l}/\text{cm}$.

3.3.4 Preparing Samples for Manual Injections

To prepare samples for manual injections:

1. Filter samples through a 0.22 or 0.45 micron filter before injection.
2. Disconnect the autoloader line from port 6 on the injection valve (top valve).
3. Screw the needle port adaptor (from the startup kit) loosely into port 6 of the injection valve (Figure 3-16).

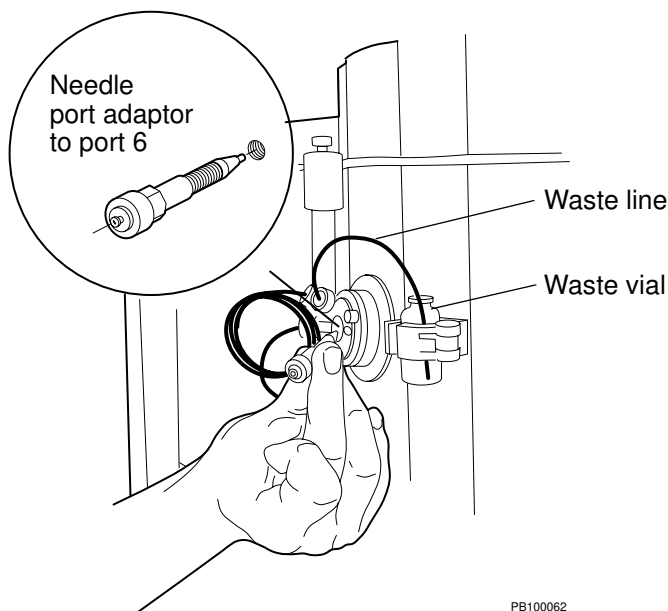


Figure 3-16 Inserting Needle Port Adaptor

4. Place a waste vial in the top right column clamp on the workstation, next to the injection valve.
5. Disconnect the line from port 1 on the injection valve (top valve).
6. Connect a waste line from port 1 on the injection valve to the waste vial.

3.3.5 Preparing Samples for Pump Injections

To prepare samples for pump injections:

1. Filter the sample through a 0.22 or 0.45 micron filter before injection.
2. Transfer the sample to large sample container.
3. Place the sample container to the right of the workstation, keeping it at the same level as the workstation.
4. Insert one of the six buffer/solvent lines into the sample container.

Hint: You may want to replace the buffer/solvent tubing with smaller diameter silicon tubing for easier handling and to minimize waste. You may need to decrease flow rate when using smaller diameter silicon tubing.

5. Prime the sample lines as described in Section 3.4.4, Priming Buffer/Solvent Sample Lines for a Pump Injection.

3.4 Priming and Flushing the System

This section describes priming and flushing:

- Pump seal wash lines
- Buffer/solvent lines
- Autoloader sample lines
- Buffer/solvent sample lines for a pump injection

NOTE: For information on priming the autosampler, see Section 3.6, Preparing an Autosampler.

3.4.1 Priming the Pump Seal Wash Lines

When to prime Prime the pump seal wash lines whenever the lines become drained or if you take the pump heads apart.

Priming To prime the pump seal wash lines:

1. Locate the two pump seal wash lines as they emerge from the pump heads in the lower right panel of the workstation. Pump seal wash lines are urethane tubing, and are not bundled together with the buffer/solvent lines (Figure 3-17).
2. Identify the inlet line (longer of two lines and connects to the bottom of the pump heads) and outlet line (shorter of the two lines and connects to the top of the pump heads).

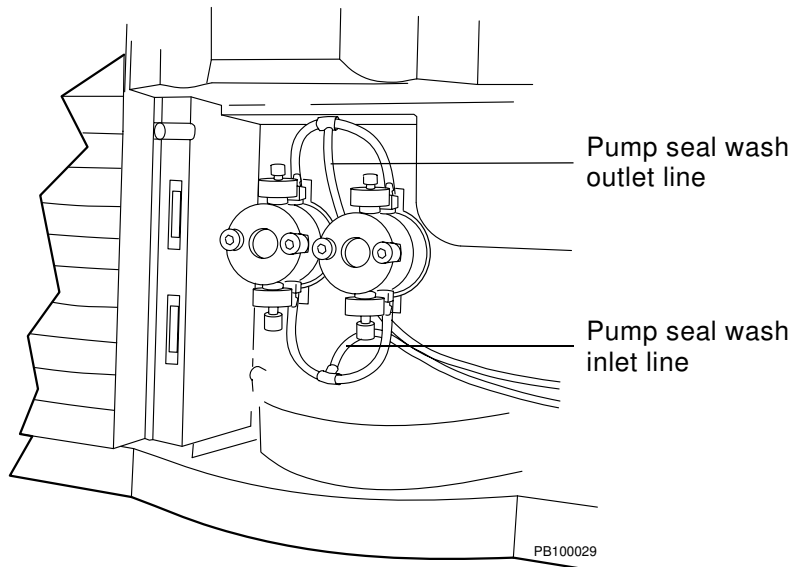


Figure 3-17 Pump Seal Wash Lines

3. Place the inlet line in the pump seal wash solution.

NOTE: A 30 percent isopropanol/water solution is recommended for the pump seal wash solution.

4. Connect a syringe to the outlet line.
5. Prime the lines by pulling pump seal wash solution into the syringe until the inlet and outlet lines are filled with fluid.
6. Remove the syringe from the outlet line and place the outlet line in the pump seal wash solution reservoir.

3.4.2 Priming the Buffer/Solvent Lines

This section describes:

- When to prime
- Priming using the Purge command
- Priming from the Control Panel

When to prime

Prime any of the six buffer/solvent lines after changing buffers/solvents or if there are air bubbles in the lines. Priming fills the line with buffer/solvent and removes air bubbles.

Hint: *If you are changing solvents, tap the old solvent out of the end of the line. This forms a bubble to prevent intermixing and helps ensure complete changeover.*

Priming using Purge command

To prime the buffer/solvent lines using the Purge command:

1. Connect the 60 ml syringe (provided in the startup kit) to the Luer-Lok® fitting at the end of the vent tube connected to the prime/purge valve (Figure 3-18). Open the valve one-half to one full turn to the left (counterclockwise).

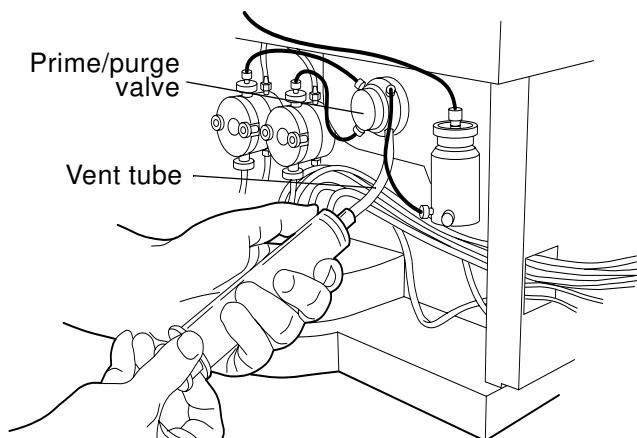


Figure 3-18 Syringe Attached to Luer-Lok Fitting on Prime/Purge Valve

2. Select **Purge System** from the Control Panel Blend menu.

NOTE: The Purge System command is not displayed if you configure Low Dispersion, Three Columns, or Direct Control column plumbing configurations. In these configurations, the column cannot be bypassed. See “Priming from Control Panel” on page 3-33, to prime with these column plumbing configurations.

The Purge Segment Setup dialog box is displayed (Figure 3-19).

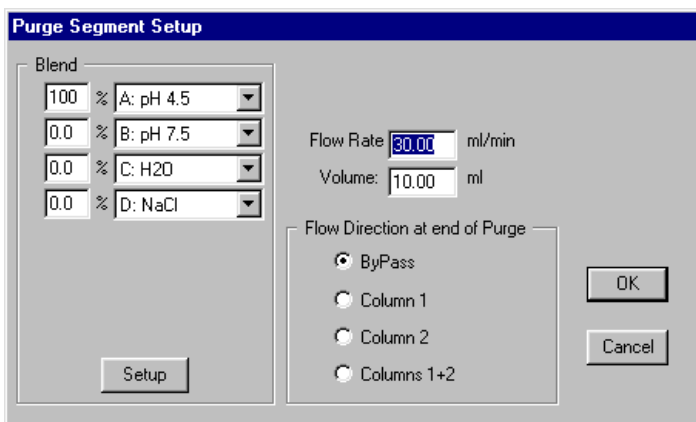


Figure 3-19 Purge Segment Setup Dialog Box

3. Select a **buffer/solvent channel** (A through F) to prime and set it for **100%**.

NOTE: If no buffers/solvents are displayed, configure buffers/solvents. For more information, see Section 4.2.2, Configuring Buffers/Solvents.

4. Type a **flow rate** of at least 20 ml/min.
5. Type a **volume** in ml sufficient to clear all air from the buffer/solvent line.

6. Select the **Flow Direction at End of Purge**. The selections depend on the current plumbing configuration.
7. Click **OK** to start the purge segment.
8. Prime the line by pulling 20 ml of buffer/solvent into the syringe you attached to the prime/purge valve.
9. Close the prime/purge valve by turning it clockwise and remove the syringe from the vent tube.
10. Stop the pump by clicking the **Stop** button on the Control Panel or by pressing the **Pump** button on the front panel of the workstation.
11. Repeat this procedure for all buffer/solvent channels. Prime each buffer/solvent line with the appropriate buffer/solvent. Prime unused lines with deionized water.

Priming from Control Panel

You can also prime buffer/solvent lines from the Control Panel:

1. Connect the 60 ml syringe (provided in the startup kit) to the Luer-Lok® fitting at the end of the vent tube connected to the prime/purge valve. See Figure 3-18 on page 3-30. Open the valve one-half to one full turn to the left (counterclockwise).
2. In the Blend section of the Control Panel, select a **buffer/solvent channel**, set it to **100%**, and click **Set**.

NOTE: *If no buffers/solvents are displayed, configure buffers/solvents. For more information, see Section 4.2.2, Configuring Buffers/Solvents.*

3. Select a **flow rate** of at least 20 ml/min in the Pump section of the Control Panel.
4. Click the **Bypass** option button in the Column section of the Control Panel to place the column out of line.

NOTE: *If you configure Low Dispersion, Three Columns, or Direct Control column plumbing configurations, the column cannot be bypassed. Physically remove the column and replace with a union before priming.*

5. Click the **Start** button to start the pump.
6. Prime the line by pulling 20 ml of buffer/solvent into the syringe you attached to the prime/purge valve.
7. Close the prime/purge valve by turning it clockwise and remove the syringe from the vent tube.
8. Click the **Stop** button to stop the pump.
9. Repeat step 2 through step 8 for all buffer/solvent channels. Prime each buffer/solvent line with the appropriate buffer/solvent. Prime unused lines with deionized water.

3.4.3 Priming and Flushing the Autoloader Sample Lines

When to prime If you make an autoloader injection on the BioCAD 700E Workstation, prime the sample lines before injecting from a sample vessel for the first time. Priming fills the entire selected sample line, up to the injection valve (Figure 3-20).

When to flush If you make an autoloader injection or direct autoloader injection on the BioCAD 700E Workstation, flush the sample lines to remove previously injected sample from the line before switching between the four sample channels. Flushing clears the sample line from the sample selection valve to the injection valve (Figure 3-20).

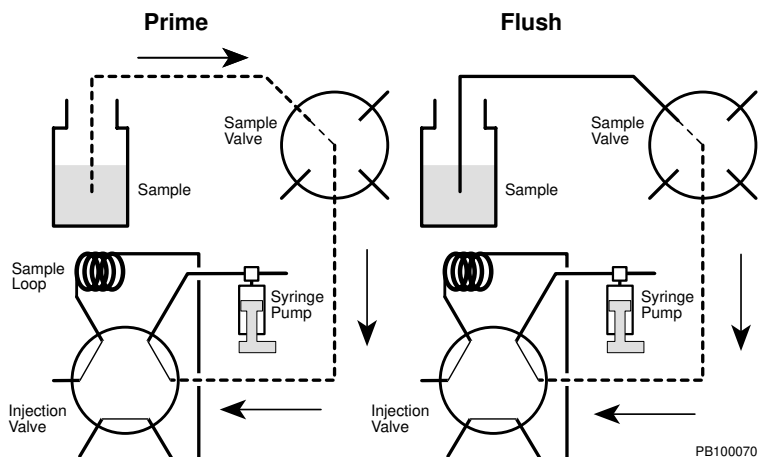


Figure 3-20 Priming and Flushing the Autoloader Sample Lines

Flow path diagrams Appendix H, Autoloader Flow Path, includes additional diagrams that illustrate the flow path of the autoloader during different operations.

Priming To prime the autoloader sample lines:

1. Prepare the samples as described in Section 3.3, Preparing Samples.

Hint: *If you are changing samples, tap old sample out of the end of the line. This forms a bubble to prevent intermixing and helps ensure complete changeover.*

2. Configure a prime volume as described in Section 4.2.3, Configuring the Autoloader.

NOTE: *The default prime volume is 350 μ l. If you have limited sample volume or are making a direct autoloader injection, decrease the prime volume accordingly by editing the system configuration. If you replumb the sample lines leading to the selection valve, increase the prime volume accordingly by editing the system configuration. For more information, see Section 4.2.3, Configuring the Autoloader.*

3. Select a **sample line** (Direct, 1, 2, 3, or 4) to prime from the Sample Loader drop-down list box in the Sample section of the Control Panel.

NOTE: *If no sample names are displayed, configure sample names. For more information, see Section 4.2.3, Configuring the Autoloader.*

4. Select **Prime** from the Control Panel Sample menu. The Prime selection on the Sample menu shows the selected channel, indicating that this channel is being primed.

The selected sample line is primed with the prime volume you set in the Configuration dialog box.

5. Repeat step 3 and step 4 for all sample lines in use.

Flushing To flush the autoloader sample lines:

1. Prepare the samples as described in Section 3.3, Preparing Samples.

Hint: *If you are changing samples, tap old sample out of the end of the line. This forms a bubble to prevent intermixing and helps ensure complete changeover.*

2. Configure a flush volume as described in Section 4.2.3, Configuring the Autoloader.

NOTE: *The default flush volume is 100 μ l. If sample carryover is a concern, increase the flush volume by editing the system configuration. For more information, see Section 4.2.3, Configuring the Autoloader.*

3. If you are making an autoloader injection, prime the sample line (1, 2, 3, or 4). For more information, see “Priming” on page 3-35.
4. Select a **sample line** (1, 2, 3, 4, or Direct) to flush from the Sample Loader drop-down list box in the Sample section of the Control Panel.
5. Select **Flush** from the Control Panel Sample menu. The Flush selection on the Sample menu shows the selected channel, indicating that this channel is being flushed.

The selected sample line is flushed with the flush volume you set in the Configuration dialog box.

6. Repeat step 3 and step 4 for all sample lines in use.

3.4.4 Priming Buffer/Solvent Sample Lines for a Pump Injection

If you use the pump to inject a sample through one of the buffer/solvent lines, use the following procedure to prime and fill the buffer/solvent line with the sample and to remove air bubbles:

1. Prepare the samples as described in Section 3.3, Preparing Samples.
2. Connect the 60 ml syringe (provided in the startup kit) to the Luer-Lok® fitting at the end of the vent tube connected to the prime/purge valve. See Figure 3-18 on page 3-30. Open the valve one-half to one full turn to the left (counterclockwise).
3. Click the **Bypass** option button in the Column section of the Control Panel to take the column out of line.

NOTE: *If you configure Low Dispersion, Three Columns, or Direct Control column plumbing configurations, the column cannot be bypassed. Physically remove the column and replace with a union before priming.*

4. In the Blend section of the Control Panel, select the **buffer/solvent channel** containing the **sample**, set it to **100%**, and click **Set**.
5. In the Pump section of the Control Panel, type a **flow rate** of at least 20 ml/min and click **Start** to start the pump.

6. Prime the line by pulling 20 ml of sample into the syringe attached to the prime/purge valve. Close the prime/purge valve by turning it clockwise and remove the syringe from the vent tube.

Hint: To conserve sample, introduce a bubble in the sample line before placing the line in the sample. Prime the line only until the bubble passes the proportioning valve.

NOTE: After priming the buffer/solvent line with sample, purge the system with equilibration buffer to remove sample that may have passed the proportioning valve. For information, see Section 5.2.4, Purging the System.

3.5 Preparing the Fraction Collector

Features The fraction collector provided (Advantec or Gilson) by Applied Biosystems includes the following features:

- Collects fractions based on time or volume.
- Performs peak cutting based on threshold and slope values.
- Performs both of the above simultaneously.
- Includes fraction collector event marks in BioCAD data files.

BioCAD software corrects for all system delay volumes and properly synchronizes all fraction collector event marks with the detector signal. If you change the length of tubing between the fraction/waste valve on the workstation and the fraction collector, you must set delay volume settings in the Configuration dialog box. For more information, see “Setting delay volumes” on page 4-13.

To prepare the fraction collector (Advantec or Gilson) provided by Applied Biosystems, you must:

- Install the fraction collector racks
- Set fraction collector mode (Gilson only)
- Prepare the fraction collector software (Advantec only)

3.5.1 Installing Fraction Collector Racks

The fraction collector is supplied with a test tube rack. Optional racks include:

- Eppendorf® rack
- Preparative funnel rack (for use with large bottles) (Advantec only)

All racks have tabs that line up with the base of the fraction collector. Make sure to push the rack all the way back against the base. Make sure all test tubes are centered in the slots and pointing straight up.

3.5.2 Setting Fraction Collector Mode

If you have a Gilson fraction collector, set the fraction collector to Manual mode and enter a delay time of 0. For more information, see the *Gilson Fraction Collector User's Guide*.

3.5.3 Preparing Fraction Collector Software

Overview If you have an Advantec fraction collector, prepare the fraction collector software by programming operating parameters on the front panel of the fraction collector. You can program the fraction collector in two modes:

- **Simple Mode**—Use the standard 120-tube configuration.
- **Bottle Mode**—Use a customized configuration. You must teach the fraction collector this customized configuration.

NOTE: After you prepare the fraction collector software, you must configure and prepare the BioCAD software for a fraction collector. See Section 4.6, Configuring and Preparing the BioCAD Software for a Fraction Collector.

Before you begin To program the fraction collector front panel, you must do one of the following:

- Deselect the Fraction Collector in the Configuration dialog box
- Disconnect the fraction collector cable from the workstation
- Exit the BioCAD software before programming

NOTE: If you select the Fraction Collector in the Configuration dialog box, the front panel of the fraction collector does not respond.

**Programming
Simple mode**

To program the fraction collector in Simple mode:

1. On the fraction collector, press **C/N** (Change and Confirm).

The mode flashes.

NOTE: *If mode does not flash, deselect Fraction Collector in the Configuration dialog box.*

2. Enter **1** to select Simple mode.
3. Enter **0000** for the Wait Time. Press **ENT/Y**.
4. Enter **3** to select Signal for the collection mode.
5. Enter **0001** count per tube for the Signals per Fraction. Press **ENT/Y**.
6. Enter **120** for the Fractionating Number. Press **ENT/Y**.

The fraction collector beeps.

**Programming
Bottle mode**

To program the fraction collector in Bottle mode:

1. On the fraction collector, press **C/N** (Change and Confirm).
2. The mode flashes.

NOTE: *If mode does not flash, deselect Fraction Collector in the Configuration dialog box.*

3. Enter **5** to select BSimple (Bottle) mode.
4. You can store up to 3 bottle arrangements in the fraction collector software. Enter **1** to select the first arrangement.
5. Enter **0000** for the Wait Time. Press **ENT/Y**.
6. Enter **3** to select Signal for the collection mode.
7. Enter **0001** count per tube for the Signals per Fraction. Press **ENT/Y**.



8. Enter the **number** of bottles you are using for the Fractionating Number. Press **ENT/Y**.

The fraction collector beeps.

After you program the fraction collector in Bottle mode, you must teach the fraction collector bottle positions. See “Teaching bottle positions” below.

Teaching bottle positions

If you program the fraction collector in Bottle mode, you must teach the fraction collector bottle positions:

1. On the fraction collector, press **C/N** (Change and Confirm).

The mode flashes.

2. Place the bottles in desired positions below the fraction collector arm. If you are using a rack, push the rack into the corner formed by the base of the fraction collector. This will help you easily reposition the rack in the correct place.
3. Enter **0** to select FC (Fraction Collector mode).
4. Enter **2** to select Bottle Arrange.
5. Enter the **number** (1, 2, or 3) of the bottle arrangement you selected above in step 4 of “Programming Bottle mode” on page 3-41.
6. Enter **0** to set the bottle arrangement.
7. The first bottle you set position for is Bottle 0 (this corresponds to Bottle 1 in the BioCAD software). Press the **arrow keys** (keys 2, 4, 6, and 8) to position the fraction collector diverter valve over the first bottle. Press **5** and an **arrow key** to move the diverter valve quickly. Press **ENT/Y** to store the position.
8. Repeat for all bottles. Make sure to press **ENT/Y** after the last bottle.
9. Press **END**.

The fraction collector beeps and automatically runs a test of bottle positions.

3.6 Preparing an Autosampler

Overview The way you prepare an autosampler depends on how you connect the autosampler to the BioCAD 700E Workstation:

- **Integrated Connection**—The BioCAD software has complete control over autosampler functions. You program sample information using the BioCAD software. You must use an AS 3500 autosampler provided by Applied Biosystems.
- **Direct Wire Connection**—The BioCAD software triggers the autosampler to make injections. You program sample information using the autosampler controls. You can use any autosampler.

NOTE: For more information on connecting an autosampler, see Section 2.4.4, Connecting the Autosampler.

Preparing To prepare an AS 3500 autosampler provided by Applied Biosystems, you must:

- Prepare the autosampler software
- Plumb the autosampler injector valve
- Attach compressed gas supply (optional)
- Prepare autosampler solvents
- Prime the autosampler
- Prepare sample vials
- Load vials in autosampler trays
- Program the autosampler method and sample set (direct wire only)

NOTE: After you prepare the autosampler, you must configure and prepare the BioCAD software for an autosampler. For more information, see Section 4.7, Configuring and Preparing the BioCAD Software for an Integrated Autosampler, or Section 4.8, Configuring and Preparing the BioCAD Software for a Direct Wire Autosampler.

3.6.1 Preparing the Autosampler Software

To prepare the autosampler software:

- Check the software version (integrated only)
- Set the autosampler to SpectraNet or Stand Alone mode
- Set the autosampler options

Checking software version

To run as an integrated autosampler, your autosampler must be installed with software version 3.36 or later. To check the current version of software installed on your autosampler:

1. When the status on the autosampler front panel is Idle, press the **Menu** key.
2. Select **Tests**.
3. Select **Software Version**.

The current version of software is displayed.

NOTE: *If your autosampler does not have software version 3.36 or later, contact Technical Support to have your autosampler upgraded for use as an integrated autosampler.*

Setting SpectraNet or Stand Alone mode

To run as an integrated autosampler, set the autosampler to SpectraNet mode. To run as a direct wire autosampler, set the autosampler to Stand Alone mode.

To set the autosampler mode:

1. When the status on the autosampler front panel is Idle, press the **Menu** key.
2. Select **Options**.
3. Select **Configurations**.
4. Use the down arrow key to select **Mode**.
5. Use the + or - key to select **SpectraNet** or **Stand Alone**.
6. Press **Enter** to accept the mode.

Setting autosampler options

Set the autosampler options (Prep Installed or TrayTemp Installed) using the front panel on the autosampler.

If you connect your autosampler as an integrated autosampler, set the options (Prep Installed or TrayTemp Installed) that correspond to the options (Prep option or Cooling option) you select in hardware setup. See Section 2.5.2, Updating BioCAD Hardware Setup Options.

To set the autosampler options:

1. When the status on the autosampler front panel is Idle, press the **Menu** key.
2. Select **Options**.
3. Select **Configurations**.
4. Use the down arrow key to select **Prep Installed**.
5. Select **Yes** if your autosampler supports the sample preparation option.
6. Use the down arrow key to select **TrayTemp Installed**.
7. Select **Yes** if your autosampler supports the sample cooling option.
8. Press **Enter** to accept the options.

NOTE: To determine if your autosampler supports the sample preparation option, look for two syringes behind the tray area or look for a heat/mix chamber to the left of the tray area. To determine if your autosampler supports the sample cooling option, look for a wick below the left-hand tray.

3.6.2 Plumbing Autosampler Injector Valve

Injection types Plumb the autosampler injector valve for one of the following injection types:

- Pull Loop
- PushLoop®
- Full Loop

For a description of the three injection types, see “Injection type” on page 4-38.

If you use an integrated autosampler, select the same injection type when you configure the autosampler. For more information, see “Configuring” on page 4-37.

Plumbing Figure 3-21 illustrates the plumbing from the BioCAD 700E Workstation to the autosampler injector valve. Figure 3-22 illustrates how to plumb the autosampler injector valve for the three injection types. Table 3-2 describes how to plumb the autosampler injector valve for the three injection types.

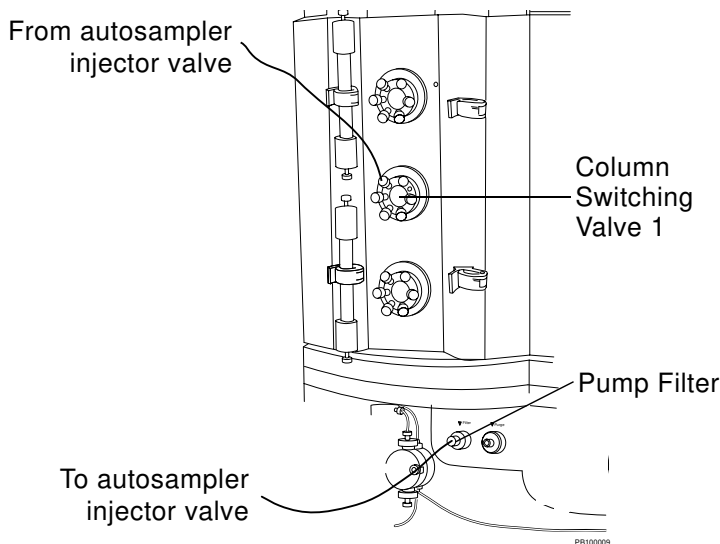


Figure 3-21 Plumbing from the BioCAD 700E Workstation to the Autosampler Injector Valve

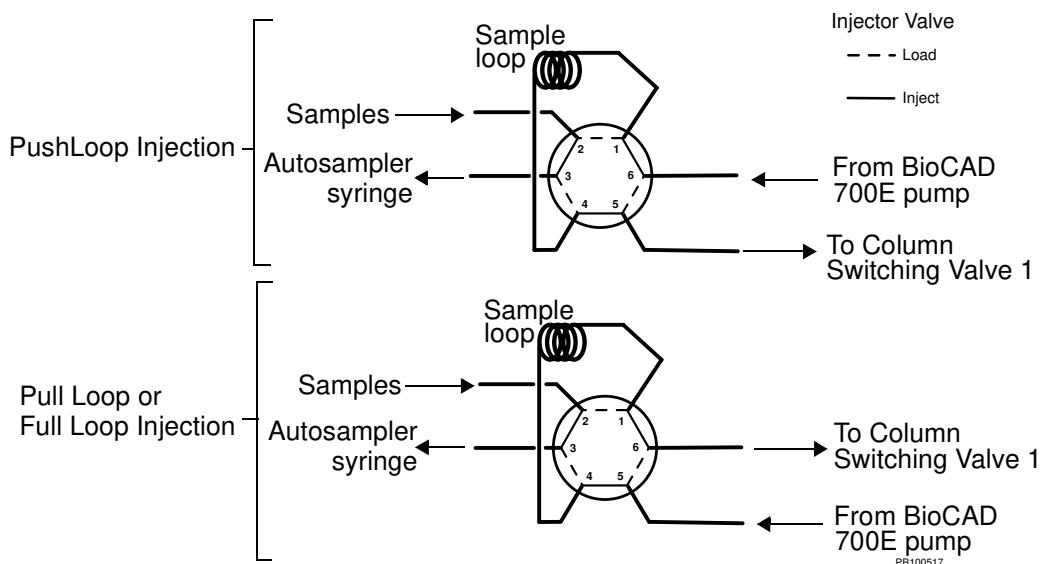


Figure 3-22 Plumbing the Autosampler Injector Valve

Table 3-2 Plumbing the Autosampler Injector Valve

Injection Type	Plumbing
PushLoop	<ul style="list-style-type: none"> • Plumb from the pump to port 6 • Plumb from port 5 to port 2 on Column Switching Valve 1 on the BioCAD 700E Workstation
Pull Loop OR Full Loop	<ul style="list-style-type: none"> • Plumb from the pump to port 5 • Plumb from port 6 to port 2 on Column Switching Valve 1 on the BioCAD 700E Workstation <p>NOTE: This plumbing ensures that sample enters and exits the sample loop from the same end, thereby preventing sample dispersion in the loop.</p>



3.6.3 Attaching the Compressed Gas Supply

If your autosampler supports the sample preparation option and you want to use the mixing feature, connect a compressed, dry gas (air or nitrogen) supply to the back of the autosampler to mix sample vials. For information on pressure settings, see the Autosampler Reference Manual.

3.6.4 Preparing Autosampler Solvents

Flush solvent Select a flush solvent appropriate for use with your autosampler solvents and your samples. One to five percent isopropanol is appropriate for most applications.

If you use an integrated autosampler, select the same flush solvent when you configure the Sample Loader section of the Configuration dialog box on page 4-37.

Insert the autosampler flush solvent line into the flush solvent reservoir. The autosampler flush solvent line, which exits from the back of the autosampler, is not labeled.

Autosampler solvents If your autosampler supports sample preparation, you can use up to four solvents with your autosampler.

If you use an integrated autosampler, select the same autosampler solvents in the Sample Preparation Configuration dialog box on page 4-42.

NOTE: The autosampler solvent lines, connected to the autosampler solvent selection valve on the back of the autosampler, are labeled S1 through S4 and correspond to lines AS1 through AS4 in the BioCAD software.

Filtering Filter all autosampler solvents through a 0.22 or 0.45 micron filter before use.

3.6.5 Priming the Autosampler

After installing the flush and autosampler solvents, prime the autosampler to fill the lines and remove air bubbles.

Priming direct wire

If you use a *direct wire* autosampler, see the *Autosampler Reference Manual* for information on priming.

Priming integrated

If you use an *integrated* autosampler, you prime the autosampler from the BioCAD software.

To prime an integrated autosampler:

1. Select **Sample Table** from the Window menu in the Control Panel.

The Sample Table is displayed.

2. Select **Prime Autosampler** from the Sample menu in the Sample Table.

If your autosampler does not support the sample preparation option, the autosampler solvent line is primed with prime volume set in the AS 3500 Autosampler dialog box. For more information, see Section 4.7, Configuring and Preparing the BioCAD Software for an Integrated Autosampler.

If your autosampler supports the sample preparation option, the Prime Autosampler dialog box is displayed (Figure 3-23).

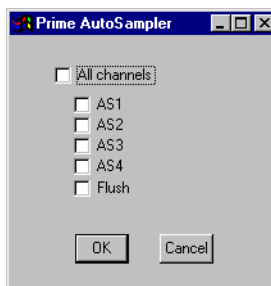


Figure 3-23 Prime Autosampler Dialog Box

3. Select the autosampler lines to prime. Selecting All Channels selects all lines.
4. Make sure the lines you selected are fully submerged in the autosampler solvents you connected to the autosampler. For more information, see Section 3.6.4, *Preparing Autosampler Solvents*.
5. Click **OK**.

The workstation primes the selected autosampler lines. It primes each line with the prime volume set in the AS 3500 Autosampler dialog box. For more information, see Section 4.7, *Configuring and Preparing the BioCAD Software for an Integrated Autosampler*. It primes for approximately 5 minutes per channel, for up to 25 minutes.

3.6.6 Preparing Sample Vials

Use vials, septa, and screw-on caps ordered from Applied Biosystems to ensure smooth autosampler operation.

Septa For septa, use either:

- An 8 mm diameter, 1.0 to 1.5 mm thick, rubber or silicon septum with a 0.1 mm thick Teflon® liner on the bottom side
- A single 0.25 mm thick Teflon liner by itself

Standard and micro vials

Vials available from Applied Biosystems include:

Vial	Volume Range	Dead Volume
Standard—1.8 ml glass	200 µl to 1.8 ml	200 µl
Micro—250 µl conical plastic	20 µl to 250 µl	20 µl

Fill vials with the appropriate volumes. Do not fill vials with less than the vial dead volume (volume below which no injection can be made).

NOTE: *If your autosampler supports the sample preparation option, do not use plastic vials if using the heat chamber. Plastic vials do not conduct heat well.*

Vial inserts for Unspecified vial shape

You can purchase 100 µl inserts with self-centering bottom springs from other vendors for use with the 1.8 ml glass vials that are available from Applied Biosystems. Examples include Alltech #98031 (glass) and Alltech #98069 (propylene).

When to use vial inserts

Use the vial inserts whenever you use the Unspecified vial shape in the BioCAD software. The vial inserts and Unspecified vial shape are useful if the following are true of your application:

- Small sample volume
- Maximum sample recovery is important
- Minimal sample loss is important
- Quantitative injection volume is not required

For more information on the Unspecified vial shape, see “Understanding Unspecified vial shape” on page 4-41.

How do vial inserts work?

The autosampler is designed so that the sample needle is at a fixed height. This design creates a clearance between the needle tip and the vial bottom. The clearance protects the needle from damage, but also creates a dead volume of liquid that cannot be sampled.

The vial insert with a self-centering bottom spring raises the vial bottom so that this dead volume is eliminated without damaging the needle. It allows you to inject nearly the complete contents of the vial.

How to use vial inserts

To use vial inserts, place the insert in a 1.8 ml glass vial and fill it with sample using an appropriate size pipet tip.

After you prepare your sample, make sure you do the following when configuring the integrated autosampler:

- Select **Pull loop** as the injection type. For more information, see “Injection type” on page 4-38.
- Select **Unspecified** as the vial shape when you enter the sample in the Sample Table. For more information, see Section 4.7.3, Entering Vials in Sample Table.

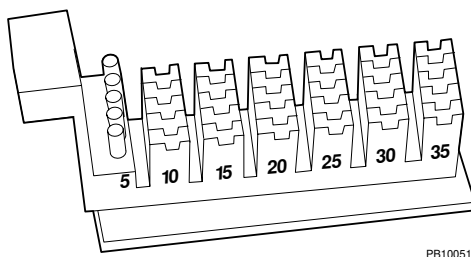
- Filtering** Filter samples through a 0.22 or 0.45 micron filter before filling.
- Sample heating and cooling** If you use an integrated autosampler that supports sample heating and cooling, you can control sample heating and cooling from two places:
- Configuration dialog box (see “Configuring sample prep” on page 4-42)
 - Sample Table window (see “Controlling sample heating and cooling” on page 5-50)

3.6.7 Loading Vials in Autosampler Trays

Tray design The AS 3500 autosampler provided by Applied Biosystems has three interchangeable trays. Trays A, B, and C are arranged in the autosampler from left to right.

Each tray holds 35 vials, arranged in seven rows of five. The first vial position is A1. The last vial position is C35. Position labels are to the left of each position (Figure 3-24).

NOTE: AS 3500 autosamplers that do not support the cooling option have trays that hold 40 vials (eight rows of five). If using an integrated autosampler, do not use vial positions A36–A40, B36–B40, or C36–C40.



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Figure 3-24 AS 3500 Autosampler Tray

Vial location If you use an AS 3500 autosampler provided by Applied Biosystems as a direct wire autosampler, place vials in the vial positions you program in your sample set. For more information, see Section 3.6.8, Programming the AS 3500 Autosampler Method and Sample Set. Place the vials consecutively, following the numbering of the vial positions.

NOTE: *In direct wire mode, the AS 3500 autosampler sampling needle can start at any vial position, but cannot skip positions. It moves forward following the numbering of the vial positions. It does not move in a serpentine pattern.*

If you use an integrated autosampler, location of vials is not important, but you must enter vials in the Sample Table that correspond to the vials you physically load in the autosampler trays. When injecting, the software ignores vial position and finds the first vial with the specified sample name.

Including empty vials

If you use a direct wire autosampler that supports sample preparation, include an appropriate number of empty vials in the autosampler trays. See the *Autosampler Reference Manual* for more information.

If you use an integrated autosampler that supports sample preparation, you can add Prep blocks to a method. If you add Prep blocks to a method for sample preparation (diluting or combining), include an appropriate number of empty vials in the autosampler trays to accommodate Derived vials that the Prep block creates. For more information, see “Creating Prep Blocks” on page 6-53.

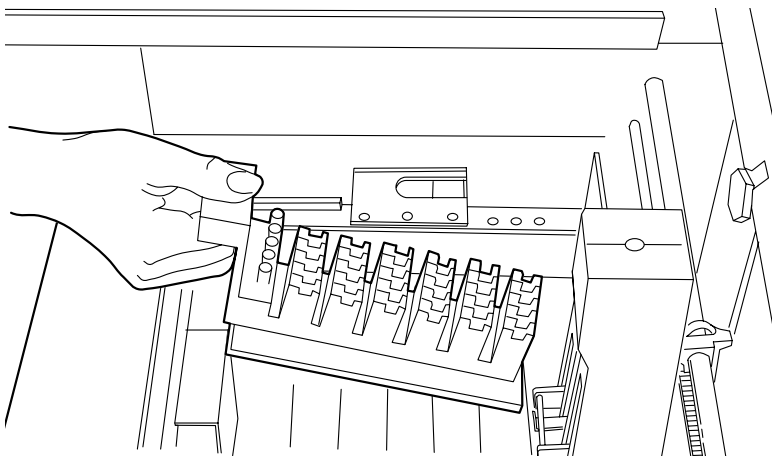
NOTE: *Include the same shape of empty vials (Standard or Micro) for the final vial and all intermediate vials that a Prep block creates. That is, if a Prep block creates a final vial that is a Micro vial, you must include empty Micro vials for the intermediate vials that the Prep block creates.*

Location of empty vials is not important, but you must enter Empty vial types in the Sample Table that correspond to the empty vials you physically load in the autosampler trays. When an empty vial is needed, the autosampler finds the first available empty vial.

**Installing
sample trays**

To install an autosampler tray:

1. Hold the tray handle, tilting the back end down.
2. Insert the runners on the tray into the slots in the appropriate position at the rear of the tub (Figure 3-25).



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**Figure 3-25 Installing Autosampler Trays
(Side View of Autosampler Tray Area)**

3. Lower the front of the tray into place and press down firmly to seat the tray. When properly seated, the upper front edge of the tray fits under the lip of the tub.

Hint: Pull the handle back toward you until the tray snaps into place. Move each tray from side-to-side to make sure it is seated properly.

4. Repeat for remaining trays. It is not necessary to install all trays before running the autosampler.

3.6.8 Programming the AS 3500 Autosampler Method and Sample Set

If you use a direct wire autosampler, you control it from the autosampler controls. The BioCAD software does not use the information you program on the autosampler front panel.

This section describes programming the AS 3500 autosampler provided by Applied Biosystems. If you are using another autosampler, see the *Autosampler Reference Manual* for programming information.

NOTE: *If you are using an integrated autosampler, skip this section. You control an integrated autosampler from the BioCAD software, not from the autosampler front panel.*

This section explains:

- Programming hints
- Programming the autosampler method
- Loading the autosampler method
- Programming the autosampler sample set

NOTE: *This is a brief introduction to programming the AS 3500 autosampler. For detailed operating information, refer to the Autosampler Reference Manual.*

Programming hints

When you program the front panel of the AS 3500 autosampler, note the following:

- To select a menu item, move the cursor with the **arrow keys**, then press **Enter**.
- To enter numeric or alphabetic values, use the **plus (+)** and **minus (-) keys** to scroll through numbers. Use the **arrow keys** to move the cursor to each digit or position.
- To display preset values (for example, On or Off), use the **+ and - keys**.
- To accept an entry and move to the next field, press **Enter**.

Programming the method

If you use a direct wire autosampler, program an autosampler method to control sample injections, preparation, and cooling.

NOTE: *The following procedure describes information you need to program the autosampler for use with the BioCAD 700E Workstation only.*

To program the autosampler method:

1. When the status on the autosampler front panel is Idle, press the **Menu** key.
2. Select **Files**.
3. Select **Edit**.
You can store up to four files (methods) in the autosampler software.
4. Use the **+** or **-** key to select the **File #** to edit.
5. Enter a **file name** a letter at a time. Use the **+** and **-** keys to display a letter. Press the **right arrow key** to move to the next position. Press **Enter** when you have entered the complete name.
6. Select **Injection**, **Injection Volume**, and **Number of Injections** per sample.
7. Enter **0** for Cycle Time.
8. If your autosampler supports the sample cooling option, turn on the autosampler tray heater/cooler. Select a temperature.

NOTE: *To determine if your autosampler supports the sample cooling option, look for a wick below the left-hand tray.*

9. When Calibration is displayed, enter information for standard injections. For information on programming calibration information, refer to the *Autosampler Reference Manual*.
10. Press **Enter** to bypass all Timed Event parameters.
11. Press **Enter** to bypass Equilibration and Gradient Delay.

12. If appropriate for your samples, change the **Sample Viscosity** setting.

NOTE: If Sample Viscosity is set to Viscous, the autosampler can take more than three minutes to inject. Set Autosampler Timeout in Configuration accordingly.

13. Leave the **Flush Volume** set at the default 400 µl.
14. Select **Injection Type**: Pull Loop, PushLoop®, or Full Loop. Press **Enter**. For more information on injection type, see Appendix F, Autosampler Injection Types, or the *Autosampler Reference Manual*.
15. Press **Enter** to bypass Injection Range and Needle Height. Press **Enter** to display the menu.

Loading the method

Load the autosampler file (method) you just created by selecting **Load**, and selecting the file.

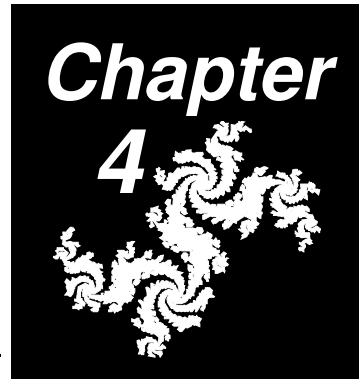
Programming the sample set

You can program up to 100 sample sets:

1. When the status on the autosampler front panel is Idle, press the **Sample** key. This key is blank and labeled below the key on the front panel.
2. Select the **file** (method) you created above.
3. Press **Enter** to accept the Injection Volume, Number of Injections, and Cycle Time you entered in the file.
4. Select the first sample **vial number**. The autosampler rack is numbered A01 through A35, B01 through B35, and C01 through C35. Numbers are on the side of the rack.
5. Enter the **number** of samples.
6. Add the sample set to the sample queue.
7. Press **Run** on the front panel of the autosampler.

NOTE: When you use a direct wire autosampler, the sampling needle can start at any vial position, but then moves forward following the numbering of the vial positions, without skipping any positions. It does not move in a serpentine pattern.

4 Configuring and Preparing the BioCAD Software



This chapter includes the following sections:

4.1	Configuration Overview	4-2
4.2	Creating a New Configuration	4-4
4.3	Saving and Printing Configurations	4-20
4.4	Loading an Existing Configuration.....	4-22
4.5	Customizing Configuration Libraries.....	4-24
4.6	Configuring and Preparing the BioCAD Software for a Fraction Collector	4-35
4.7	Configuring and Preparing the BioCAD Software for an Integrated Autosampler.....	4-36
4.8	Configuring and Preparing the BioCAD Software for a Direct Wire Autosampler.....	4-53

4.1 Configuration Overview

What is a configuration?

A configuration is a software file that specifies the physical setup of the BioCAD 700E Workstation, including:

- Buffers/solvents
- Samples
- Sample loop size
- Syringe size
- Injection mode
- Columns
- Plumbing
- Optional external devices
- Data collection
- System pressure

NOTE: A configuration does not alter the physical setup of the BioCAD 700E Workstation. You must create the configuration file to match the physical setup.

You control the configuration by changing settings in the Configuration dialog box (Figure 4-1).

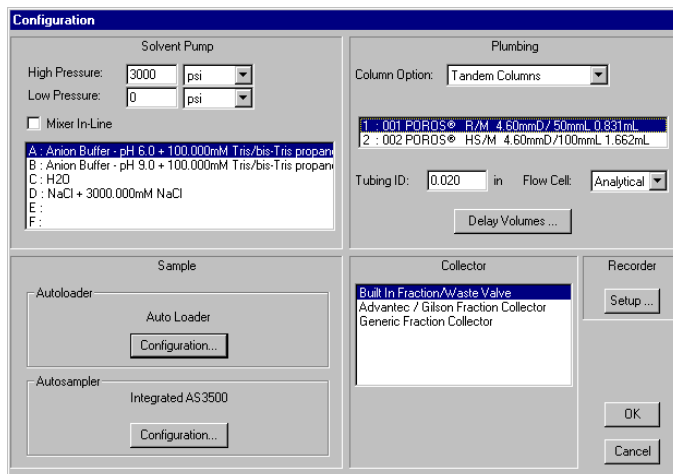


Figure 4-1 Configuration Dialog Box

You can create configurations, save configurations, and load configurations at a later time.

System versus method configuration

It is important that you understand the difference between the system configuration and the method configuration.

- **System configuration** is the configuration information currently set in the Configuration dialog box. The system configuration is *always* used when you run the BioCAD 700E Workstation. You can think of the system configuration as the *active* configuration.

NOTE: *The system configuration is stored in the SYSCONF.CFG file. Editing the Configuration dialog box overwrites this file.*

- **Method configuration** is the configuration information stored with a method file (.MET). It represents the configuration information set in the Configuration dialog box when the method was run or saved. The method configuration may or may not be the same as the current system configuration. You can think of the method configuration as the *inactive* configuration.

NOTE: *The system configuration (not the method configuration) is used when you run a method. To update the system configuration to a specific method configuration, you must load the configuration from the method. For more information, see Section 4.4, Loading an Existing Configuration.*

CAUTION

Changing the settings in the Configuration dialog box during a method run will abort the run.

4.2 Creating a New Configuration

Displaying Configuration dialog box

You use the Configuration dialog box to create a configuration and configure the various components of the workstation. To display the Configuration dialog box (Figure 4-2), select **Edit Config** from the Config menu in the Control Panel.

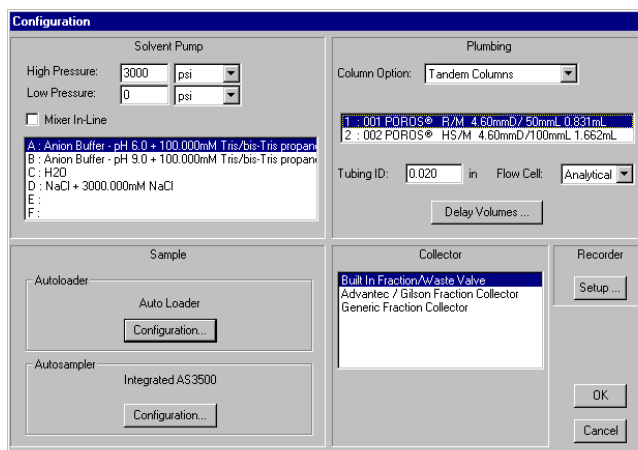


Figure 4-2 Configuration Dialog Box

The following sections describe configuring:

- Pump
- Buffers/solvents
- Autoloader
- Columns and plumbing
- Detectors and Strip Chart Recorder

NOTE: For information on configuring a fraction collector, see Section 4.6, Configuring and Preparing the BioCAD Software for a Fraction Collector.

For information on configuring an autosampler, see Section 4.7, Configuring and Preparing the BioCAD Software for an Integrated Autosampler, or Section 4.8, Configuring and Preparing the BioCAD Software for a Direct Wire Autosampler.

4.2.1 Configuring the Pump

To configure the pump:

1. Display the Configuration dialog box by selecting **Edit Config** from the Config menu in the Control Panel.
2. In the Solvent Pump section of the Configuration dialog box, type high and low **pressure limits** in the High Pressure and Low Pressure text boxes.

***Hint:** Set the high pressure limit to the pressure limit of the column with the lowest pressure limit.*

3. Click the down arrow to the right of the text box to select one of the following **pressure units**:
 - psi (pounds per square inch)
 - bar
 - MPa (megapascal)

1 bar = approximately 15 psi = 0.1 MPa

The pressure maximum is 3,000 psi (204 bar, 20.4 MPa). The pressure maximum does not vary with flow rate.

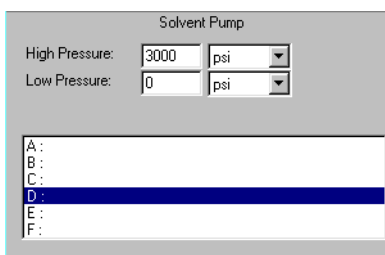
The pump stops if the system pressure exceeds the high pressure limit or falls below the low pressure limit.

4.2.2 Configuring Buffers/Solvents

You can configure up to six buffers/solvents in the Configuration dialog box. After you configure them, you can select up to four of the six buffers/solvents for blending from the Control Panel or when creating a method.

To configure buffers/solvents:

1. Display the Configuration dialog box by selecting **Edit Config** from the Config menu in the Control Panel.
2. Double-click a **buffer/solvent channel (A through F)** in the Solvent Pump section of the Configuration dialog box (Figure 4-3).



Double-click a
buffer/solvent
channel

Figure 4-3 Buffer/Solvent Channels

The Buffer/Solvent Library is displayed.

3. Select a **buffer/solvent**, then click **OK**.
The buffer/solvent is added to the Configuration dialog box.
4. To clear a buffer/solvent channel, double-click the **channel (A through F)** to display the Buffer/Solvent Library, then click the **Clear** button.
5. Repeat step 2 and step 3 for all buffer/solvent channels.

For information on adding items to the Buffer/Solvent Library, refer to Section 4.5.1, Customizing the Buffer/Solvent Library.

4.2.3 Configuring the Autoloader

When to configure the Autoloader

Configure the Autoloader before making the following types of injections:

- Autoloader injection
- Direct autoloader injection

Do not configure the Autoloader when making the following types of injections:

- Manual injection
- Pump injection
- Autosampler injection

Configuring

To configure the autoloader:

1. Display the Configuration dialog box by selecting **Edit Config** from the Config menu in the Control Panel.
2. In the Sample section of the Configuration dialog box (Figure 4-4), click the **Configuration** button under **Autoloader**.

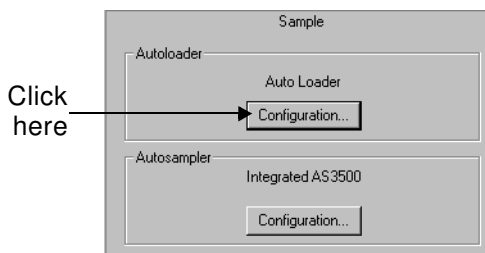


Figure 4-4 Autoloader Configuration

The Autoloader dialog box is displayed (Figure 4-5).

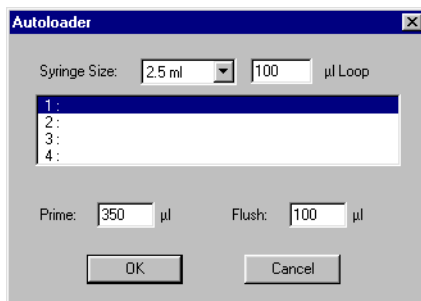


Figure 4-5 Autoloader Dialog Box

- Syringe** 3. Select the **syringe size** (0.1, 0.5, 1.0, 2.5, 5, 10, or 25 ml) from the Syringe Size drop-down list box.

The BioCAD 700E Workstation is provided with 500 µl and 5 ml syringes.

- Sample loop** 4. Type the **sample loop size** in microliters.

The BioCAD 700E Workstation is provided with provided with 100 µl, 2 ml, and 5 ml sample loops.

NOTE: You can create your own sample loop by coiling an appropriate length of tubing.

Install the appropriate syringe and sample loop on the workstation. For instructions on selecting and installing the syringe and sample loop, see Section 3.1.4, Changing the Syringe and Sample Loop.

NOTE: You can also change the syringe size or sample loop size without accessing the Configuration dialog box. Select **Change Syringe** or **Change Loop Size** from the Sample menu on the Control Panel.

Sample names 5. Select up to four **sample names** from the Sample Library.
Sample names are not required, but provide information in your reports.

- Double-click a **sample channel (1 through 4)** to display the Sample Library.
- Select a **sample**, then click **OK** to add the sample name to the Configuration dialog box.
- To clear a sample channel, double-click the channel (**1 through 4**) to display the Sample Library, then click the **Clear** button.
- Repeat the above procedure for each sample you want to configure.

For information on adding names to the Sample Library, refer to Section 4.5.2, Customizing the Sample Library.

Prime and flush volumes 6. Type **Prime** and **Flush** volumes as needed (Figure 4-6). Default volumes are acceptable for most applications.

Parameter	Default Volume	Description
Prime volume	350 μ l	Volume required to fill the entire sample line from the sample vessel to the injection valve. NOTE: Modify the prime volume if you replumb the sample lines leading to the selection valve. For more information, see Section 3.3.2, Preparing Samples for Autoloader Injections (Large Vessels).
Flush volume	100 μ l	Volume required to flush the sample line between sample selection valve and the injection valve. Flushing removes the previously injected sample from the sample line before loading from a different sample channel. NOTE: Use a larger flush volume if sample carryover is a concern. Use a smaller flush volume if sample volume is limited. Use a smaller flush volume if making a direct autoloader injection. For more information, see Section 3.3.3, Preparing Samples for Direct Autoloader Injections.

4

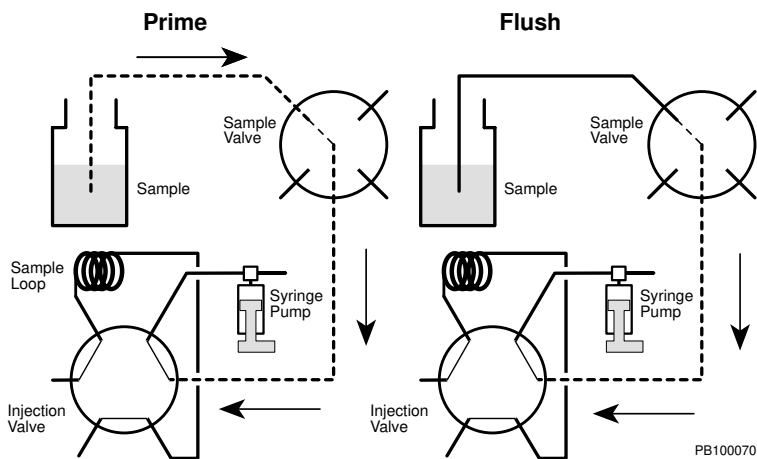


Figure 4-6 Priming and Flushing the Autoloader Sample Lines

4.2.4 Configuring Columns and Plumbing

To configure columns and plumbing, you must:

- Configure the column plumbing option
- Set the delay volumes

Configuring the column plumbing option

Configure a column plumbing option that matches the physical plumbing configuration. For more information, see Section 3.1.1.3, Plumbing Tubing and Columns.

NOTE: After you configure the column plumbing option, you can control the flow path from the Control Panel or when creating a method. See Section 5.2.5, Controlling Columns, or “Plumbing Events” on page 6-36.

To configure the column plumbing option:

1. Display the Configuration dialog box by selecting **Edit Config** from the Config menu in the Control Panel.

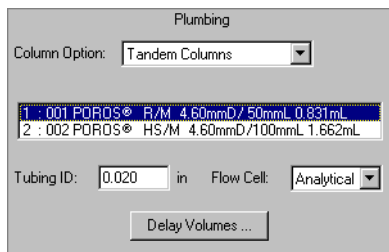


Figure 4-7 Column Plumbing Configuration

2. Select a **column plumbing option** from the Column Option drop-down list box (Figure 4-7). Choices are:
 - Low Dispersion
 - Single Column
 - Tandem Columns
 - Three Columns
 - Direct Control

For more information on each column plumbing option, see “Column plumbing configurations” on page 1-15.

3. Select up to three **columns** from the Column Library. If no column is configured, the column is designated with question marks and zeroes.
 - Double-click a **column line** to display the Column Library.
 - Select a **column**, then click **OK** to add the column to the Configuration dialog box.
 - To clear a column, double-click the column entry, then click the **Clear** button.
 - Repeat the above procedure to configure each column.

For information on adding columns to the Column Library, refer to Section 4.5.3, Customizing the Column Library.

4. Type the **tubing inner diameter** (in inches) in the Tubing ID text box. Use the following table to select the minimum diameter tubing for your flow rate.

Flow Rates	Tubing		Volume	
	ID	Color	µl/ft	µl/cm
0 to 20 ml/min	0.010"	Blue	15	0.51
20 to 60 ml/min, or for optimal system pressure	0.020"	Orange	62	2.03
0 to 60 ml/min, low pressure columns	0.030"	Green	139	4.56

NOTE: All tubing has a 1/16-inch OD.

NOTE: It is important to enter correct tubing ID because delay volumes are calculated based on tubing ID.

4

- Select the type of **flow cell** (Analytical, Semi-prep, or Other) installed in the UV/Vis detector from the Flow Cell drop-down list box.

Path length, volume, and maximum flow rates for these flow cells are:

- Analytical**—6 mm path length, 9 μ l volume (45 ml/min maximum flow rate)
- Semi-prep**—3 mm path length, 4.5 μ l volume (100 ml/min maximum flow rate)

CAUTION

If you have an analytical flow cell installed in the UV/Vis detector, do not exceed a 45 ml/min flow rate.

Setting delay volumes

Delay volumes are used to:

- Perform calculations of column efficiency parameters when exporting data
- Set a delay before collecting fractions to synchronize collected fractions with the UV/Vis detector signals

When to set

Set delay volumes if you:

- Change the length of tubing between the pump filter and the injection valve.
- Change the length of any tubing plumbed to the injection valve or column switching valves. See Section 3.1.1, Selecting Tubing Diameter and Length, for recommended tubing lengths.
- Change the length of tubing between the fraction/waste valve on the workstation and the fraction collector supplied with the BioCAD 700E Workstation. This tubing is supplied with the fraction collector.

NOTE: *Do not modify delay volumes if you use different diameter tubing. Delay volumes are automatically updated for tubing diameter changes, based on the value you type in the Tubing ID text box.*

Setting To set the delay volumes:

1. Click the **Delay Volumes** button in the Plumbing section of the Configuration dialog box.

The Estimate Delay Volumes dialog box is displayed (Figure 4-8).

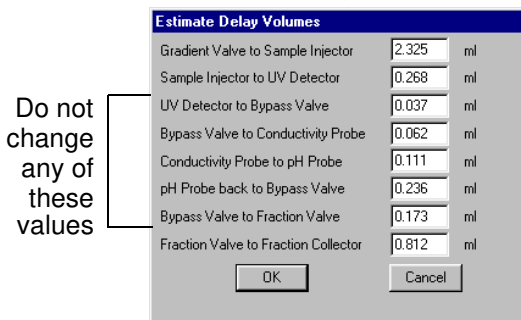


Figure 4-8 Estimate Delay Volumes Dialog Box

2. Change the following **delay volumes** as needed:
 - **Gradient Valve to Sample Injector**—Tubing between proportioning valve and injection valve.
 - **Sample Injector to UV Detector**—Tubing plumbed to injection valve or column switching valves.
 - **Fraction Valve to Fraction Collector**—Tubing connected between the fraction/waste valve on the workstation and a fraction collector. You can calculate this volume based on tubing dimensions or determine it experimentally by creating and timing an air bubble in the tubing. For more information, contact Biosystems Technical Support.

NOTE: All other delay volumes in this dialog box are optimized for the standard tubing provided on the workstation. Do not change these delay volumes.

3. Click **OK**.

4

4.2.5 Configuring Detectors

To configure detectors, you must:

- Select wavelength mode
- Configure data channels for UV/Vis, fluorescence, pH, conductivity, pressure, and auxiliary detector
- Configure the auxiliary detector

Selecting wavelength mode

The wavelength mode you select for UV/Vis detection depends on the detectors you enable in hardware setup. For more information, see Section 2.5.2, Updating BioCAD Hardware Setup Options.

The following table summarizes the wavelength modes available based on the detectors you enable in hardware setup.

If you enable ...	Then ...
Internal UV/Vis Detector (Model 201)	UV/Vis single-wavelength mode only is available
Internal UV/Vis Detector (Model 205)	UV/Vis single- or dual-wavelength mode is available
External UV/Vis Detector (Model 201, 204, or 205)	UV/Vis dual-wavelength mode is only available
External Fluorescence Detector (Model 304/305)	UV/Vis single-wavelength mode only is available

If your internal UV/Vis detector is Model 205, and you do not enable an external UV/Vis detector, you must select single- or dual-wavelength mode:

- To select single-wavelength mode, select **UV/VIS Single Wavelength** from the Detector menu in the Control Panel.
- To select dual-wavelength mode, select **UV/VIS Dual Wavelength** from the Detector menu in the Control Panel.



Configuring data channels

To configure data channels:

1. Click the **Setup** button in the Recorder section of the Configuration dialog box.

The Data Recorder Configuration dialog box is displayed (Figure 4-9).

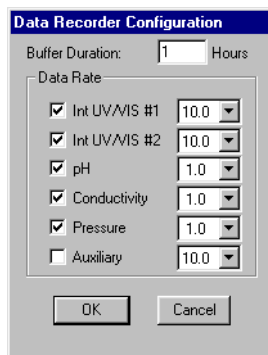


Figure 4-9 Data Recorder Configuration Dialog Box

CAUTION

Changing the settings in the Data Recorder Configuration dialog box deletes the contents of the Strip Chart Recorder.

Buffer duration

2. Type the **Buffer Duration** (acquisition time) for the electronic Strip Chart Recorder.

The default is one hour. The maximum is 24 hours.

The number you type depends on the data rates selected, the amount of memory, and the amount of disk space on your computer.

Set the buffer duration to accommodate your longest run, but note that when you make changes to the Strip Chart Recorder (by zooming or changing options), it takes longer to update when you have a long buffer duration set.

Data is collected in the electronic Strip Chart Recorder whenever the pump is running, independent from data that is stored as part of running a method.

CAUTION

When the buffer duration expires, the oldest data is lost as new data is added.

- Enabling channels* 3. Enable the data channels for the detector signals you want to collect in data files. Choices are:

Channel	Displays Signal/Trace From ...
Int UV/VIS #1	Internal UV/Vis detector first wavelength
Int UV/VIS #2	Internal UV/Vis detector second wavelength NOTE: This channel is available only if you do not enable an external detector in hardware setup.
Ext UV/VIS	External UV/Vis detector NOTE: This channel is available only if you enable an external UV/Vis detector in hardware setup.
Ext Fluor	External fluorescence detector NOTE: This channel is available only if you enable an external fluorescence detector in hardware setup.
pH	pH monitor
Conductivity	Conductivity monitor
Pressure	System pressure trace
Auxiliary	Auxiliary detector

NOTE: If you do not enable a data channel, the corresponding signal or trace is not stored in data files.



Setting data rates 4. Set the **data rate** for acquisition of each channel in points per second. Data rate ranges are:

Channel	Data Rate Ranges (points per second)
UV/Vis #1 and UV/Vis #2 (Model 201 UV/Vis, Model 205 UV/Vis, and Model 304/305 Fluorescence)	0.1–20.0
UV/Vis #2 (Model 204 UV/Vis only)	0.1–10.0
pH, Conductivity, and Pressure	0.1–1.0
Auxiliary	0.1–10.0
NOTE: Default data rates are displayed in Figure 4-9.	

Guidelines for data rates

Use the following guidelines to determine appropriate data rates:

- Default settings are recommended for most applications.
- Use a data rate of 1 or 2 points per second for very long runs or runs with conventional (non-perfusion) columns. Adjust the rate for UV/Vis Channel #1 and UV/Vis Channel #2 if you are operating in dual-wavelength mode.
- Lower data rates allow longer buffer duration.
- Higher data rates can create large files that take longer to display and analyze.
- Set data rates for the UV/Vis and auxiliary channels high enough to collect at least 10 data points per peak.

Configuring the auxiliary detector

To configure an auxiliary detector:

1. Enable the auxiliary channel and set the data rate as described in “Configuring data channels” on page 4-16.
2. Select **Auxiliary Detector Setup** from the Detector menu.



The Auxiliary Setup dialog box is displayed (Figure 4-10).

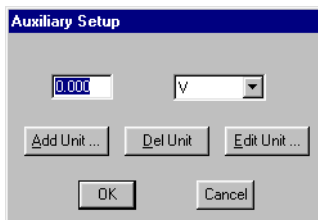


Figure 4-10 Auxiliary Setup Dialog Box

3. Select **conversion units** from the drop-down list box to convert to from volts. Enter a **maximum value** for the units you selected.

NOTE: Refer to the reference manual for your auxiliary detector for the conversion units and maximum value.

This information is not stored in the configuration, but it is saved in the Control Panel. Change the value only if you change the voltage-to-units conversion on the auxiliary detector.

4. If necessary, modify the list of available units for calibration using **Add Unit**, **Del Unit**, or **Edit Unit**, then give a conversion factor for volts to the desired units (Figure 4-11).

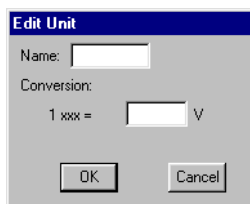


Figure 4-11 Edit Unit Dialog Box

NOTE: Refer to the reference manual for your auxiliary detector for the conversion factor.

The auxiliary detector channel on the Strip Chart Recorder and other data displays will be available in the added units as well as V (volts).

4.3 Saving and Printing Configurations

Saving configuration information

There are two ways to save configuration information:

- **Manually**—You can manually save configuration information as a configuration file (.CFG). For more information, see “Manually saving” below.
- **Automatically**—When you run a method, the system configuration is automatically saved in method files (.MET) and data files (.B*). When you save a method after creating it, the system configuration can be saved as the method configuration in the method file (.MET). The configuration information is for documentation purposes and can be included in reports. This does not create a .CFG file. For more information, see “Automatically saving” on page 4-21.

Manually saving

To manually save a configuration for future use:

1. After you enter all configuration information in the Configuration dialog box, click **OK**.
2. Select **Save Configuration** from the File menu in the Control Panel.

The Save File dialog box is displayed.
3. Type a **file name** for the configuration file in the File Name text box.
4. Specify a **directory** in the Directories list box.
5. Click **OK**.

The configuration is save as a configuration file (.CFG).

NOTE: *If you do not manually save the configuration, you can recall it at a later time by loading a method or data file that used the configuration. For more information, see “Loading from a method (.MET) or data (.B*) file” on page 4-23.*

Automatically saving

When you create or edit a method, and then save the method, the current configuration settings are saved with the method file. You can control whether the system configuration is saved as the method configuration in the method file (.MET).

If you want the method configuration to be the same as the system configuration, do one of the following:

- Do not alter the settings in the Configuration dialog box after creating the method.
- Alter the settings in the Configuration dialog box by accessing it from the Control Panel after creating the method. Select **Update Configuration** from the Method Editor Options menu.

If you want the method configuration to be different from the system configuration, do one of the following:

- Alter the settings in the Configuration dialog box by accessing it through the Method Editor Options menu. This action changes the method configuration, but does not change the system configuration.
- If you alter the settings in the Configuration dialog box by accessing it through the Control Panel after creating the method, do not select **Update Configuration** from the Method Editor Options menu. This action changes the system configuration, but does not change the method configuration.

Printing

To print the current system configuration, select **Print Configuration** from the File menu in the Control Panel.

Print setup

Menus that contain a Print command also contain a Print Setup command.

Print Setup allows you to select a printer, as well as other printer options. Print Setup is a standard feature with all Microsoft Windows applications. For more information on Print Setup and connecting printers to your computer, refer to the *Microsoft Windows User's Guide*.

4.4 Loading an Existing Configuration

There are two ways to load an existing configuration as the system configuration:

- Load from a configuration file (.CFG)
- Load from a method (.MET) or data (.B*) file

Loading from a configuration file (.CFG)

To load an existing configuration you saved previously as a configuration file:

1. Select **Open Configuration** from the File menu in the Control Panel.

The Open File dialog box is displayed. A list of existing configuration files (.CFG) is also displayed.

2. Select a configuration file (.CFG), then click **OK**.

The new configuration settings are loaded in the Configuration dialog box. This is now the *system* configuration.

3. Check the physical setup of the BioCAD 700E Workstation and make sure it matches the system configuration. Change the physical setup if necessary. For more information, see Chapter 3, Preparing the Workstation and External Devices.

Loading from a method (.MET) or data (.B*) file

You can also load a configuration from a method or data file:

1. Select **Open Configuration** from the Control Panel File menu.

The Open File dialog box is displayed (Figure 4-12).

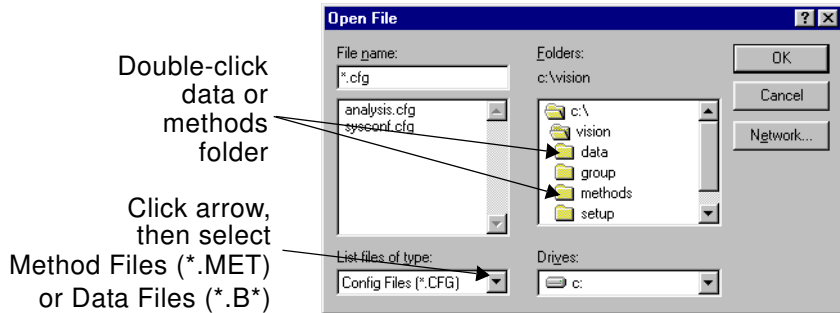


Figure 4-12 Open File Dialog Box

2. Select **Method Files (*.MET)** or **Data Files (*.B*)** from the List Files of Type drop-down list box.

No files are listed because the methods or data directories are not open.

3. Double-click the **methods folder** or **data folder**.

All method (.MET) files or data (.B*) files are listed.

4. Select a **method file** or **data file**. Click **OK**.

The configuration settings associated with the method file or data file are loaded in the Configuration dialog box. This is now the *system* configuration.

5. Check the physical setup of the workstation and make sure it matches the system configuration. Change the configuration if necessary. For more information, see Chapter 3, Preparing the Workstation and External Devices.

4.5 Customizing Configuration Libraries

The BioCAD software lets you customize the following configuration libraries:

- Buffer/Solvent Library
- Sample Library
- Column Library

Each configuration library allows you to create, edit, and save information about buffer/solvents, samples, or columns that you expect to use frequently. You can quickly and easily retrieve information for a specific buffer/solvent, sample, or column when you create future configurations.

4.5.1 Customizing the Buffer/Solvent Library

To customize the Buffer/Solvent Library:

1. Select **Edit Config** from the Config menu in the Control Panel to display the Configuration dialog box.
2. Double-click any of the **buffer/solvent channels (A through F)** in the Solvent Pump section (Figure 4-13).

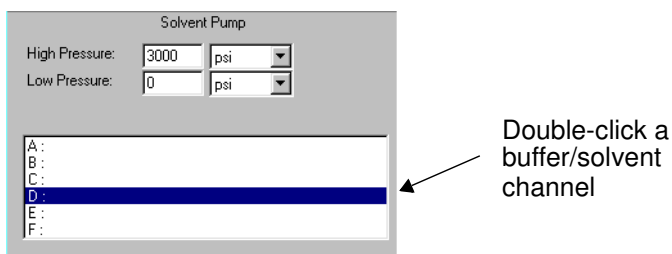


Figure 4-13 Buffer/Solvent Channels

The Buffer/Solvent Library dialog box is displayed (Figure 4-14).

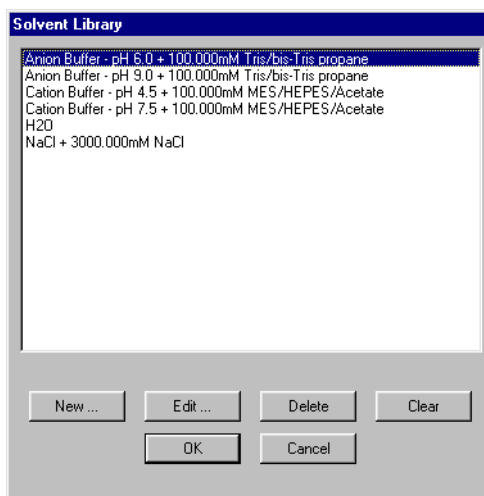


Figure 4-14 Buffer/Solvent Library



Creating or editing

3. To create a buffer/solvent channel, click the **New** button. To edit a buffer/solvent channel, select the channel and click the **Edit** button.

The Specify Channel dialog box is displayed (Figure 4-15).



Figure 4-15 Specify Channel Dialog Box

NOTE: If you are editing a buffer that has been calibrated, the bottom of the Specify Channel dialog box displays a *Calibrated With* text box that lists the solution used to calibrate the buffer.

4. Enter or modify the following information:

Parameter	Description
Name	Describes the buffer/solvent for documentation purposes.
Short Name	Eight characters displayed on the Control Panel next to the channel letter.
Component Name	Identifies the component in the buffer or eluent. The component name is: <ul style="list-style-type: none"> • Used in pH and Eluent blend mode • Not required in Percent blend mode
Concentration	Concentration of the component in the buffer or eluent. The concentration is: <ul style="list-style-type: none"> • Used in pH and Eluent blend mode • Not required in Percent blend mode <p>NOTE: <i>When you program in pH or Eluent blend mode, the software uses the component concentration in the eluent or buffer to calculate the percent of eluent or buffer in the final blend.</i></p>

Continued

Parameter	Description
Type	<p>Choices are:</p> <ul style="list-style-type: none"> • None (default)—Used in Percent mode or as the base buffer/solvent in Eluent mode. • Buffer—Used for pH mode. Enter the buffer concentration. • Eluent—Column eluent for Eluent and pH modes. • H2O—Water used in the pH mode to adjust buffer and eluent strength. • Sample—Select when you are making pump injections to load large-volume samples through the pump. Enter concentration for export to Group Analysis software. <p><i>Hint: If you are programming in Eluent blend mode, specify a buffer/solvent, such as 3 M NaCl, as Eluent.</i></p> <p><i>If you are programming in pH blend mode, specify pH buffers as Buffer, water as H2O, and a salt solution as Eluent.</i></p> <p><i>For information on the pH Map template, see Section 7.1.1, pH Map Template.</i></p>

- Deleting**
5. To delete an entry from the Buffer/Solvent Library, select a **buffer/solvent**, then click **Delete**.

NOTE: Before clicking Delete, make sure you select the correct item. After you click Delete, you cannot retrieve the item.

4.5.2 Customizing the Sample Library

To customize the Sample Library:

1. Select **Edit Config** from the Config menu in the Control Panel to display the Configuration dialog box.
2. In the Sample section of the Configuration dialog box, click the **Configuration** button under **Autoloader**.

The Autoloader dialog box is displayed (Figure 4-16).

3. Double-click any of the **sample channels** in the Autoloader dialog box.

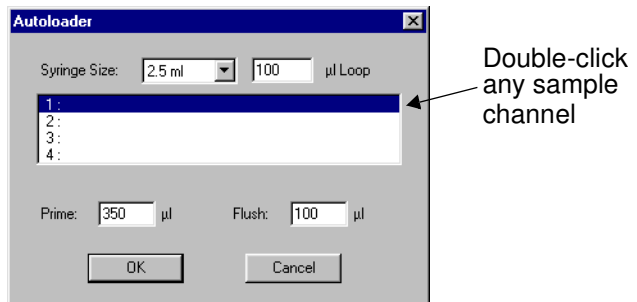


Figure 4-16 Sample Channels

The Sample Library dialog box is displayed (Figure 4-17).

NOTE: If you configure an integrated autosampler, you can also display the Sample Library dialog box from the Sample Table. See Section 4.7, Configuring and Preparing the BioCAD Software for an Integrated Autosampler.

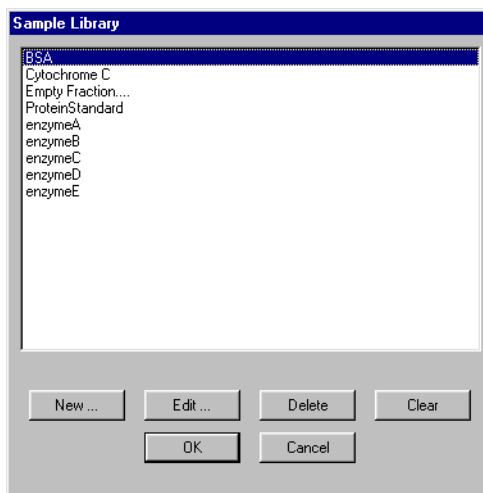


Figure 4-17 Sample Library

Creating or editing

4. To create a new sample, click the **New** button. To edit a sample, select the sample and click the **Edit** button. The Sample Editor dialog box is displayed (Figure 4-18).

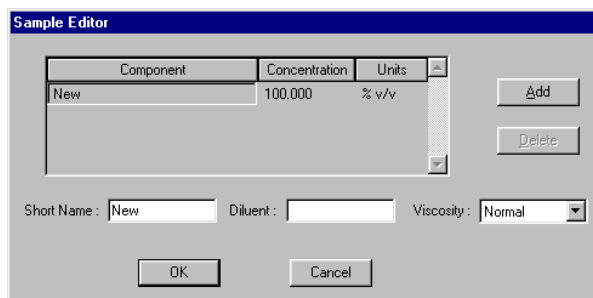


Figure 4-18 Sample Editor Dialog Box

NOTE: Samples with the same Short Name, but different attributes are treated as different samples. In most locations in the software, all attributes are listed for a sample. However, Short Name only is displayed in error codes, Inject segments, and in the Control Panel.

5. Enter or modify the following information:

Parameter	Description
Component	<p>Enter all component names in the sample. You must enter at least one component name.</p> <p>To add additional components, click the Add button and enter information on the line provided. To delete a component, select the component and click Delete.</p> <p>Component Names are used for documentation purposes only.</p>
Concentration and Units	<p>Enter concentrations and units for all components. The first component defaults to 100% v/v.</p> <p>Concentration is used for documentation purposes only.</p>
Short Name	<p>Enter up to eleven characters. The Short Name is displayed in error codes, in Inject segments in a method, and on the Control Panel next to the sample number.</p> <p>NOTE: Use unique Short Names for different samples. Samples that have the same Short Name but different attributes are treated as different samples.</p>
Diluent	<p>Enter the diluent that a sample contains. This diluent is for documentation purposes only.</p>
Viscosity	<p>Not functional at this time.</p>

6. When all components are added, click **OK**.

- Deleting** 7. To delete an entry from the Sample Library, select a **sample**, then click **Delete**.

NOTE: Before clicking Delete, make sure you select the correct item. After you click Delete, you cannot retrieve the item.

4.5.3 Customizing the Column Library

To customize the Column Library:

1. Select **Edit Config** from the Config menu in the Control Panel to display the Configuration dialog box.
2. Double-click any of the **column options** in the Plumbing section of the Configuration dialog box.

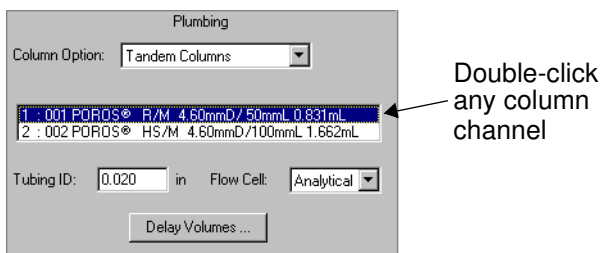


Figure 4-19 Column Options

The Column Library dialog box is displayed (Figure 4-20).

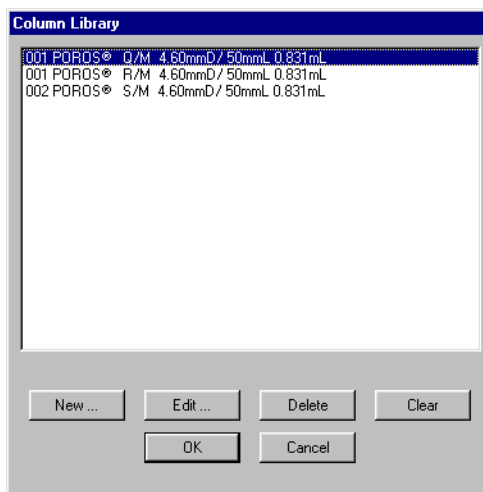


Figure 4-20 Column Library

4

Creating or editing

- To create a new column entry, click the **New** button. To edit a column entry, select the column and click the **Edit** button. The Column Editor dialog box is displayed (Figure 4-21).

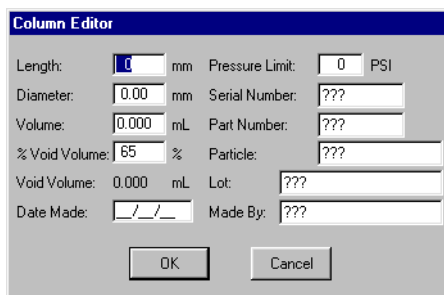


Figure 4-21 Column Editor Dialog Box

- Enter or modify the following information:

NOTE: If you enter a valid part number for your POROS column, the software automatically fills in all fields except Serial Number, Lot, Date Made, and Made By. If you do not enter column dimensions, you cannot use CV (column volumes) or cm/hr (linear velocity) when programming volumes or flow rates.

Parameter	Description
Length	Column length in mm, from column label.
Diameter	Inside diameter of column in mm, from column label.
Volume	Column volume. Software calculates value based on column length and diameter.

Continued

Parameter	Description
% Void Volume	(Total Particle Volume ÷ Column Volume) x 100 NOTE: % Void Volume is dependent on the packing characteristics of your media.
Void Volume	Column void volume. Software calculates estimated value based on column length, diameter, and % void volume.
Pressure Limit	Column pressure limit. If system exceeds column pressure limit while the column is inline, the pump shuts down.
Serial Number	From column label.
Part Number	Valid part number, from column label.
Particle	Column chemistry and particle size, from column label.
Lot	From column label.
Date Made	From column label.
Made By	From column label.

- Deleting**
- To delete an entry from the Column Library, select a **column**, then click **Delete**.

NOTE: Before clicking Delete, make sure you selected the correct item. After you click Delete, you cannot retrieve the item.

4.6 Configuring and Preparing the BioCAD Software for a Fraction Collector

- Before you begin** Before you configure and prepare the BioCAD software for a fraction collector, do the following:
- Connect the fraction collector to the BioCAD 700E Workstation as described in Section 2.4.3, Connecting the Fraction Collector.
 - Program operating parameters on the front panel of the fraction collector as described in Section 3.5.3, Preparing Fraction Collector Software.

- Configuring the fraction collector** To configure and prepare the BioCAD software for a fraction collector:
1. Select **Edit Config** from the Config menu in the Control Panel to display the Configuration dialog box.
 2. Select **Advantec/Gilson Fraction Collector** from the Collector list box (see Figure 4-2 on page 4-4).
 3. Type the **number of tubes** in the fraction collector tube rack.
 4. Type the **tube volume** (ml/tube).
 5. If you replumb the tubing from the fraction waste valve to the fraction collector, set the **delay volume** for the new tubing length. See “Setting delay volumes” on page 4-13.

NOTE: After you configure the fraction collector, you can control it from the Control Panel or in a method. For more information, see Section 5.4.1, Controlling the Fraction Collector, or “Fraction Collector Events” on page 6-41.

4.7 Configuring and Preparing the BioCAD Software for an Integrated Autosampler

To configure and prepare the BioCAD software for an integrated autosampler, you must:

- Configure an integrated autosampler
- Customize the Sample Library
- Enter vials in the Sample Table

4.7.1 Configuring Integrated Autosampler

Overview There are two ways you can configure an autosampler:

- **Integrated**—The BioCAD software has complete control over autosampler functions. You program sample information using the BioCAD software. You must use an AS 3500 autosampler provided by Biosystems.
- **Direct Wire**—The BioCAD software triggers the autosampler to make injections. You program sample information using the autosampler controls. You can use any autosampler.

Before you begin Before you configure an integrated autosampler:

- Power up the autosampler.
- Start the BioCAD software. See Section 2.6, Starting Up and Shutting Down, for more information.
- Make sure your BioCAD software is version 2.05 or later by selecting **About BioCAD** from any Window menu.
- Make sure your autosampler software is version 3.36 or later. See “Checking software version” on page 3-44.

Configuring To configure an integrated autosampler:

1. Make sure your autosampler has an integrated connection to the workstation and is powered up. See “Connecting integrated autosampler” on page 2-21.
2. Select **Edit Config** from the Config menu to display the Configuration dialog box.
3. In the Autosampler section of the Configuration dialog box, click the **Configuration** button under **Integrated AS3500**.

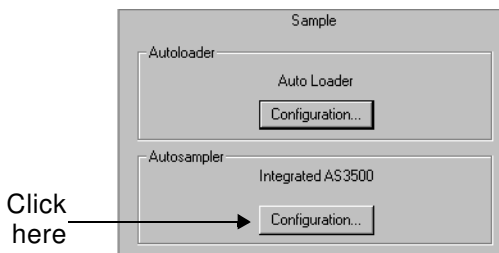


Figure 4-22 Integrated Autosampler Configuration

NOTE: If Integrated AS 3500 is not displayed in the Autosampler section, specify AS 3500 as autosampler in the hardware setup. See Section 2.5.2, Updating BioCAD Hardware Setup Options.

The AS 3500 dialog box is displayed (Figure 4-23).

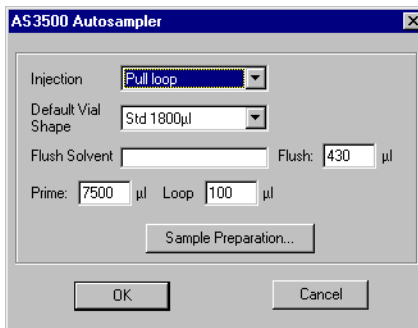


Figure 4-23 AS 3500 Autosampler Dialog Box

Injection type 4. Select the **injection type** from the Injection drop-down list box. Choices are:

Injection Type	When to Use	Injection Volume Range	Volume Drawn
Pull Loop (small) (default setting)	To conserve sample. Only desired sample amount is pulled into sample loop.	0.1 µl to 200 µl	Injection volume + 1.1 µl
Pull Loop* (large)	To make large injections.	200 µl to 1.5 ml	Injection volume + 45 µl
PushLoop	<ul style="list-style-type: none"> To make low-volume injections To make variable-volume injections with high precision 	0.1 µl to 200 µl	Injection volume + 14 µl
Full Loop	<ul style="list-style-type: none"> To make routine injections of the same volume To make fixed-volume injections with high precision 	Volume of sample loop, up to a maximum of 1.2 ml	<ul style="list-style-type: none"> (1.33 x loop vol) + 70 µl (for injections ≤ 100 µl) (2 x loop vol) + 45 µl (for injections > 100 µl)
*You can make Pull Loop (large) injections only if your autosampler supports the sample preparation option.			



For a description of how different injection types are made, see Appendix F, Autosampler Injection Types.

- Default vial shape** 5. Select a **vial shape** from the Default Vial Shape drop-down list box.

Default Vial Shape	Dead Volume	Maximum Capacity	*Available Sample Volume	When to Use
Std 1800 µl	200 µl	1800 µl	Up to 1600 µl	When using the 1.8 ml glass vial.
Std 2000 µl	200 µl	2000 µl	Up to 1800 µl	When using the 1.8 ml glass vial. NOTE: This vial shape allows you to override the maximum capacity suggested by the vendor, thereby increasing the available sample volume*.
Micro	20 µl	250 µl	Up to 230 µl	When using the 250 µl conical plastic vial.
Unspecified	None	2000 µl	Contents of vial	When handling small volume samples and using the 1.8 ml glass vial with a vial insert. For applications where sample recovery is more important than injection volume accuracy. NOTE: For more information, see “Vial inserts for Unspecified vial shape” on page 3-52, and “Understanding Unspecified vial shape” on page 4-41.
<p>*NOTE: Available sample volume equals the volume of liquid in the vial minus the dead volume for the vial.</p> <p>NOTE: The needle height is the same for all of the above vial shapes.</p>				

NOTE: This selection sets the default vial shape for other parts of the software. You can change the vial shape in other parts of the software, if desired.

- Flush reagent* 6. Select a reagent to use for the autosampler **Flush Solvent**:
- Double-click on the **Flush Solvent** field. The Buffer/Solvent Library is displayed.
 - Select a reagent and click **OK**.

NOTE: Select a flush solvent that is appropriate for use with your autosampler solvents and your samples. One to five percent isopropanol is appropriate for most applications.

- Flush volume* 7. Type the **Flush** volume. The default and recommended volume is 430 μ l.
- The Flush volume is used to flush the needle and the line between the needle and injection valve on an integrated autosampler. The Flush volume is used when you:
- Flush an integrated autosampler from the Sample Table. For more information, see Section 5.4.2, Controlling an Integrated Autosampler.
 - Flush an integrated autosampler during an Autosampler Inject segment in a method. For more information, see “Autosampler Inject Segments” on page 6-30.

- Prime volume* 8. Type the **Prime** volume.
- Prime volume is used when you prime the autosampler solvent and flush lines. See Section 3.6.5, Priming the Autosampler.

NOTE: Type a prime volume of at least 7,500 μ l to ensure complete priming of autosampler lines.

- Loop size* 9. Type the **Loop** size.
- You must physically install the correct sample loop on the autosampler. See the *Autosampler Reference Manual*.

**Understanding
Unspecified
vial shape**

The Unspecified vial shape is a feature of the BioCAD software that allows for maximum flexibility when working with small volume samples. It has no dead volume limitation and a maximum volume of 2.0 ml in the software.

It allows you to inject nearly the complete contents of the vial if you use a micro vial insert inside a 1.8 ml glass vial. The micro vial insert eliminates the dead volume. For more information on micro vial inserts, see “Vial inserts for Unspecified vial shape” on page 3-52.

**When to use
Unspecified
vial shape**

Use Unspecified vial shape if the following are true of your application:

- Small sample volume
- Maximum sample recovery is important
- Minimal sample loss is important
- Quantitative injection volume is not required

**How to use
Unspecified
vial shape**

To use the Unspecified vial shape:

- Prepare your sample in a micro vial insert inside of a 1.8 ml glass vial. For more information, see “Vial inserts for Unspecified vial shape” on page 3-52.
- Select **Pull loop** as the injection type when you configure the integrated autosampler. For more information, see “Injection type” on page 4-38.
- Select **Unspecified** as the vial shape when you enter the sample in the Sample Table. For more information, see Section 4.7.3, Entering Vials in Sample Table.

CAUTION

Always use micro vial inserts when using the Unspecified vial shape. Other vials do not eliminate dead volume and will cause you to aspirate air if you sample below the dead volume for that vial type.

Configuring sample prep

The integrated autosampler that you use with your BioCAD Workstation may support sample heating and cooling or sample preparation. If so, you need to configure sample preparation:

1. Click the **Sample Preparation** button in the AS 3500 Autosampler dialog box (see Figure 4-23).

NOTE: If the *Sample Preparation* button is not displayed, select autosampler 310 or 311 in hardware setup. See Section 2.5.2, Updating BioCAD Hardware Setup Options.

The Sample Preparation Configuration dialog box is displayed (Figure 4-24).

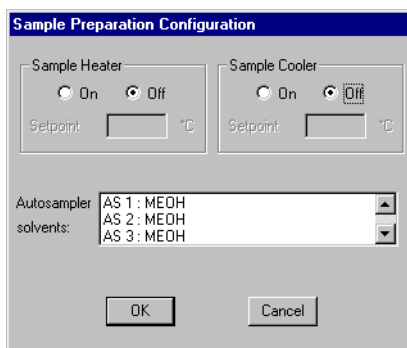


Figure 4-24 Sample Preparation Configuration

Sample heating (heat chamber)

2. If desired, turn on the autosampler heat chamber by clicking the **On** button and typing a setpoint temperature. Valid range is from ambient temperature to 100°C (212°F) in 1°C increments.

The autosampler *heat chamber* is set to this temperature.

NOTE: You can set the temperature as low as 0°C, but the actual setpoint does not go below ambient temperature.

NOTE: To heat a sample vial, you add a *Heat/Mix* segment to a method, which places the sample vial in the heat chamber. See “Heat/Mix Segments” on page 6-64.

*Sample cooling
(tray area)*

3. If desired, turn on the autosampler cooler by clicking the **On** button and typing a setpoint temperature. Valid range is from 0 to 60°C (32 to 140°F) in 1°C increments.

The autosampler *tray area* is set to this temperature.

NOTE: Allow 30 minutes for heating and cooling devices to reach temperature.

NOTE: You can also control autosampler sample heating and cooling from the Sample Table. See Section 5.4.2, Controlling an Integrated Autosampler. The settings you make last are active.

*Autosampler
solvents*

4. To configure autosampler solvents, double-click an autosampler solvent line, **AS1** through **AS4**. Lines AS1 through AS4 correspond to the physical lines S1 through S4 on the autosampler. Autosampler solvents you configure are available for use in Dilute and Combine segments in a method. For more information, see “Dilute Segments” on page 6-57, and “Combine Segments” on page 6-61.

The Buffer/Solvent Library is displayed.

5. Select a buffer/solvent, then click **OK**.

For information on adding to the Buffer/Solvent Library, refer to Section 4.5.1, Customizing the Buffer/Solvent Library.

6. Repeat step 4 and step 5 for each autosampler solvent line.

To clear an autosampler solvent channel, double-click it to display the Buffer/Solvent Library, then click the **Clear** button.

7. Click **OK**.

4.7.2 Customizing the Sample Library

If you configure an integrated autosampler, the Sample Library is available. The Sample Library allows you to create, edit, and save samples for use with an integrated autosampler.

To customize the Sample Library:

1. Select **Sample Table** from the Window menu in the Control Panel.

The Sample Table window is displayed (Figure 4-25).

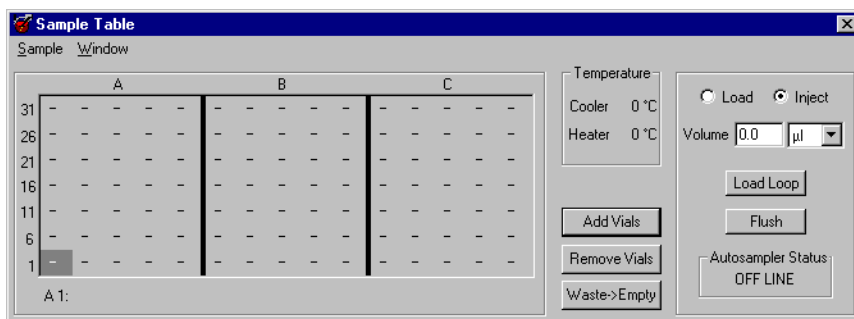


Figure 4-25 Sample Table Window

NOTE: If Sample Table is not displayed in the Window menu, select an AS 3500 autosampler in hardware setup. See Section 2.5.2, Updating BioCAD Hardware Setup Options.

2. Double-click any **vacant vial position** in the grid.
The Vial Editor dialog box is displayed (Figure 4-26).

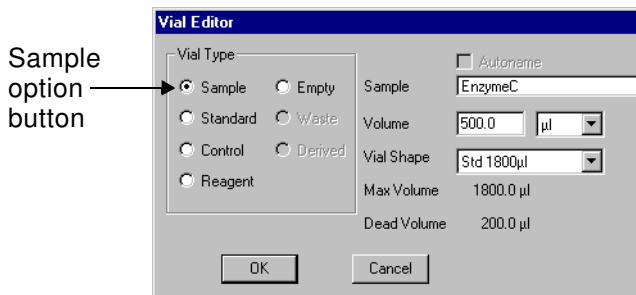


Figure 4-26 Vial Editor Dialog Box

3. Click the **Sample** option button.
The Sample Library dialog box is displayed (Figure 4-27).

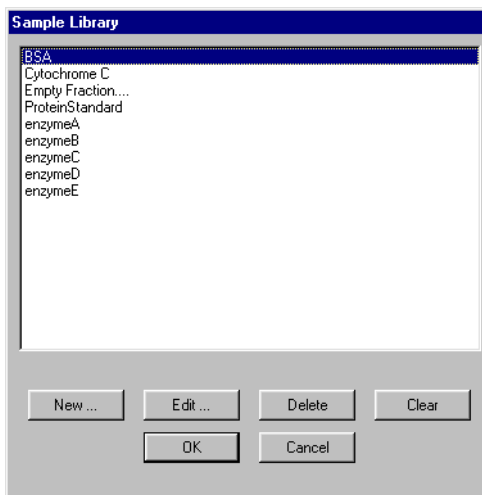


Figure 4-27 Sample Library Dialog Box

4. Create, edit, or delete samples as described in “Creating or editing” on page 4-30, and “Deleting” on page 4-31.

4.7.3 Entering Vials in Sample Table

Overview If you use an integrated autosampler, enter vials in the Sample Table to correspond to the vials you physically loaded in the autosampler trays. When you enter sample vials in the Sample Table window, the vials are automatically added to the current *system* configuration.

When you run a method, the BioCAD software compares the sample names and volumes requested by the method to the sample names and volumes entered in the Sample Table. If all requested sample names and volumes are not listed in the Sample Table, a “Method’s configuration does not match ...” error is displayed.

NOTE: *You cannot alter the Sample Table when a method is running. However, you can make an injection from the Sample Table when a method is running.*

This section describes:

- Entering single vials or batches of vials
- Autonaming batch-entered vials
- Adding base name and suffix to Sample Library
- Clearing vials
- Saving Sample Table settings in a configuration

Entering single vials or batches of vials

To enter single vials or batches of vials in the Sample Table:

1. Physically load sample vials in the autosampler trays. See Section 3.6.7, Loading Vials in Autosampler Trays.
2. Select **Sample Table** from the Window menu in the Control Panel.

The Sample Table window is displayed (Figure 4-28).

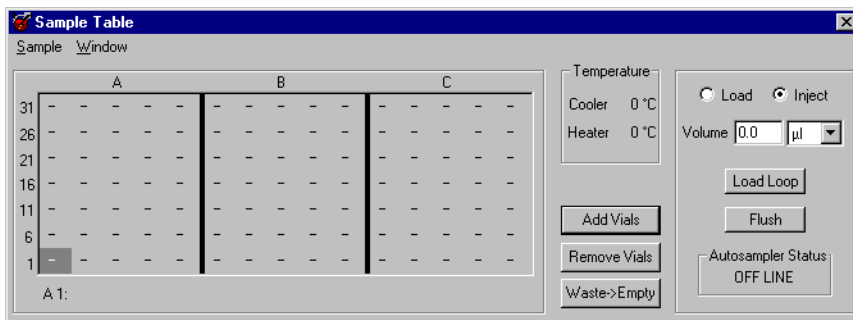


Figure 4-28 Sample Table Window

3. To enter a single vial, double-click a vial position in the Sample Table. To enter a batch of vials, click-drag over the vial positions and click **Add Vials**.

The Vial Editor dialog box is displayed (Figure 4-29).

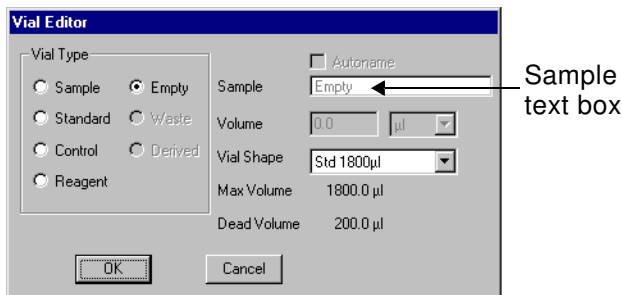


Figure 4-29 Vial Editor Dialog Box



4. Select the **vial type**:

Vial Type	Description
Sample, Standard, Control, or Reagent	Vial is listed anywhere you select a Sample vial in a method, for example, in a Dilute, Combine, Heat/Mix, or Inject Segment Setup dialog box.
Empty*	Vial will be used as the destination vial in a method Prep block segment, such as Dilute or Combine.
Derived*	<p>Derived vial types are display-only and reflect the state of an entered vial (Sample, Standard, Control, Reagent, or Empty). Derived vial types are the result of some action by the autosampler. For example, an Empty vial changes to a Derived vial after reagents and samples are added to the vial during the execution of a Prep block segment, such as Dilute or Combine.</p> <p>Derived vial types are listed anywhere you select a Derived vial in a method, for example, in a Dilute, Combine, Heat/Mix, or Inject Segment Setup dialog box.</p>
Waste*	Waste vial types are display-only and reflect the state of an entered vial. For example, intermediate vials created by a Prep block are changed to Waste vials at the end of the Prep block.
<p>*Empty, Derived, and Waste vial types are available only if your autosampler supports the sample preparation option.</p>	



If you select Empty, “Empty” is displayed in the Sample text box, and the vial is entered.

If you select Sample, Standard, Control, or Reagent, the Sample Library is displayed. Select a **sample name** or add a new sample name and click **OK**. The Vial Editor dialog box is displayed, with the name you selected from the Sample Library displayed in the Sample text box.

If the vial has already been entered as Sample, Standard, Control, or Reagent, double-click the **Sample text box** to display the Sample Library. Select a **sample name** or add a new sample name and click **OK**. The Vial Editor dialog box is displayed again, with the name selected from the Sample Library displayed in the Sample text box.

For information on adding names to the Sample Library, see Section 4.7.2, Customizing the Sample Library.

5. Select a **vial shape** (Std 1800 μ l, Std 2000 μ l, Micro, or Unspecified) from the Vial Shape drop-down list box.

For information on vial shapes, see “Default vial shape” on page 4-39.

6. Type a **volume** for Sample, Standard, Control, or Reagent vial types. Allowable fill volumes for each vial shape are:
 - **Std 1800 μ l**—200 μ l to 1.8 ml
 - **Std 2000 μ l**—200 μ l to 2.0 ml
 - **Micro**—20–250 μ l
 - **Unspecified**—0–2.0 ml

NOTE: Always type an accurate volume to avoid injecting from a vial with insufficient volume.

NOTE: As injections are made, the software recalculates the volume remaining in the vial. It accounts for volume used for injections and waste associated with making injections.

7. Click **OK**.

- Repeat step 3 through step 7 for all vials you want to enter in the Sample Table.

The Sample Table window is displayed again, with all selected vial positions labeled with the designated vial type: Sample (S), Standard (St), Control (C), Reagent (R), or Empty (E). You can click any vial position, and the sample name selected from the Sample Library and the vial volume are displayed at the bottom of the window.

Hint: Because you cannot alter the Sample Table during a method run, you may want to enter “dummy” vials in the Sample Table to account for any vials you may need to add to the autosampler tray during a method run.

Type an appropriate volume and name (such as “unknown#”) for the “dummy” vials. Be aware that you cannot update the sample name of the “dummy” vials in the final data files. This technique is not advisable for quality assurance procedures.

Autonaming batch-entered vials

You can use the Autaname feature when you batch-enter sample vials to specify a base name for the vials. The BioCAD software adds a suffix (-1, -2, -3, and so on) to the base name. You can also add the base name and suffix to the Sample Library.

To autaname batch-entered sample vials:

- Physically load sample vials in the autosampler trays. See Section 3.6.7, Loading Vials in Autosampler Trays.
- Select **Sample Table** from the Window menu in the Control Panel.

The Sample Table window is displayed (see Figure 4-28 on page 4-47).

- Select the sample vials to enter by click-dragging over the vial positions.

The maximum number of sample vials you can add is 105 (total number of vials in autosampler trays A, B, and C). Selected vials are highlighted.

- Click the **Add Vials** button.

The Vial Editor dialog box is displayed (Figure 4-30).

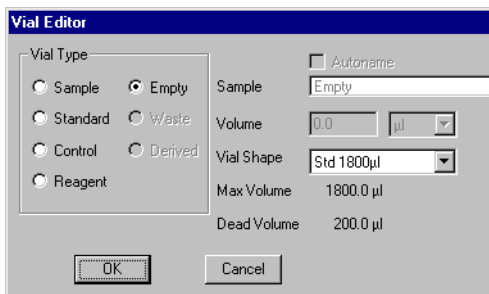


Figure 4-30 Vial Editor with Autaname Check Box

5. Click the **Autaname** check box.

The Sample text box changes to Base Name text box (Figure 4-31).

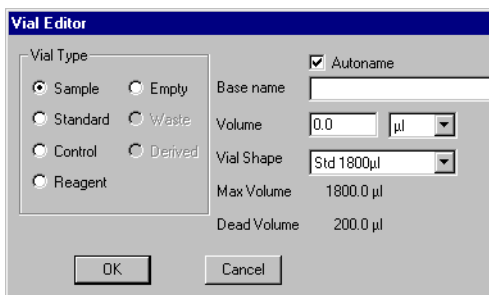


Figure 4-31 Vial Editor with Base Name Text Box

6. Type a **base name** (up to approximately 16 characters) for the vials.
7. Select the **vial type**, **vial shape**, and **volume**. See step 4 on page 4-48 to determine vial type. Click **OK**.

The Sample Table window is displayed again, with all selected vial positions labeled with the designated vial type: Sample (S), Standard (St), Control (C), Reagent (R), or Empty (E). You can click any vial position to display the base name, suffix, and vial volume. The sample name for each vial is the base name plus the suffix.

Adding base name and suffix to Sample Library

When you batch-enter vials using Autaname, you can also add the base name and suffix (sample name) to the Sample Library:

1. In the Sample Table window, double-click a vial position that was added using the Autaname feature.

The Vial Editor dialog box is displayed.

2. Double-click the base name and suffix in the Sample text box.

The Sample Library is displayed, with the base name and suffix added to the library list, with the following information:

- **Short Name**—Base name and suffix (for example, SAMPLE-1)
- **Sample Viscosity**—Normal
- **Component Name**—Base name and suffix (for example, SAMPLE-1)
- **Concentration/Units**—100%

To change this information, see Section 4.7.2, Customizing the Sample Library.

Clearing vials

To clear sample vials from the Sample Table window:

1. Select the vial positions to clear. Click-drag to select multiple vials.
2. Click the **Remove Vials** button.

Vials are cleared from the Sample Table window and from the current system configuration. Sample name information is not deleted from the Sample Library.

Saving Sample Table settings in a configuration

You can save the settings in the Sample Table to a configuration file that you can call up and use at a later time.

Select **Save Configuration As** from the File menu in the Control Panel. Type a file name for the configuration file. The Sample Table settings are saved to the configuration.

If you want to use this configuration and Sample Table settings again, you can load the configuration by selecting **Open Configuration** from the File menu in the Control Panel.

4.8 Configuring and Preparing the BioCAD Software for a Direct Wire Autosampler

Overview There are two ways you can configure an autosampler:

- **Integrated**—The BioCAD software has complete control over autosampler functions. You program sample information using the BioCAD software. You must use an AS 3500 autosampler provided by Applied Biosystems.
- **Direct Wire**—The BioCAD software triggers the autosampler to make injections. You program sample information using the autosampler controls. You can use any autosampler.

Before you begin Before you configure a direct wire autosampler:

- Program an autosampler method using the controls of the direct wire autosampler. For more information, see Section 3.6, *Preparing an Autosampler*, or the *Autosampler Reference Manual*.
- Power down the direct wire autosampler.
- Start the BioCAD software. For more information, see Section 2.6, *Starting Up and Shutting Down*.

After configuring After you configure a direct wire autosampler, you can power it up and control it from the autosampler controls. For more information, see the *Autosampler Reference Manual*.

From the Control Panel, you can trigger the direct wire autosampler to make an injection. For more information, see Section 5.4.3, *Controlling a Direct Wire Autosampler*.

You can create a BioCAD method to trigger the autosampler method. For more information, see Section 6.3.7, *Making Direct Wire Autosampler Injections in a Method*.

Configuring To configure and prepare the BioCAD software for a direct wire autosampler, you must:

- Set contact closure information
- Set timeout period
- Set starting vial and injections per vial

To configure a direct wire autosampler:

1. Make sure your autosampler has a direct wire connection to the workstation and is powered down. See “Connecting direct wire autosampler” on page 2-22.
2. Select **Edit Config** from the Config menu to display the Configuration dialog box.
3. In the Autosampler section of the Configuration dialog box, click the **Configuration** button under **Direct Wire Autosampler**.

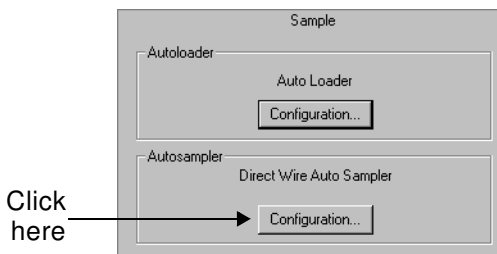


Figure 4-32 Direct Wire Autosampler Configuration

NOTE: If Direct Wire Autosampler is not displayed in the Autosampler section, specify a direct wire autosampler in the hardware setup. See Section 2.5.2, Updating BioCAD Hardware Setup Options.

The Direct Wire Autosampler dialog box is displayed (Figure 4-33).

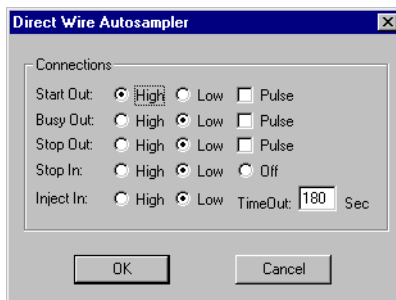


Figure 4-33 Direct Wire Autosampler Dialog Box

- Contact closure* 4. If you are using the autosampler provided by Biosystems, use the default contact closure information already selected in the Connections section.

If you are using another autosampler, specify contact closure information to allow the BioCAD software to control the autosampler. Refer to your autosampler manual for information.

- Timeout period* 5. Type the **Timeout** period.

The timeout period is the length of time the BioCAD software waits for an injection before stopping pump flow and generating an error message. Default is 180 seconds.

NOTE: Set the *Timeout* long enough to allow the autosampler to make an injection. When using a slow sample speed, injecting a viscous sample, or performing sample preparation, the autosampler may require more than 180 seconds for an injection.

6. Click **OK** to save the configuration and return to the Control Panel.

Starting vial and injections per vial

7. Select **Set Vial** from the Control Panel Sample menu. The Set Vial Number dialog box is displayed (Figure 4-34).

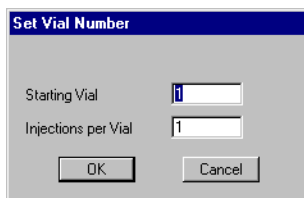


Figure 4-34 Set Vial Number Dialog Box

8. Type the **starting vial number** and the **number of injections** to make from each vial. Click **OK**.

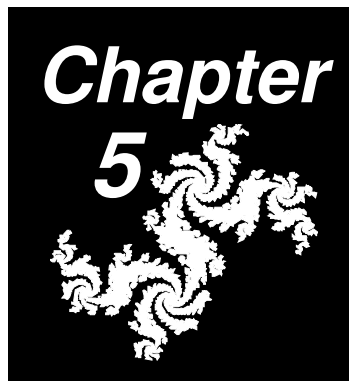
In the AS 3500 autosampler provided by Applied Biosystems, vials are labeled A1 through A35, B1 through B35, and C1 through C35. In the BioCAD software, vials are designated 1 through 105. To specify any of the B or C positions in the BioCAD software, determine the actual vial number.

Rack	Rack Vial #	Vial # in BioCAD Software
Rack A	A1–A35	1–35
Rack B	B1–B35	36–70
Rack C	C1–C35	71–105

NOTE: *The starting vial number and injections per vial information does not control a direct wire autosampler. You must control a direct wire autosampler from the autosampler controls.*

The starting vial number and injections per vial information is for documentation purposes only and is stored in the data file. If you do not enter this information, no starting vial number or injection number is reported.

5 Controlling the Workstation From the Control Panel



This chapter includes the following sections:

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5.2	Controlling Workstation Components and Systems	5-4
5.2.1	Changing the Syringe and Sample Loop	5-4
5.2.2	Controlling Buffers/Solvents and Blending	5-8
5.2.3	Controlling the Pump and Flow Rate	5-13
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5.2.5	Controlling Columns	5-18
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5.1 Overview

Use the Control Panel and Sample Table to manually control the BioCAD 700E Workstation. Any actions you perform from the Control Panel or Sample Table override a running method.

NOTE: All operations described in this chapter can also be performed using a method.

Displaying Control Panel

The Control Panel (Figure 5-1) is displayed when you start the BioCAD software. If you are using the BioCAD software, but the Control Panel is not displayed, do one of the following:

- Select **Control Panel** from the Window menu in the BioCAD software.
- Double-click the **Control icon** at the bottom of the screen, if you have minimized the Control Panel.

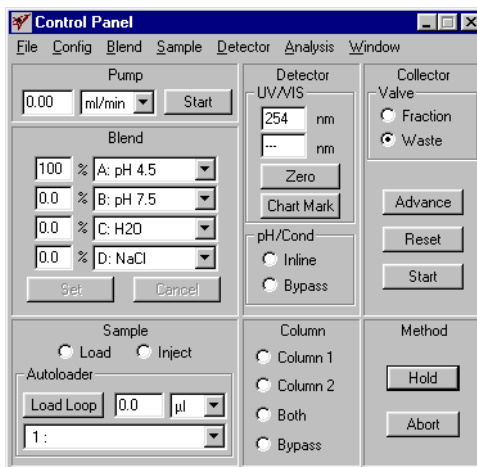


Figure 5-1 Control Panel

Displaying Sample Table

If you configure an integrated autosampler, the Sample Table (Figure 5-2) is available. Display the Sample Table by selecting **Sample Table** from the Window menu.

NOTE: If Sample Table is not displayed in the Window menu, select an AS 3500 autosampler in hardware setup. See Section 2.5.2, Updating BioCAD Hardware Setup Options.

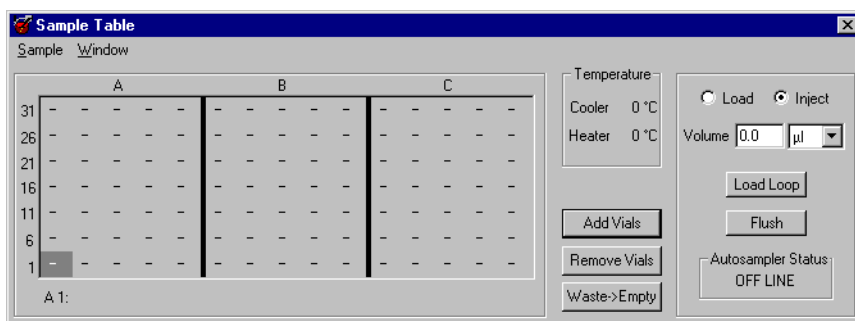


Figure 5-2 Sample Table

Adding events to a running method

When you perform an operation from the Control Panel or Sample Table, such as turning off the pump, an event is added to the method that is stored with the data file (.BIO or .B##). These added events are flagged with an asterisk (*) and a plus sign (+) and appear only in methods contained in data files. No event is added to the method file (.MET).

If you retrieve the method from the data file (.BIO or .B##) and run the method, events that control hardware are executed. Events that do not control hardware, such as Event Mark, or events that are specific for the original data, such as Collect Fraction to Tube 1, are ignored.

5.2 Controlling Workstation Components and Systems

You can manually control all workstation components and systems from the Control Panel. This section describes:

- Changing the syringe and sample loop
- Purging the system
- Controlling buffers/solvents and blending
- Controlling the pump and flow rate
- Controlling columns
- Controlling detectors
- Controlling the fraction/waste valve
- Running a gradient
- Calibrating custom pH blend buffers
- Programming event outputs
- Stopping and resuming a method

5.2.1 Changing the Syringe and Sample Loop

Overview You can change the syringe size and sample loop size from the Control Panel. The changes you make update the syringe and sample loop sizes in the Configuration dialog box.

Selecting syringe and sample loop size Injection volume determines the syringe and sample loop size. To achieve maximum injection volume accuracy and precision, follow these guidelines when specifying injection volumes and selecting sample loop sizes:

- For injection volumes less than 50 percent of the sample loop volume, enter the injection volume in the Control Panel or method.
- For injection volumes equal to the sample loop volume, enter a volume of three times the sample loop volume in the Control Panel or method. For example, you can accurately inject a 100 μ l sample using the 100 μ l sample loop if you use an injection volume of 300 μ l.
- Do not inject volumes between 50 percent and 100 percent of the sample loop volume.

Changing syringe

Change the syringe to accommodate different injection volumes.

Syringes provided with the BioCAD 700E Workstation include:

- 500 µl—Injection volume range 0.001 to 0.5 ml
- 5 ml—Injection volume range 0.01 to 5 ml

To change the syringe, you must:

- Remove the existing syringe
- Attach a new syringe

Removing

To remove the syringe:

1. Select **Change Syringe** from the Sample menu in the Control Panel.

NOTE: If *Change Syringe* is not displayed in the *Sample menu*, configure an autoloader. See Section 4.2.3, *Configuring the Autoloader*.

The syringe plunger retracts and the Set Syringe Size dialog box is displayed (Figure 5-3).

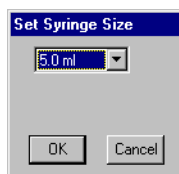


Figure 5-3 Set Syringe Size Dialog Box

CAUTION

Do not remove the syringe before retracting the plunger. The syringe will break.

2. Unscrew and remove the knurled knob at the base of the plunger.
3. Unscrew the body of the syringe (turn to the left) and remove.

Attaching To attach a new syringe:

1. Place the hole in the base of the plunger over the pin on the syringe piston.

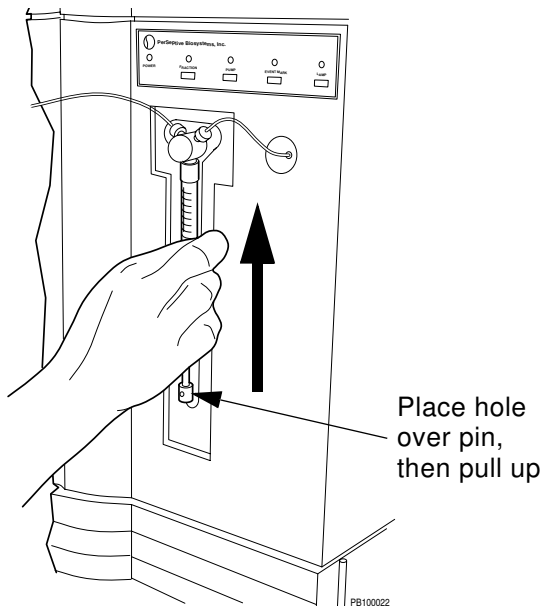


Figure 5-4 Replacing the Syringe

2. Pull up on the syringe body until it reaches the fitting.
3. While pushing up gently, turn the syringe body to the right and screw into place.
4. Replace the knurled knob in the plunger base.
5. Select the **size** of the syringe you installed from the drop-down list box in the Set Syringe Size dialog box. Click **OK**.

The syringe plunger returns to the home position.

NOTE: The home position varies with different syringe sizes. The syringe size you select updates the syringe size in the Configuration dialog box.

Changing sample loop

Change the sample loop to accommodate different injection volumes.

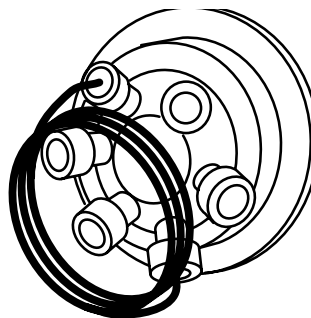
Sample loops provided with the BioCAD 700E Workstation include:

- 100 μ l
- 2 ml
- 5 ml

To change the sample loop:

1. Unscrew the installed loop from positions 2 and 5 on the sample injection valve.
2. Replace with the appropriate sample loop.

Connect loop to positions 2 and 5 on injection valve



PB100046

Figure 5-5 Installing a Sample Loop

3. Select **Change Loop Size** from the Sample menu on the Control Panel.

NOTE: If *Change Loop Size* is not displayed in the *Sample* menu, configure an autoloader. See Section 4.2.3, Configuring the Autoloader.

The Set Sample Loop Size dialog box is displayed.

4. Type the **size** of the loop you installed and click **OK**.

NOTE: The sample loop size you type updates the sample loop size in the Configuration dialog box.

5.2.2 Controlling Buffers/Solvents and Blending

In the Control Panel, you can select and blend up to four of the six buffers/solvents configured.

Selecting buffers/solvents

To select different buffers for blending:

1. Click the down arrow to the right of a buffer/solvent channel in the Blend section of the Control Panel (Figure 5-6).

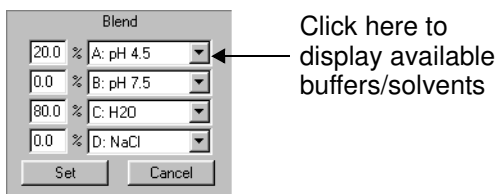


Figure 5-6 Blend Section of Control Panel

A list of available buffers/solvents is displayed.

2. Double-click a buffer/solvent to select it for the channel. If the desired buffer/solvent is not available, change the buffer/solvent configuration. For more information, see Section 4.2.2, Configuring Buffers/Solvents.

Blending To control blending of buffers/solvents:

1. Select a **blend mode** (Percentage, Eluent, or pH) from the Blend menu.

Blend Mode	Description
Percentage	Conventional % (v/v) technique used on most HPLC instruments. Allows you to select a percentage of solvent and blend up to four of the six configured solvents.
Eluent	Allows you to specify an eluent concentration, and select a buffer and an eluent to accomplish the blend. NOTE: <i>Eluent mode is available only if you configure a buffer/solvent as type Eluent. For more information, see Section 4.2.2, Configuring Buffers/Solvents, and Section 4.5.1, Customizing the Buffer/Solvent Library.</i>
pH	Uses two concentrated buffers at different pH values, water, and a concentrated eluent such as salt. This mode is required for running a pH Map template. NOTE: <i>pH mode is available only if you configure two pH buffers as type Buffer, a salt solution as type Eluent, and water as type H2O. For more information, see Section 4.2.2, Configuring Buffers/Solvents, and Section 4.5.1, Customizing the Buffer/Solvent Library.</i>
NOTE: <i>When using either the pH or Eluent blend modes, you can switch back to the Percentage blend mode to view the actual calculated blend.</i>	

The Blend section of the Control Panel changes accordingly (Figure 5-7, Figure 5-8, and Figure 5-9).

2. Type **values** for the blends and click **Set** to activate the blending.

Percentage mode

If you select Percentage mode, select the **buffers/solvents** to blend and type a **percentage** for each.

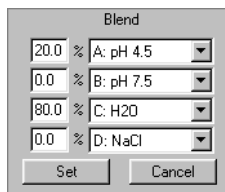


Figure 5-7 Percentage Blend Mode

NOTE: After you type a percentage for the first solvent, the percentages for additional solvents set automatically to equal a total of 100%. You can change the percentage of any solvent by entering a value.

For example, if you enter 20% for solvent A, when you click on solvent B, it sets to 80%. If you click on solvent C and enter a percentage, solvent B is again adjusted to make the total 100%.

Eluent mode

If you select Eluent mode, enter or select the following:

- Base buffer/solvent (for diluting)
- Eluent concentration (mM)
- Eluent

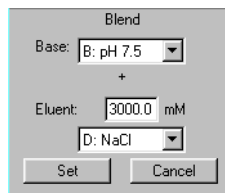


Figure 5-8 Eluent Blend Mode

pH mode

If you select pH mode, enter or select the following:

- Blend concentration (mM)
- Blend pH or %
- Two pH buffers
- Eluent concentration (mM)
- Eluent

The screenshot shows a 'Blend' dialog box with the following fields: Blend concentration set to 100.00 mM, Buffer A set to pH 6.0, Buffer B set to pH 9.0, Eluent concentration set to 0.000 mM, and Eluent type set to D: NaCl. There are 'Set' and 'Cancel' buttons at the bottom.

Using pH blend buffers
or calibrated custom buffers

The screenshot shows a 'Blend' dialog box with the following fields: Blend concentration set to 10.000 mM, Buffer A set to pH 8, Buffer B set to pH 12, Eluent concentration set to 0.000 mM, and Eluent type set to D: NaCl. There are 'Set' and 'Cancel' buttons at the bottom.

Using uncalibrated
custom buffers

Figure 5-9 pH Blend Modes

Valid eluent concentration range is determined by:

- Requested concentration for the blend entered in the Control Panel
- Initial concentration of the buffers entered in the Buffer/Solvent Library
- Initial concentration of the eluent entered in the Buffer/Solvent Library

For example, assume the initial concentration of each buffer is 200 mM. If you set the blend concentration to 100 mM of buffer, the system uses 50 percent of the buffer to achieve this concentration ($100 \text{ mM}/200 \text{ mM} = 50 \text{ percent}$).

Assume the initial concentration of the eluent is 3,000 mM. If 50 percent of the final solution is made up of buffer, the remaining 50 percent is made up of eluent and water. The valid range for the eluent in this example is 0 to 1,500 mM ($50 \text{ percent of } 3,000 \text{ mM} = 1,500 \text{ mM}$).

When using the following buffers, type a pH value:

- pH blend buffers (described in Section 3.2.2, Preparing pH Blend Mode Buffers)
- Calibrated custom pH blend buffers (described in Section 3.2.2, Preparing pH Blend Mode Buffers, and Section 5.2.9, Calibrating Custom pH Blend Buffers)

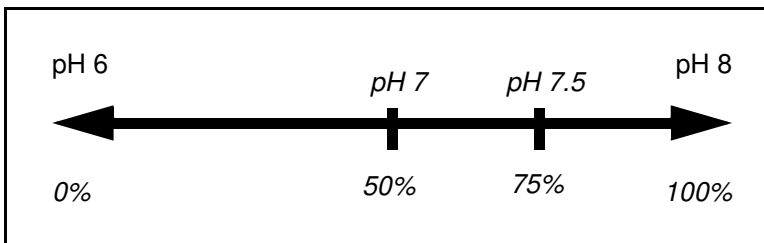
The BioCAD software automatically calculates the percent of each buffer needed to achieve the desired pH and eluent concentrations.

When using uncalibrated custom pH blend buffers, type a **percent** value that regulates the percentages of the two buffers. For more information, see “Understanding percent value in pH blending” below.

Understanding percent value in pH blending

The percent (%) value you type in the Blend section of the Control Panel corresponds to the pH of the two buffers used.

For example, you use two buffers of pH 6 and pH 8. To set the pH blend map for pH 7, type 50% (50% of pH 6 and pH 8 is approximately pH 7). The titration curve of the buffers affects the actual pH achieved.



Below is another example using buffers from pH 4 to pH 5:

Buffers	Type % ...	To achieve pH ...
pH 4 and pH 5	10%	4.1
	20%	4.2
	30%	4.3

5.2.3 Controlling the Pump and Flow Rate

To enter a flow rate and start and stop the pump:

1. Type a **flow rate** in the text box in the Pump section of the Control Panel (Figure 5-10). The flow rate range is 0.2 to 100.0 ml/min.

NOTE: *If you have an analytical flow cell installed in the UV/Vis detector, do not exceed a 45 ml/min flow rate.*

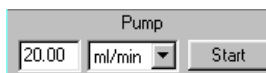


Figure 5-10 Pump Section of Control Panel

2. Select **flow rate units** (ml/min, CV/min, or cm/hr) from the drop-down list box. For more information, see “Flow rate units” on page 5-14.

NOTE: *CV/min and cm/hr are available only if you enter column dimensions in the Configuration dialog box.*

3. Click **Start** to start the pump.

The pump begins flowing, and the flow rate is displayed in the Status window.

4. To change the flow rate, type a new flow rate value, then click on any other area of the Control Panel to initiate the change.
5. Click **Stop** to stop the pump.

Flow rate units You can specify flow rates in:

- **ml/min**—Conventional flow rate units.
- **cm/hr**—A unit of linear velocity, equal to the volumetric flow in ml/hr divided by the cross-sectional area of the column in cm^2 .

Linear velocity is useful when considering scale-up. If column length is held constant, then pressure drop, run time, and column performance are usually constant with linear velocity, and independent of column diameter. Cm/hr is available only when you enter column dimensions in the Configuration dialog box and a column is inline.

- **CV/min** (column volumes)—Equal to the volumetric flow in ml/min divided by the total column bed volume in ml.

CV/min is useful for gauging the rate of turnover in the column. CV/min is available only when you enter column dimensions in the Configuration dialog box and a column is inline.

NOTE: *If you use a multi-column plumbing configuration, CV (column volume) calculations include the combined volumes of all inline columns, including columns installed on the SCOUT device.*

5.2.4 Purging the System

When to purge Purge the system before making an injection to flush and equilibrate the system with your initial buffer/solvent blend.

The Purge feature:

- Takes the columns out of line
- Changes the buffer/solvent blend to the set blend
- Increases the flow rate to the set rate
- Runs for a set volume duration
- Resets to the original flow rate
- Resets the columns to the specified position

Purging using Purge command

To purge the system:

1. Select **Purge System** from the Blend menu in the Control Panel.

NOTE: The Purge System command is not available if you configure Low Dispersion, Three Columns, or Direct Control column plumbing configurations. In these configurations, the column cannot be bypassed. See “Purging from Control Panel” on page 5-17, to purge with these column plumbing configurations.

The Purge Segment Setup dialog box is displayed (Figure 5-11).

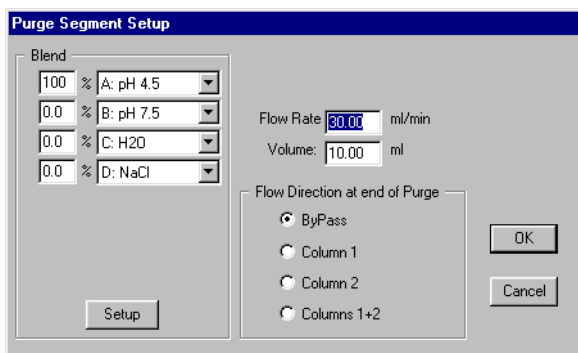


Figure 5-11 Purge Segment Setup Dialog Box

2. Select a **buffer/solvent blend** for purging the system. To change the blend mode (Percent, Eluent, or pH) click **Setup**.

For more information on entering blends in different modes, see Section 5.2.2, Controlling Buffers/Solvents and Blending.

3. Type a **flow rate** for purging. The default is 30 ml/min.

CAUTION

If you are using an analytical flow cell and 0.010-inch ID blue tubing, reduce the purge flow rate to 20 ml/min to avoid exceeding the system pressure limit.

4. Type a **purge volume** sufficient to purge the system. The default is 10 ml.
5. Select the **Flow Direction at End of Purge**. The selections depend on the current plumbing configuration.

NOTE: *If you do not set flow direction, the column is not placed back inline after the Purge segment.*

6. Click **OK** to begin the system purge.

Hint: *To include the sample loop in the purge, click the **Inject** option button in the Sample section of the Control Panel. To bypass the sample loop during the purge, click the **Load** option button in the Sample section of the Control Panel.*

The columns are taken out of line, and the selected buffer/solvent blend is pumped through the system at the specified flow rate for the specified volume.

At the end of the purge cycle, the flow rate is restored to the original rate and the columns are switched to the specified position.

Purging from Control Panel

You can also purge the system directly from the Control Panel:

1. Select a **buffer/solvent blend** for purging the system in the Blend section of the Control Panel.
2. Select a **flow rate** for purging in the Pump section of the Control Panel.
3. Click the **Bypass** option button in the Column section of the Control Panel to take the column out of line.

NOTE: *If you configure Low Dispersion, Three Columns, or Direct Control column plumbing configurations, the column cannot be bypassed. Physically remove the column and replace with a union before purging.*

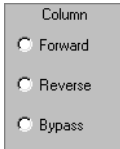
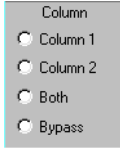
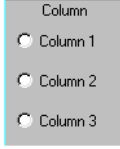
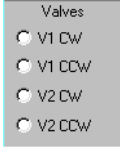
4. Click the **Start** button to start the pump. Allow the pump to run for a time sufficient to purge the system.
5. Click the **Stop** button to stop the pump.

5.2.5 Controlling Columns

Flow path options

You can control the flow path by using the option buttons in the Column section of the Control Panel. The option buttons available in the Column section depend on the column option you select in the Configuration dialog box. To change the column plumbing configuration, see Section 4.2.4, Configuring Columns and Plumbing.

The options for each column plumbing configuration are:

Column Plumbing Configuration	Flow Path Options
Single Column	
Tandem Columns	
Three Columns	
Direct Control	
Low Dispersion	None

Single column Single-column configuration allows you to:

- Operate Column 1 alone
- Bypass Column 1 directly to the detectors
- Backflush Column 1 for washing, regeneration, or reversed elution

Tandem column Tandem-column configuration allows you to:

- Operate Column 1 alone
- Operate Column 2 alone
- Operate Column 1 and Column 2 in series
- Bypass both columns directly to the detectors

Three column Three-column configuration allows you to operate any one of the three columns individually. You cannot operate more than one column at a time. You cannot bypass all of the columns. One column is inline at all times.

Direct control Direct control configuration allows you to customize plumbing in configurations other than those directly supported by the software. You plumb columns and column switching valves any way you desire. You must specify flow rate in ml/min.

You control flow direction by specifying column switching valve positions (clockwise or counterclockwise) in the Valves section of the Control Panel:

- **V1 CW**—Column Switching Valve 1 clockwise
- **V1 CCW**—Column Switching Valve 1 counterclockwise
- **V2 CW**—Column Switching Valve 2 clockwise
- **V2 CCW**—Column Switching Valve 2 counterclockwise

For a diagram of the ports connected for each valve position (clockwise or counterclockwise), see Figure 3-10 on page 3-12.

Low dispersion Low-dispersion configuration is a single-column configuration. It shortens the flow path and minimizes delay volume by bypassing the two column switching valves. However, only forward flow is available, and the column is inline at all times.

Displaying plumbing diagrams To display plumbing diagrams to assist you when you change column plumbing, select **Show Column Plumbing** or **Show System Plumbing** from the Edit menu, or refer to Figure 3-6 through Figure 3-10 on page 3-10 through page 3-12.

5.2.6 Controlling Detectors

You can use the Control Panel to:

- Enable and select UV/Vis detector mode and wavelengths
- Enable and select fluorescence detector wavelengths
- Zero the detector signal
- Add a chart mark to the Strip Chart Recorder
- Bypass the pH and Conductivity monitors

NOTE: You can use both the internal UV/Vis detector and an external fluorescence detector. However, you enable and select the wavelengths for each detector separately. For more information, see the following sections.

UV/Vis wavelengths

To enable and select the UV/Vis detector wavelength:

1. Select **UV/VIS Single Wavelength** or **UV/VIS Dual Wavelength** from the Detector menu in the Control Panel.

NOTE: If you enable an external fluorescence detector in hardware setup, only single wavelength mode is available for the UV/Vis detector. If you enable an external UV/Vis detector in hardware setup, only dual wavelength mode is available for the UV/Vis detector.

2. If you enable single wavelength, type a **wavelength** in the top text box in the Detector section of the Control Panel (Figure 5-12).

Valid range is 190 to 800 nm. Operating wavelengths for the lamps are:

- **Deuterium (UV)**—190 to 365 nm
- **Tungsten (Vis)**—366 to 800 nm

3. Click elsewhere on the Control Panel to enable the wavelength setting.

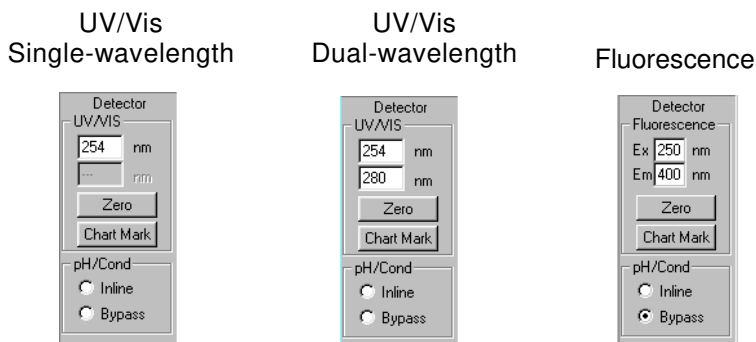


Figure 5-12 Detector Section of Control Panel

4. If you enable dual-wavelength, type **wavelengths** in both text boxes in the Detector section of the Control Panel (Figure 5-12).

If you are using the *internal* detector in dual wavelength mode, valid ranges are 190 to 450 nm **OR** 366 to 700 nm, because the detector cannot switch between the two lamps. Operating wavelengths for each lamp are:

- **Deuterium (UV)**—190 to 450 nm
- **Tungsten (Vis)**—366 to 700 nm

If you are using an *external* detector for dual wavelength mode, you can type any wavelength combination.

5. Click elsewhere on the Control Panel to enable the wavelength settings.

NOTE: When you run a method with dual wavelength enabled, the setting changes to single wavelength at the end of a method to reduce wear of the detector grating.

Fluorescence wavelengths

To enable and select the fluorescence detector wavelengths:

1. Select **Fluorescence Control** from the Detector menu in the Control Panel.

NOTE: *The Fluorescence Control command is available only if you enable an external fluorescence detector in hardware setup. For more information, see Section 2.5.2, Updating BioCAD Hardware Setup Options.*

2. Type **Excitation** and **Emission wavelengths** in the Detector section of the Control Panel (see Figure 5-12).

Valid entries ranges are (even values only):

- **Excitation**—200 to 650 nm
- **Emission**—200 to 800 nm

3. Click elsewhere on the Control Panel to enable the wavelength settings.

NOTE: *To enhance sensitivity when using emission wavelengths above 650 nm, install a PMT that is sensitive to longer wavelengths. For more information, see the Applied Biosystems Model 304/305 Fluorescence Detectors Operating Manual.*

Zeroing detector

Click the **Zero** button in the Detector section of the Control Panel to zero the detector signal (see Figure 5-12).

Adding chart mark

Click the **Chart Mark** button in the Detector section of the Control Panel to add a mark to the Strip Chart Recorder (see Figure 5-12). This action also adds a Chart Mark event to a running method.

Bypassing monitors

Click the **Bypass** option button in the Detector section of the Control Panel to take the pH and conductivity monitors out of line. Click the **Inline** option button to place the pH and conductivity monitors inline (see Figure 5-12).

NOTE: *You can bypass only the pH and conductivity monitors. UV/Vis detectors are always inline.*

5.2.7 Controlling the Fraction/Waste Valve

Collecting single fractions

You can control the fraction/waste valve on the BioCAD 700E Workstation to collect single fractions without using a fraction collector. For information on using a fraction collector to collect fractions, see Section 5.4.1, Controlling the Fraction Collector.

To collect single fractions:

1. Place the fraction (top) line in a container to collect fractions.
2. Place the waste (bottom) line in a waste container.

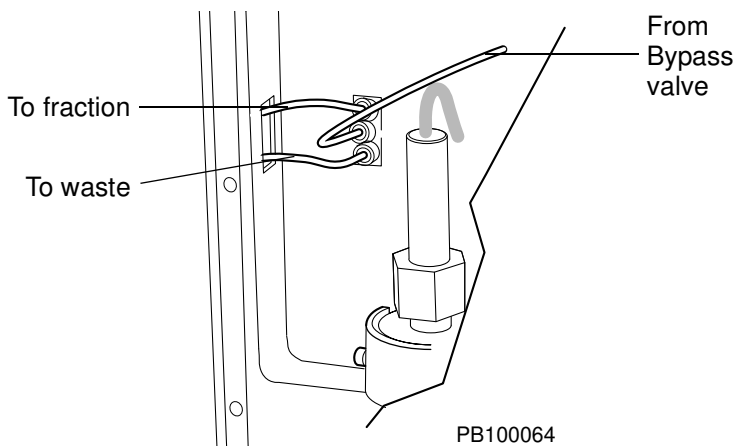


Figure 5-13 Fraction/Waste Valve

3. Divert flow to the fraction container or the waste container by clicking the valve option buttons in the Control Panel (Figure 5-14).

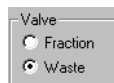


Figure 5-14 Valve Section of Control Panel

- **Fraction**—Diverts flow out the top port of the valve
- **Waste**—Divert flow out the bottom port of the valve

5.2.8 Running a Gradient

After you inject a sample from the Control Panel, you can run a gradient from the Control Panel to elute the sample.

To run a gradient manually from the Control Panel:

1. Select the **Blend** menu, then select the **blend mode** (Percentage, Eluent, or pH) for the gradient.

For more information on blend modes, see Section 5.2.2, Controlling Buffers/Solvents and Blending.

2. Type the **initial conditions** for the gradient in the Blend section of the Control Panel.

Select the buffers/solvents to use in the gradient. If the buffer/solvent you want is not shown, click the arrow to the right of one of the channels. A list of available channels appears. Select the channel you want by clicking it. Click **Set**.

Hint: If you double-click a channel in the drop-down list box, 100% is entered automatically in the % text box.

3. Type a **flow rate** in the Pump section of the Control Panel and click the **Start** button to start the pump.
4. When the column is equilibrated, select **Run Gradient** from the Blend menu.

The Gradient Edit dialog box appears (Figure 5-15). The initial conditions you entered on the Control Panel are listed.

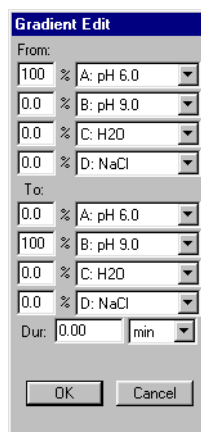


Figure 5-15 Gradient Edit Dialog Box

5. Type the **end conditions** for the gradient.
6. Type the **duration** for the gradient and specify **minutes**, **ml**, or **CV** (column volumes for current column).

NOTE: If CV units are not displayed in the drop-down list box, place the column inline by clicking the appropriate option button in the Column section of the Control Panel.

7. Click **OK** to begin the gradient.

5.2.9 Calibrating Custom pH Blend Buffers

Overview If you run in pH blend mode using your own custom pH blend buffers (instead of the pH blend buffers), you can calibrate your custom pH blend buffers.

For more information on custom and pH blend buffers, see Section 3.2.2, Preparing pH Blend Mode Buffers.

pH calibration curve When you calibrate custom pH blend buffers, the software generates a pH calibration curve. The pH calibration curve allows you to specify a pH value in the Control Panel, method segments, or the pH Map template. The software then calculates the percent of each buffer needed to achieve the desired pH.

NOTE: *If you do not calibrate custom buffers, you can still run in pH blend mode. However, you must specify a percent value (instead of a pH value) in the Control Panel, method segment, or the pH Map template.*

The pH calibration curve is permanently associated with the buffers you calibrate and is used any time you use the buffer pair. If you recalibrate the buffer pair, a new calibration curve is associated with the buffers.

CAUTION

If you recalibrate a buffer pair, the new calibration curve overwrites the original calibration curve. You cannot have more than one calibration curve for a buffer pair.

**Before
calibrating**

Before you calibrate custom pH blend buffers:

1. Prepare and connect the pair of pH buffers to the workstation.

See Section 3.2.1, Preparing Buffers/Solvents.

2. Prime the buffer/solvent lines.

See Section 3.4.2, Priming the Buffer/Solvent Lines.

3. Configure the buffers as type Buffer in the Configuration dialog box.

See Section 4.2.2, Configuring Buffers/Solvents, and Section 4.5.1, Customizing the Buffer/Solvent Library.

4. Configure and plumb columns. If you use Single or Tandem Column plumbing configuration, take the column out of line by clicking the Bypass option button in the Column section of the Control Panel. If you use Low Dispersion, Three Column, or Direct Control configuration, install a union in place of the column.

See Section 4.2.4, Configuring Columns and Plumbing.

5. Calibrate the pH probe.

See Section 9.5.2, Calibrating the pH Probe.

Calibrating To calibrate pair of custom pH blend buffers:

1. Select **Calibrate pH Mode Buffers** from the Blend menu in the Control Panel.

The Calibrate pH Mode Buffers dialog box is displayed (Figure 5-16).

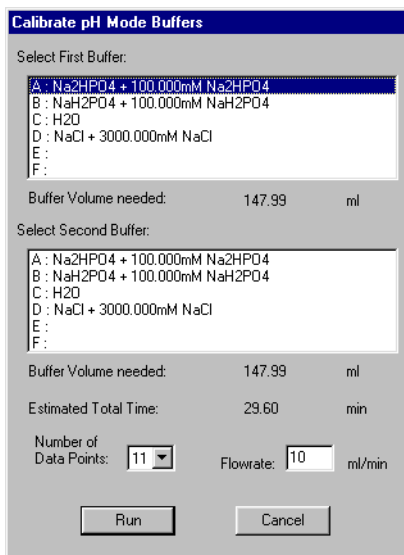


Figure 5-16 Calibrate pH Mode Buffers

2. Select a **First** and **Second Buffer** by clicking on the lists of configured buffers. Selected buffers are highlighted.
3. Select the **Number of Data Points** for the calibration curve. The recommended number is 11.
4. Type a **flow rate** that is comparable to the rate you will use when running in pH blend mode. The default flow rate is the optimum flow rate calculated by the software, based on the column configuration.
5. Note the **Buffer Volume Needed** and **Estimated Total Time** displayed for each buffer. Make sure you have enough buffer before starting the calibration.
6. Click **Run** to start the calibration.

During the calibration

A calibration method runs. The method contains a Step segment for each data point you specified in the Calibrate pH Mode Buffer dialog box (see Figure 5-16). The buffer blend changes with each Step segment, starting with 100 percent of the first buffer, and ending with 100 percent of the second buffer.

Each Step segment:

- Samples the buffer blend
- Measures the pH
- Records the pH

Calibration complete

When the calibration is complete, the software creates a file called PHCALIB.B##, and stores the file in the C:\BIOCAD\DATA directory.

NOTE: The PHCALIB.B## file contains the data and method associated with the calibration. It does not contain the calibration curve. Calibration curve information is stored internally with the calibrated buffers. You can delete the PHCALIB.B## file without losing the calibration curve.

When the calibration is complete, the pH Buffer Calibration Curve dialog box is displayed (Figure 5-17).

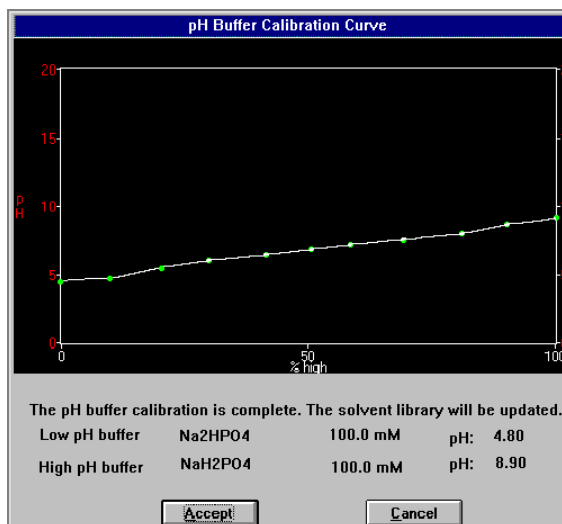


Figure 5-17 pH Buffer Calibration Curve

The pH Buffer Calibration Curve dialog box displays:

- The curve for the calibration (quadratic fit), with the x-axis scaled in percent of the higher pH buffer
- The final pH for each calibrated buffer

Calibration acceptable

If the calibration curve is acceptable, click **Accept** to store the calibration with the buffers in the Buffer/Solvent Library for future use. Calibrated buffers are labeled with the solution used for calibration in the Specify Channel dialog box in the Buffer/Solvent Library. For more information, see Section 4.5.1, Customizing the Buffer/Solvent Library.

Calibration not acceptable

If the calibration curve is not acceptable, click **Cancel**. To determine the problem with the calibration, you can:

- Examine the data file created by the calibration (the latest PHCALIB.B## file) in Data Analysis
- Examine the method
- Recalibrate the pH probe

When you have corrected the problem, calibrate the buffers again.

Running a pH Map template

For information on running a pH Map template using calibrated custom pH blend buffers, see Section 7.1.1, pH Map Template.

5.2.10 Programming Event Outputs

If you connect an auxiliary device to the BioCAD 700E, you may need to program event outputs from the workstation to provide the necessary power control for the device.

To program event outputs:

1. Select **Auxiliary Output** from the Control Panel Detector menu.

The Auxiliary Out Event dialog box is displayed (Figure 5-18).

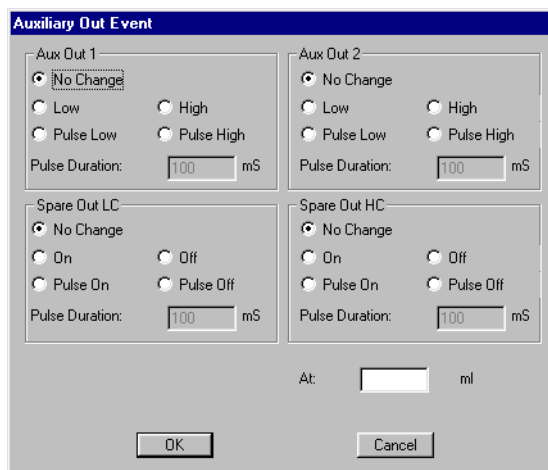


Figure 5-18 Auxiliary Out Event Dialog Box

2. Set the appropriate **pulse levels** and **pulse durations**. See the reference manual for the auxiliary device for its specific power control requirements.

You can use:

- Aux Out 1 and Aux Out 2 signals (TTL logic) to trigger another device
- Spare Out LC (low current 0.25 Amp) signal to power a 12 V device
- Spare Out HC (high current 1 Amp) signal to power a 24 V device

3. Make the following connections to the Auxiliary connector on the left side panel of the workstation. Connect only to the pins that transmit the signals you need.

NOTE: When connecting a device to the Auxiliary connector, use a shielded cable with a ferrite bead to maintain CE compliance. Recommended source and part number for the ferrite bead is:
 Fair-Rite Products Corporation, No. 2643164151.

Auxiliary Pin	Assignment	Signal
10	Output #1–TTL	Aux Out 1
11	Output #2–TTL	Aux Out 2
12	Logic output ground	TTL Ground
5	Output valve power #2 (+ 12 V, 0.25 Amp)	Spare Out LC (12 V)
6	Output valve switch #2	LC Return
3	Output valve power #1 (+ 24 V, 1.0 Amp)	Spare Out HC (24 V)
4	Output valve switch #1	HC Return

NOTE: Always use pins 3 and 4 together to supply power and ground for a 24 V device. Always use pins 5 and 6 together to supply power and ground for a 12 V device.

4. Click **OK** to send the signal.



5.2.11 Stopping and Resuming a Method

There are three ways to stop a method from the Control Panel:

- Hold
- Stop pump
- Abort

To stop a method ...	Description	To resume the method ...
Click the Hold button in the Method section of the Control Panel	Suspends the method and freezes the workstation in its present state, with the pump running. If a gradient is running, conditions do not change. You can perform manual actions from the Control Panel, such as replenishing buffers and priming lines.	Click the Resume button in the Method section of the Control Panel.
Stop the pump by clicking the Stop button in the Pump section of the Control Panel or by pressing the Pump button on the front panel of the workstation	Suspends the method and freezes the workstation in its present state, with the pump off.	Start the pump to resume the method. Click the Start button in the Pump section of the Control Panel or press the Pump button on the front panel of the workstation.
Click the Abort button in the Method section of the Control Panel or click the Abort button in the Method Queue	Clears the method and shuts down the pump. All methods are cleared from the Method Queue. Data files contain data acquired up to the time you click Abort. NOTE: To delete unwanted data files after clicking Abort, use Windows Explorer. For more information, refer to the Microsoft Windows User's Guide.	You cannot resume a method after aborting it. You must run the method again.

5.3 Making Injections From the Control Panel

You can make injections from the Control Panel without creating a method. This section describes how to make the following types of injections from the Control Panel:

- Autoloader injections
- Direct autoloader injections
- Manual injections
- Pump injections

For information on making the following types of injections from the Control Panel, see the appropriate section:

- Integrated Autosampler Injections—Section 5.4.2, Controlling an Integrated Autosampler
- Direct Wire Autosampler Injections—Section 5.4.3, Controlling a Direct Wire Autosampler

NOTE: To determine which injection mode to use, see “Selecting an injection mode” on page 1-12.

Injection volume considerations

Injection volume determines the syringe and sample loop size. To achieve maximum injection volume accuracy and precision, follow these guidelines when specifying injection volumes and selecting sample loop sizes:

- For injection volumes less than 50 percent of the sample loop volume, enter the injection volume in the Control Panel or method.
- For injection volumes equal to the sample loop volume, enter a volume of three times the sample loop volume in the Control Panel or method. For example, you can accurately inject a 100 μ l sample using the 100 μ l sample loop if you use an injection volume of 300 μ l.
- Do not inject volumes between 50 percent and 100 percent of the sample loop volume.

NOTE: The actual volume injected onto the column never exceeds the sample loop size.

**Before
you begin**

Before you make an injection from the Control Panel:

1. Prepare the sample as described in Section 3.3, Preparing Samples.
2. Prime or flush the sample lines as described in Section 3.4.3, Priming and Flushing the Autoloader Sample Lines, or Section 3.4.4, Priming Buffer/Solvent Sample Lines for a Pump Injection.
3. Configure the workstation as described in Chapter 4, Configuring and Preparing the BioCAD Software.
4. Purge the system as described in Section 5.2.4, Purging the System.
5. Set the buffer/solvent blends for equilibrating as described in Section 5.2.2, Controlling Buffers/Solvents and Blending.
6. Set the appropriate flow rate and start the pump as described in Section 5.2.3, Controlling the Pump and Flow Rate.
7. Click the **Inject** button in the Sample section of the Control Panel to place the Injection valve in the Inject position and fill the sample loop with buffer.
8. Place the column inline to equilibrate as described in Section 5.2.5, Controlling Columns.
9. Set the wavelength and zero the detector as described in Section 5.2.6, Controlling Detectors.
10. Allow the pressure to stabilize by checking the pressure display in the Status window.

Hint: When you make injections from the Control Panel, press the Chart Mark button at the time of injection to mark the Strip Chart Recorder with an event mark.

5.3.1 Making Autoloader Injections

Flow path diagrams

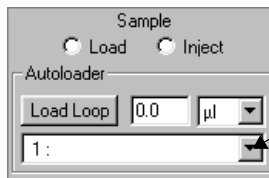
Appendix H, Autoloader Flow Path, includes diagrams that illustrate the flow path of the autoloader during different operations.

Making injections

To make an autoloader injection from the Control Panel:

1. Complete the tasks listed in “Before you begin” on page 5-35.
2. Select a **sample channel** to inject from by clicking the down arrow to the right of the list box at the bottom of the Sample section (Figure 5-19).

If you entered sample names when you configured the Autoloader, the sample names appear next to the number of the sample channel.



Click here to select a sample channel

Figure 5-19 Sample Section of Control Panel

3. Type an **injection volume** in the text box next to the Load Loop button.

For accurate injection volume delivery, follow the guidelines in “Injection volume considerations” on page 5-34.

4. Click the **Load Loop** button.

The workstation automatically places the injection valve in the load position and loads the loop with sample from the selected channel.

NOTE: Clicking the **Load** option button instead of the **Load Loop** command button places the sample loop in the load position, but does not load the loop.

5. After the syringe withdraws sample and returns to the home position, click the **Inject** option button.

The injection valve switches to the inject position and injects the sample onto the column.

Hint: After the injection is complete and before running a gradient, click the **Load** option button to place the injection valve in the load position. This step reduces delay time and volume when you run a gradient.

6. Run a gradient or change the buffer/solvent blend to elute the sample. For more information, see Section 5.2.8, Running a Gradient.
7. When the run is complete, stop the pump by clicking the **Stop** button in the pump section of the Control Panel.
8. Save the data from the run by selecting **Save Data As** from the File menu in the Strip Chart Recorder window. Type a **file name** and select a **directory** in the Save File dialog box. Click **OK**.

The data is saved to a data file. Method information is not saved in the data file.

5.3.2 Making Direct Autoloader Injections

Flow path diagrams

Appendix H, Autoloader Flow Path, includes additional diagrams that illustrate the flow path of the autoloader during different operations.

Making injections

To make a direct autoloader injection from the Control Panel:

1. Complete the tasks listed in “Before you begin” on page 5-35.
2. Select **Direct** from the drop-down list box at the bottom of the Sample section (Figure 5-20).

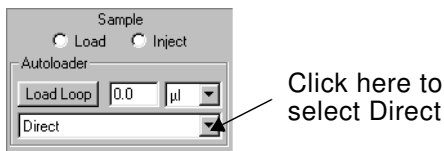


Figure 5-20 Sample Section of Control Panel

The Enter Sample Information dialog box is displayed (Figure 5-21).

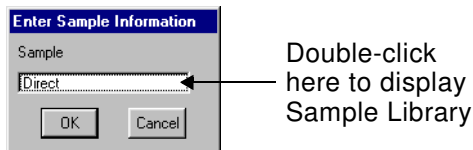


Figure 5-21 Enter Sample Information Dialog Box

3. Double-click on **Direct** to display the Sample Library. Select or add a sample. For information on adding sample names to the Sample Library, see Section 4.5.2, Customizing the Sample Library.
4. Click **OK** to close both dialog boxes.

5. Type an **injection volume** in the text box next to the Load Loop button.

For accurate injection volume delivery, follow the guidelines in “Injection volume considerations” on page 5-34.

6. Click the **Load Loop** button.

The workstation automatically places the injection valve in the load position and loads the loop with sample from the Direct mode channel.

NOTE: Clicking the **Load** option button instead of the **Load Loop** command button places the sample loop in the load position, but does not load the loop.

7. After the syringe withdraws sample and returns to the home position, click the **Inject** option button.

The injection valve switches to the inject position and injects the sample onto the column.

Hint: After the injection is complete and before running a gradient, click the **Load** option button to place the injection valve in the load position. This step reduces delay time and volume when you run a gradient.

8. Run a gradient or change the buffer/solvent blend to elute the sample. For more information, see Section 5.2.8, Running a Gradient.
9. When the run is complete, stop the pump by clicking the **Stop** button in the pump section of the Control Panel.
10. Save the data from the run by selecting **Save Data As** from the File menu in the Strip Chart Recorder window. Type a **file name** and select a **directory** in the Save File dialog box. Click **OK**.

The data is saved to a data file. Method information is not saved in the data file.

5.3.3 Making Manual Injections

To make a manual injection from the Control Panel:

1. Complete the tasks listed in “Before you begin” on page 5-35.

NOTE: *When making a manual injection, the Sample Loader configuration does not matter and there are no sample lines to prime.*

2. Click the **Load** option button in the Sample section of the Control Panel.

The workstation places the injection valve in the load position.

3. Insert the needle port adaptor in port 6 of the injection valve.
4. Load a syringe with your sample.

For accurate injection volume delivery, follow the guidelines in “Injection volume considerations” on page 5-34.

5. Insert the syringe in the needle port adaptor and inject the sample (Figure 5-22).

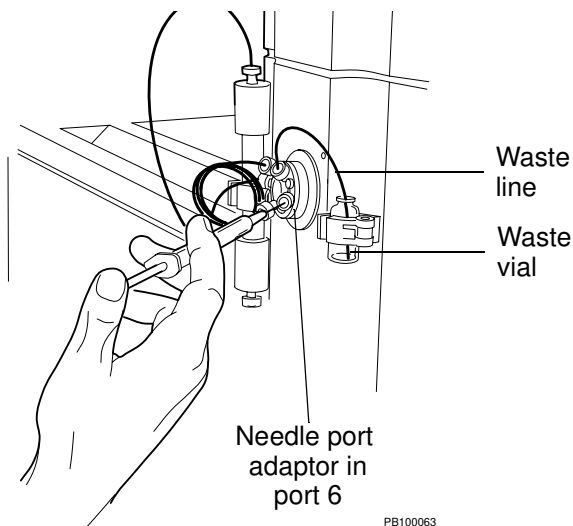


Figure 5-22 Making a Manual Injection

6. Click the **Inject** option button.

The injection valve switches to the inject position and injects the sample onto the column.

Hint: After the injection is complete and before running a gradient, click the **Load** option button to place the injection valve in the load position. This step reduces delay time and volume when you run a gradient.

7. Run a gradient or change the buffer/solvent blend to elute the sample. For more information, see Section 5.2.8, Running a Gradient.
8. When the run is complete, stop the pump by clicking the **Stop** button in the Pump section of the Control Panel.
9. Save the data from the run by selecting **Save Data As** from the File menu in the Strip Chart Recorder window. Type a **file name** and select a **directory** in the Save File dialog box. Click **OK**.

The data is saved to a data file. Method information is not saved in the data file.

5.3.4 Making Pump Injections

To make a pump injection from the Control Panel:

1. Complete the tasks listed in “Before you begin” on page 5-35.

NOTE: When making a pump injection, the Sample Loader configuration does not matter.

NOTE: When making a pump injection, create a buffer/solvent channel of type Sample. For more information, see Section 4.5.1, Customizing the Buffer/Solvent Library.

2. Click the **Stop** button in the Pump section of the Control Panel to stop the pump.
3. Type a **flow rate** in the Pump section of the Control Panel.
4. Select the **buffer/solvent channel** that you are using for your sample in the Blend section of the Control Panel. Set to **100%**. Click the **Set** button.
5. Click the **Start** button to start pumping your sample.
6. Allow the pump to run for a time sufficient to introduce the desired volume of sample onto the column.
7. Run a gradient or change the buffer/solvent blend to elute the sample. For more information, see Section 5.2.8, Running a Gradient.
8. When the run is complete, stop the pump by clicking the **Stop** button in the pump section of the Control Panel.
9. Save the data from the run by selecting **Save Data As** from the File menu in the Strip Chart Recorder window.

The data is saved to a data file. Method information is not saved in the data file.

5.4 Controlling External Devices

This section describes how to control the following external devices from the Control Panel:

- Fraction collector
- Integrated autosampler
- Direct wire autosampler

5.4.1 Controlling the Fraction Collector

Controlling a fraction collector

If you configure a fraction collector, you can control it from the Collector section of the Control Panel:

1. Select **Fraction Collector Setup** from the Config menu in the Control Panel.

NOTE: If *Fraction Collector Setup* is not displayed, configure a fraction collector. For more information, see Section 4.6, Configuring and Preparing the BioCAD Software for a Fraction Collector.

The Fraction Collector Setup dialog box is displayed (Figure 5-23). The tube size you selected in the Configuration dialog box is also displayed.

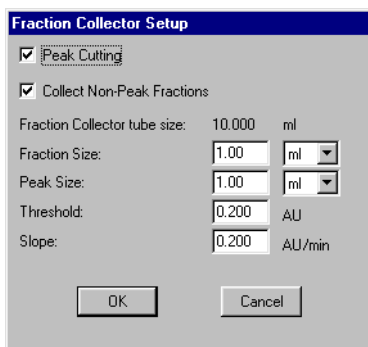


Figure 5-23 Fraction Collector Setup Dialog Box

2. Select and type fraction collector setup **parameters** in the dialog box. For more information, see “Setting fraction collection parameters” on page 6-47. Click **OK** to accept the settings and return to the Control Panel.
3. If you change the length of the tubing between the BioCAD 700E Workstation and the fraction collector, change the **delay volume** by clicking the Delay Volumes button in the Configuration dialog box. Type the correct delay volume in the Fraction Valve to Fraction Collector text box. For more information, see “Setting delay volumes” on page 4-13.

NOTE: *The fraction collector response is not immediate. The delay volume set in the configuration must elapse before the commands you select execute.*

4. Click one of the following option buttons to divert the flow:
 - **Fraction**—Diverts flow to fraction collection tubes
 - **Waste**—Diverts flow to waste



Figure 5-24 Collector Section of Control Panel

5. Click the following buttons to control fraction collection:
 - **Start/Stop**—Starts fraction collection according to the parameters you set in step 2. Stops fraction collection and diverts flow to waste if the fraction collector is collecting fractions.
 - **Advance**—Advances the diverter valve to the next tube in the rack sequence and collects flow. Use this button to control fraction collection if you do not set parameters in step 2.
 - **Reset**—Moves the fraction collector diverter valve to the home position (tube 1) in the rack and diverts flow to waste. Use when pooling fractions from multiple runs.

5.4.2 Controlling an Integrated Autosampler

If you configure an integrated autosampler, you can control the autosampler from the Sample Table. You can also change the sample loop size and control heating and cooling from the Sample Table.

Before you begin

Before you make an integrated autosampler injection from the Control Panel:

1. Connect an integrated autosampler as described in Section 2.4.4, Connecting the Autosampler.
2. Prepare the integrated autosampler and samples as described in Section 3.6, Preparing an Autosampler.
3. Configure and Prepare the BioCAD software for an integrated autosampler as described in Section 4.7, Configuring and Preparing the BioCAD Software for an Integrated Autosampler.
4. Purge the system as described in Section 5.2.4, Purging the System.
5. Set the buffer/solvent blends for equilibrating as described in Section 5.2.2, Controlling Buffers/Solvents and Blending.
6. Set the appropriate flow rate and start the pump as described in Section 5.2.3, Controlling the Pump and Flow Rate.
7. Place the column inline to equilibrate as described in Section 5.2.5, Controlling Columns.
8. Set the wavelength and zero the detector as described in Section 5.2.6, Controlling Detectors.
9. Allow the pressure to stabilize by checking the pressure display in the Status window.

Making an injection

To make an injection from the Sample Table:

1. Complete the tasks listed in “Before you begin” on page 5-46.
2. Select **Sample Table** from the Window menu in the Control Panel.

The Sample Table window is displayed (Figure 5-25).

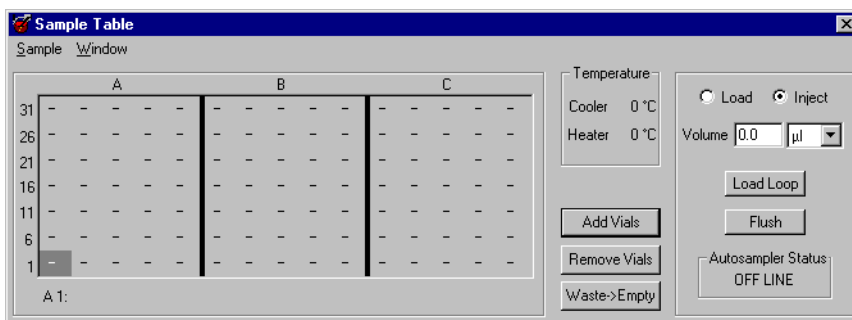


Figure 5-25 Sample Table Window

NOTE: If Sample Table is not displayed in the Window menu, select an AS 3500 autosampler in hardware setup. See Section 2.5.2, Updating BioCAD Hardware Setup Options.

3. Select a **sample vial** from the Sample Table by clicking it.
4. Type the **volume** to inject in the Volume text box.
5. If desired, flush the autosampler needle:
 - Click the **Flush** button to display the Set Flush Amount dialog box.
 - Change the flush volume, if desired (the default volume is the volume you set in Configuration).
 - Click **OK** to flush the autosampler needle with the flush solvent set in Configuration.

6. Click the **Load Loop** button.

The autosampler injector valve switches to the load position, and the autosampler picks up the selected vial, withdraws the specified volume, and loads the sample in the autosampler loop.

7. Click the **Inject** option button.

The autosampler injector valve switches to the inject position and injects the sample onto the column.

NOTE: *The autosampler injects from the vial you select in the Sample Table. If the sample volume in the vial is insufficient to make the injection, a warning is displayed. Click **OK** to reduce the injection volume or select a different vial. Click **Cancel** to cancel the injection.*

8. Run a gradient or change the buffer/solvent blend to elute the sample. For more information, see Section 5.2.8, Running a Gradient.
9. When the run is complete, stop the pump by clicking the **Stop** button in the pump section of the Control Panel.
10. Save the data from the run by selecting **Save Data As** from the File menu in the Strip Chart Recorder window. Type a **file name** and select a **directory** in the Save File dialog box. Click **OK**.

The data is saved to a data file. Method information is not saved in the data file.

Changing sample loop size

You can change sample loop size from the Sample Table without changing Configuration:

1. Select **Change Loop Size** from the Sample menu in the Sample Table.

The Set Sample Loop Size dialog box is displayed (Figure 5-26).

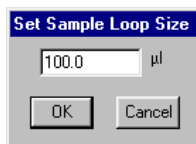


Figure 5-26 Set Sample Loop Size Dialog Box

2. Type a new sample loop size and click **OK**.
3. Change the sample loop installed on the autosampler. For more information, see the *Autosampler Reference Manual*.

Controlling sample heating and cooling

The integrated autosampler that you use with the BioCAD 700E Workstation may support the sample preparation (heat chamber) and sample cooling (tray area) options. If so, you can control sample heating and cooling from the Sample Table without changing Configuration.

1. To turn on the integrated autosampler heat chamber, select **Sample Heater** from the Sample menu in the Sample Table window. Click the **On** button and type a setpoint temperature. Valid range is from ambient temperature to 100°C (212°F) in 1°C increments.

The autosampler *heat chamber* is set to this temperature.

NOTE: You can set the temperature as low as 0°C, but the actual setpoint does not go below ambient temperature.

NOTE: To heat a sample vial, you place the sample vial in the heat chamber by adding a Heat/Mix segment to a method. See “Heat/Mix Segments” on page 6-64.

2. To turn on the autosampler cooler, select **Sample Cooler** from the Sample menu in the Sample Table window. Click the **On** button and type a setpoint temperature. Cooling range is from 0 to 60°C (32 to 140°F) in 1°C increments.

The autosampler *tray area* is set to this temperature.

Heater and cooler temperatures are displayed in the Sample Table window.

NOTE: Allow 30 minutes for the heat chamber or tray area to reach temperature.

NOTE: If your autosampler supports sample preparation, you can also control autosampler heating (heat chamber) and cooling (tray area) from the Configuration dialog box. See “Configuring sample prep” on page 4-42. The settings you make last are active.

5.4.3 Controlling a Direct Wire Autosampler

Before you begin

Before you make a direct wire autosampler injection:

1. Connect a direct wire autosampler as described in Section 2.4.4, Connecting the Autosampler.
2. Prepare the direct wire autosampler, including the samples and autosampler method, as described in Section 3.6, Preparing an Autosampler.
3. Configure and prepare the BioCAD software for a direct wire autosampler as described in Section 4.8, Configuring and Preparing the BioCAD Software for a Direct Wire Autosampler.
4. Purge the system as described in Section 5.2.4, Purging the System.
5. Set the buffer/solvent blends for equilibrating as described in Section 5.2.2, Controlling Buffers/Solvents and Blending.
6. Set the appropriate flow rate and start the pump as described in Section 5.2.3, Controlling the Pump and Flow Rate.
7. Place the column inline to equilibrate as described in Section 5.2.5, Controlling Columns.
8. Set the wavelength and zero the detector as described in Section 5.2.6, Controlling Detectors.
9. Allow the pressure to stabilize by checking the pressure display in the Status window.

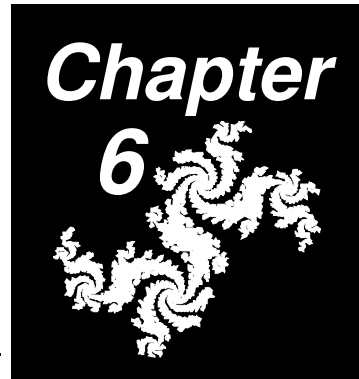
Making an injection

Use the autosampler controls to program an autosampler method with an injection sequence. For more information on programming the AS 3500 autosampler, see Section 3.6.8, Programming the AS 3500 Autosampler Method and Sample Set, or the *Autosampler Reference Manual*.

To make the first injection, click the **Inject** option button in the Sample section of the Control Panel. The workstation signals the autosampler with a contact closure. The autosampler makes the injection and signals the workstation with a contact closure.

After the injection is complete, run a gradient or change the buffer/solvent blend to elute the sample. For more information, see Section 5.2.8, Running a Gradient.

6 Creating and Running Methods



This chapter includes the following sections:

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6.1 Using the Method Editor

This section describes:

- Method structure
- Method views
- Method units

Overview Use the Method Editor to create, edit, and run separation methods. The Method Editor is designed to make method programming easy to learn and implement.

To display the Method Editor window (Figure 6-1), select **Method Editor** from the Window menu in any window.

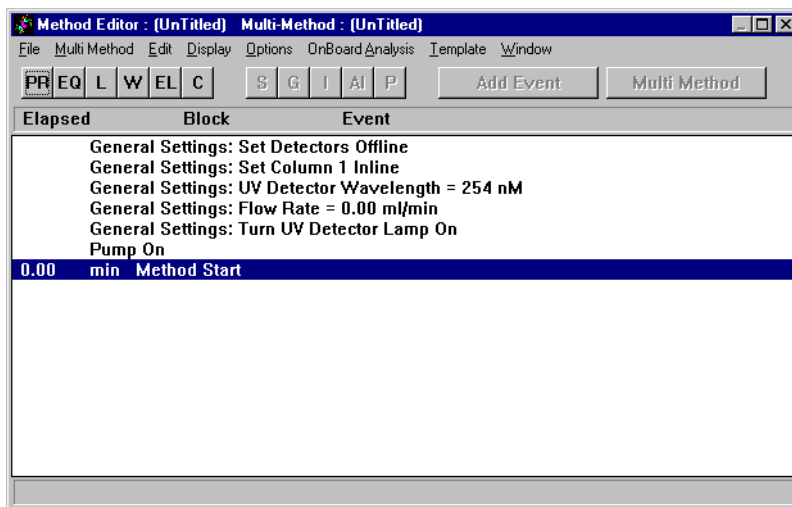


Figure 6-1 Method Editor

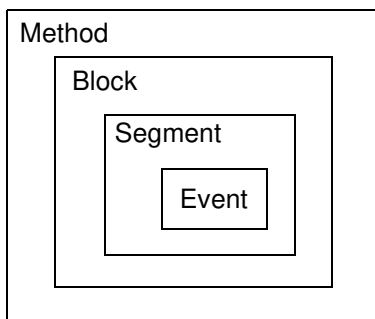
6.1.1 Method Structure

The BioCAD method structure makes method programming easy and intuitive.

Blocks, segments, and events

A BioCAD method is made up of blocks, segments, and events.

- **Blocks**—Basic chromatographic steps, for example, Equilibrate or Elute
- **Segments**—Flow operations within a block, for example, Inject or Purge
- **Events**—Individual operations within a segment, for example, Set Flow Rate



6.1.1.1 Blocks

Blocks are the building blocks you use to create a method. Blocks are basic chromatographic steps.

Blocks fall into two categories:

- **Separation**—Perform basic chromatographic steps. Equil, Load, Wash, Elute, and Clean are separation blocks.
- **Preparative**—Prepare sample using the autosampler (available only if your autosampler supports the sample preparation option).

You can include six types (one preparative, five separation) of blocks in a method. You can use one type of block more than once in a method. You do not need to use all six types of blocks in a method.

You typically add blocks to a method in the following order:

- **Equil**—Equilibrates the column and system for a separation
- **Prep**—Prepares samples (available only if you configure an integrated autosampler that supports sample prep)
- **Load**—Loads the sample onto the column
- **Wash**—Washes non-retained or weakly-retained contaminants off the column
- **Elute**—Removes retained molecules from the column as purified components
- **Clean**—Regenerates the column and system so that another separation can be performed

6.1.1.2 Segments

Segments are flow operations within a block.

Separation block segments

You can include five types of segments in separation blocks (Equil, Load, Wash, Elute, Clean):

- **Step**—Specifies a constant buffer/solvent blend to pass over the column for a specified duration or until thresholds are reached, for example, to wash or equilibrate the column
- **Gradient**—Defines a buffer/solvent blend that changes over time or volume, for example, a linear elution gradient
- **Inject**—Injects a sample onto the column using the autoloader injection valve and sample loop
- **Autosampler Inject**—Injects a sample onto the column using an optional integrated autosampler
- **Purge**—Flushes the system with a buffer/solvent blend while the column is bypassed

Prep block segments

You can include three types of segments in Prep blocks:

- **Dilute**—Adds a diluent and sample in a specified ratio to an empty vial when using an integrated autosampler
- **Combine**—Add multiple samples and reagents to an empty vial when using an integrated autosampler
- **Heat/Mix**—Heats or mixes a sample vial when using an integrated autosampler

**Types of
segments in
blocks**

A block can contain only segments that are appropriate for that block. For example, a Load block cannot contain a Gradient segment. The segments you can use with each block are:

Block	Allowable Segments
Prep	Dilute, Combine, Heat/Mix
Equilibrate	Step, Gradient, Purge
Load	Step, Inject, Autosampler Inject, Purge
Wash	Step, Gradient, Purge
Elute	Step, Gradient, Purge
Clean	Step, Gradient, Inject, Autosampler Inject, Purge

6.1.1.3 Events

Events are individual operations within a segment, such as:

- Set Flow Rate = 5.00 ml/min
- Turn Pump On

You can add events to separation segments (Step, Gradient, Inject, Autosampler Inject, and Purge). You cannot add events to preparative segments (Dilute, Combine, and Heat/Mix).

There are four types of events in a method:

- **Automatic Events**—Predefined events that are part of a segment. They are automatically added to a method when you add a segment to a method.
- **Added Events**—Miscellaneous events you can add to a method segment individually, to meet your application needs. Added events are flagged with an asterisk (*).
- **Control Panel Added Events**—Events added to a method as a result of an action you make from the Control Panel, such as stopping the pump. These added events are flagged with an asterisk (*) and a plus sign (+) and appear only in methods contained in data files.

If you use Save As to save the method contained in the data file to a method file, these added events remain in the method, marked with *, but the + is removed.

- **Documentation Added Events**—Events added to a method during the method run. These events document the result of other actions and are not executable. Examples include Method Canceled, Peak End, and Threshold Expired. These added events are flagged with an asterisk (*) and a minus sign (-) and appear only in methods contained in data files.

If you use Save As to save the method contained in the data file to a method file, these added events remain in the method, marked with * and -. When you run a method, events flagged with - are not executed.

6.1.2 Method Views

Scrolling If a method occupies more than one screen, use the scroll bars on the right side of the window to view the entire method.

Views The Method Editor lets you look at the method in two views. To change views, select **Block View** or **Sequence View** from the Display menu:

Block view Block view (Figure 6-2) is a summary view that includes:

- Each block, listed numerically (01, 02,...)
- Each segment, listed alphabetically (A, B,...)
- Type of each segment (S, G, I, AI, or P)
- Duration of each segment in minutes, ml, and CV
- Total duration of the method in minutes, ml, and CV

		Type	min	ml	CV
General Settings: Wavelength = 280 nM, FlowRate = 5.00 ml/min, Set Column 1 Inline, Se					
[01]	Equil Block				
	[A] Purge at pH 5	P	0.58	15.42	0.00
	[B] Equilibrate Column	S	3.32	16.60	10.00
[02]	Load Block				
	[A] Inject standards	I	0.33	1.67	1.00
[03]	Elute Block				
	[A] Gradient	G	4.98	24.90	15.00
	[B] Hold end of gradient	S	2.00	10.00	6.02
Totals:			11.22	68.58	32.03

Figure 6-2 Block View of a Method

NOTE: If you use a multi-column plumbing configuration, CV (column volume) readings include the combined volumes of all inline columns.

Sequence view Sequence view (Figure 6-3) is a detailed view that includes:

- Block name and number
- Segments within each block
- Automatic events within each segment
- Added events, flagged with an asterisk (*)
- Control Panel added events, flagged with a plus sign (+). These added events appear only in methods contained in data files.
- Duration of each block, segment, and event in minutes, ml, or CV
- Total duration of the method in minutes, ml, or CV

Elapsed	Block	Event
	General Settings: Set Detectors Inline	
	General Settings: Set Column 1 Inline	
	General Settings: UV Detector Wavelength = 280 nM	
	General Settings: Flow Rate = 5.00 ml/min	
	Pump On	
0.00	min	Method Start
0.00	min	[01] Equil Block
0.00	min	(A) Purge at pH 5
0.00	min	0.00 ml Set Column Offline
0.00	min	0.00 ml Set Solvent Blend 50.000 mM A:pH 4.5 + B:pH 7.5 @ pH
0.00	min	0.00 ml Set Flow Rate = 30.00 ml/min
0.50	min	15.00 ml Set Flow Rate = 5.00 ml/min
0.58	min	15.42 ml Set Column 1 Inline
0.58	min	
0.58	min	(B) Equilibrate Column
0.58	min	0.00 CV Set Solvent Blend 50.000 mM A:pH 4.5 + B:pH 7.5 @ pH

Figure 6-3 Sequence View of a Method

NOTE: If you use a multi-column plumbing configuration, CV (column volume) readings include the combined volumes of all inline columns.

6.1.3 Method Units

Displaying units In Block view, the method displays segments in minutes, ml, and CV.

In Sequence view, you can display method duration in one of three different units by selecting one of the following from the Display menu:

- **Time Units**—Displays each event in minutes
- **Volume Units**—Displays each event in ml
- **CV Units**—Displays each event in column volumes

NOTE: If CV Units is dimmed, enter column dimensions in the Configuration dialog box. For more information, see Section 4.2.4, Configuring Columns and Plumbing.

Programming units When you create a method, you program segments and events in minutes, ml, or CV (column volumes). Use the following guidelines to select a unit:

- CV units are useful because column equilibration and gradient slope are primarily a function of the total number of column volumes of eluent passed through the column.
- CV units make the method scalable because the BioCAD software automatically adjusts for changes in column sizes.
- CV and ml units make the method effectively independent of flow rate.

You can program a method in CV units only if you enter column dimensions in the Configuration dialog box. See Section 4.2.4, Configuring Columns and Plumbing.

If you use a multi-column plumbing configuration, CV (column volume) calculations include the combined volumes of all inline columns.

CV calculations are performed only for those segments for which a column is inline. If the column is bypassed, the CV duration is listed as zero. If the flow rate is set to zero in the General Settings, the volume-based segment duration is also listed as zero.

6.2 Creating a New Method

Overview A summary of the basic steps for creating a new method appear below. For additional information on these steps, see the appropriate sections.

To create a new method:

1. Display the Method Editor. See Section 6.2.1, Displaying the Method Editor.
2. Create, edit, or load a **configuration** for your method. See Section 6.2.2, Selecting a Configuration.
3. Specify **General Settings**. See Section 6.2.3, Specifying General Settings.
4. Create the **blocks**. See Section 6.2.4, Creating Separation Blocks.
5. Create **segments** for each block. See Section 6.2.5, Creating Separation Block Segments.
6. Add additional **events** to the segments, if necessary. See Section 6.2.6, Adding Events.
7. Display the **Blend Graph** to verify that blend and gradient information is set properly. See Section 6.2.7, Using the Blend Graph.
8. Specify **data storage options** for the data files the method will create. See Section 6.2.10, Specifying Data Storage Options.
9. Specify **print settings**. See Section 6.2.11, Specifying Print Settings.
10. Specify **analysis and printing after run**. See Section 6.2.12, Specifying Analysis and Printing After Run.
11. Name and **save** the method. See Section 6.2.13, Saving Methods.

NOTE: For an example of how to create a simple method, see Section 6.3, Creating Typical Methods.

6.2.1 Displaying the Method Editor

Display the Method Editor by selecting the Window menu from any window, then selecting **Method Editor**. The Method Editor window is displayed (Figure 6-4).

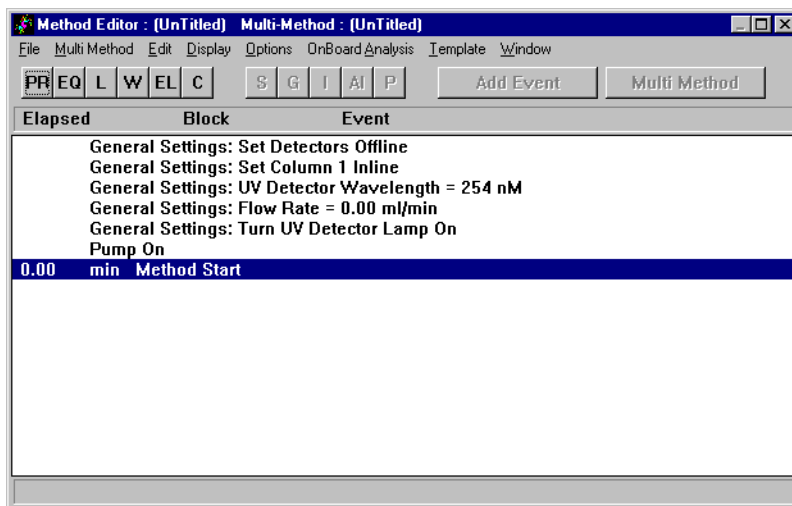


Figure 6-4 Method Editor Window (New Method)

6.2.2 Selecting a Configuration

When you create a new method, the current *system* configuration is used as the *method* configuration.

NOTE: For definitions of method and system configurations, see “System versus method configuration” on page 4-3.

To create a specific method configuration, you can:

- Edit the system configuration before creating your method.
- Load a specific configuration as the system configuration before creating your method. For more information, see Section 4.4, Loading an Existing Configuration.
- Change the method configuration after creating your method. For more information, see “Changing the method configuration” on page 6-14.

Changing the method configuration After you start creating a method, there are two ways to change the *method* configuration:

To change the method configuration ...	Description
Select Edit Configuration from the Method Editor Options menu to display the Configuration dialog box.	This changes the <i>method</i> configuration. It does not change the <i>system</i> configuration. The method configuration is not used when you run the method unless you load the method configuration as the system configuration before running. See Section 4.4, Loading an Existing Configuration. <i>Hint: Use Edit Config when you create a method to run at a later time, using a configuration different from the current system configuration.</i>
Make changes to the system configuration by selecting Edit Config from the Edit menu in the Control Panel. Select Update Configuration from the Method Editor Options menu.	This loads the current <i>system</i> configuration as the <i>method</i> configuration. <i>NOTE: If you change the system configuration from the Control Panel Config menu, it does not affect the method configuration until you select Update Config from the Method Editor Options menu.</i> <i>Hint: Use Update Config when you create a method to run using the current system configuration.</i>

If you plan to run your method immediately, check that the physical configuration of the BioCAD 700E Workstation, matches the *system* configuration. The system configuration is used when you run a method.

6.2.3 Specifying General Settings

The General Settings section of a method allows you to set basic settings for your system including:

- Flow rate
- UV/Vis wavelengths
- Fluorescence wavelengths
- Initial column state
- Initial detector state

General settings do not usually change during a method, so you set them once at the beginning of the method.

NOTE: You can change General Settings later in a method by adding events.

To specify the General Settings for your method:

1. At the top of the Method Editor window, below the Block and Segment buttons, double-click the **General Settings** line.

The General Settings dialog box is displayed (Figure 6-5).

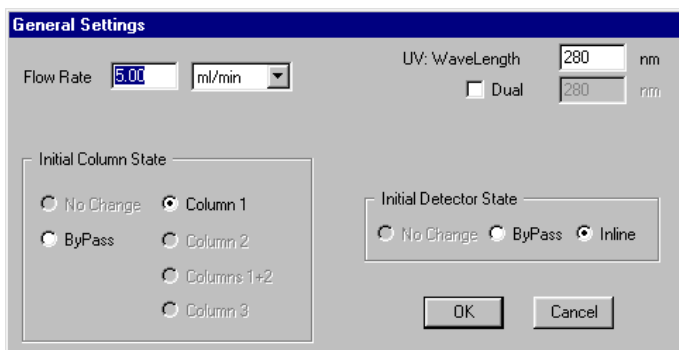


Figure 6-5 General Settings Dialog Box

2. Type or select the following parameters:

Parameter	Description
Flow rate	<p>Valid range is 0.20 to 100.00 ml/min. Available units include ml/min, cm/hr, and CV/min. For more information, see “Flow rate units” on page 5-14.</p> <p>NOTE: <i>If you have an analytical flow cell installed in the UV/Vis detector, do not exceed a flow rate of 45 ml/min.</i></p>
UV/Vis wavelengths	<p>Valid ranges are:</p> <ul style="list-style-type: none"> • Single wavelength—190 to 800 nm. • Dual-wavelength—190 to 450 nm OR 366 to 700 nm, because the detector cannot switch between the deuterium and tungsten lamps. <p>Lamps used in these ranges are:</p> <p>Deuterium (UV)—190 to 450 nm</p> <p>Tungsten (Vis)—366 to 700 nm</p> <p>NOTE: <i>If you enable an external fluorescence detector in hardware setup, dual wavelength mode is not available for the UV/Vis detector. If you enable an external UV/Vis detector in hardware setup, only dual wavelength mode is available for the UV/Vis detector.</i></p> <p>NOTE: <i>When you run a method with dual wavelength enabled, the setting changes to single wavelength at the end of a method to reduce wear of the detector grating.</i></p>
Fluorescence wavelengths	<p>Valid ranges are (even values only):</p> <ul style="list-style-type: none"> • Excitation—200 to 650 nm • Emission—200 to 800 nm
Initial Column State	<p>Initial setting for the flow direction. Choices available depend on column configuration.</p>

Parameter	Description
Initial Detector State	Initial state for the pH and conductivity monitors: <ul style="list-style-type: none">• Bypass• Inline NOTE: <i>UV/Vis detectors are always inline. Detector bypass affects only the pH and conductivity detectors.</i>

CAUTION

*If you use organic solvents, select **Bypass** before running your samples to take the pH and conductivity monitors out of line.*

3. Click **OK** to return to the Method Editor.

6.2.4 Creating Separation Blocks

Overview At the top of the Method Editor window, click a block button to add the appropriate separation block:

- **EQ**—Equil
- **L**—Load
- **W**—Wash
- **EL**—Elute
- **C**—Clean/Regenerate

NOTE: If block buttons are dimmed, select the General Settings line or the separator line below an existing block.

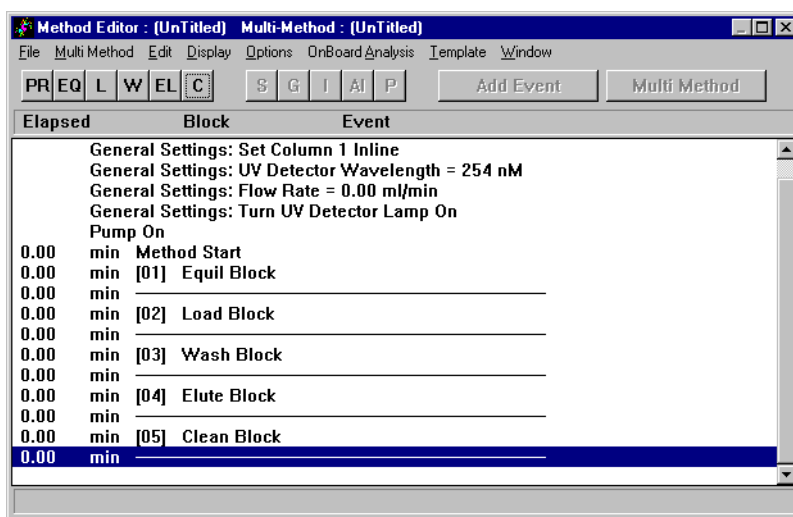


Figure 6-6 Selected Blocks

Each block is sequentially numbered. The column headings (Type, min, ml, CV) above the General Settings line designate the type and duration of the segments within each block.

Duration of each block is initially zero because no segments are selected. Do not add a block without adding a segment to it.

6.2.5 Creating Separation Block Segments

Overview Segment buttons, at the top of the Method Editor window, are dimmed until you select a block. Click a block line in the method, and the appropriate segment buttons are activated.

Click a segment button to add the appropriate segment to the block:

- **S**—Step
- **G**—Gradient
- **I**—Inject
- **AI**—Autosampler Inject
- **P**—Purge

NOTE: Segment buttons are dimmed until you select a block. By default, the line below the block is selected, instead of the block. Display the method in Sequence view to get a more detailed look at the method.

You set the duration of a segment in minutes, ml, or CV. You can set different segments within a method to have different duration units.

6.2.5.1 Step Segments

You can add a Step segment to all separation block types (EQ, L, W, EL, C). A Step segment sets the buffer/solvent blend to a particular blend and holds it for a set duration (ml, min or CV) or until thresholds you set are reached.

Add a Step segment to a Load block to make:

- Pump injections
- Direct wire autosampler injections

NOTE: If a method does not include an Inject segment, data collection begins at the beginning of a run. To trigger data collection later in a run without an Inject segment, add a Data Collection Start event.

To add a Step segment:

1. Select a separation block in the method by clicking it.
2. Click the Segment **S** button to display the Step Segment Setup dialog box (Figure 6-7).

The dialog box is titled "Step Segment Setup". It contains the following fields and controls:

- Blend:** Four rows of percentage blend settings. Each row has a text input field, a percentage sign, and a dropdown menu.

Percentage	Component
100	A: pH 6.0
0.0	B: pH 9.0
0.0	C: H2O
0.0	D: NaCl
- Dur:** A text input field with "0.00" and a dropdown menu with "min".
- Segment Name:** A text input field containing "Step Segment".
- Thresholds:** A table with columns for "Signal" and "Threshold".

Signal	Threshold
<input type="checkbox"/> UV	0.000 AU
<input type="checkbox"/> Cond	0.00 mS
<input type="checkbox"/> pH	0.00 pH
<input type="checkbox"/> Aux	0.00 Volts
- Delay:** A text input field with "0.00" and a dropdown menu with "min".
- Buttons:** "Setup", "OK", and "Cancel".

Figure 6-7 Step Segment Setup Dialog Box (Percentage Blend Mode)

The default blend settings are the blend settings specified in a previous segment.

3. Select a **buffer/solvent blend** for the Step segment. To change the blend mode (Percent, Eluent, or pH), click **Setup**.

For more information on entering blends in different modes see Section 5.2.2, Controlling Buffers/Solvents and Blending.

4. Type the **duration** of the segment in minutes, ml, or CV.

NOTE: *Using CV (Column Volume) units makes the method scalable. That is, it operates the same way with different column sizes. Using ml or CV units makes the segment operate the same way independent of flow rate.*

CV units are available only if you enter column dimensions in the Configuration dialog box.

5. Type a **Segment Name** to identify the function of the Step segment. The default name is Step Segment. The segment name appears in the Status window when a segment is running.
6. Set **thresholds** and type a **delay duration** as needed. See below for information on thresholds and delay durations.
7. Click **OK** to add the Step segment to the block.
8. When you have added all segments to the method, save the method by selecting **Save** from the File menu.

Setting thresholds and delay durations

You can use thresholds to end a segment before the specified duration. For example, you can use thresholds to:

- End an equilibration block when the conductivity or UV/Vis signal returns to baseline
- Collect a single peak with the fraction/waste valve

As a method runs, a segment continues for the specified duration, unless thresholds are reached.

NOTE: *All threshold conditions must be met before the segment stops.*

For each detector signal, you can set a threshold maximum or minimum. To set a maximum threshold, click the > option button and type a value. To set a minimum threshold, click the < option button and type a value.

When using delay duration and thresholds, note the following:

- Always enter a delay duration when using thresholds. If you do not, the detector signal at the start of the segment may be outside the threshold criteria, and the segment will not run.
- When setting a threshold, make sure the detector signal exceeds the threshold value after the set delay. If, for example, you set the UV/Vis threshold to < 0.01 to mark the end of a peak and the peak has not started to elute after the set delay duration, the segment will not run because the threshold value is reached.

CAUTION

Do not add events to a Step segment with threshold settings. The added events cause the segment to ignore the threshold settings and continue to run.

6.2.5.2 Gradient Segments

You can add a Gradient segment to all block types except a Prep or Load block. A Gradient segment sets the buffer/solvent blend to a starting blend and continuously changes the blend in a linear fashion to a final blend over a set duration.

You can create more complex gradients by combining appropriate Step and Gradient segments.

To add a gradient segment:

1. Select a Wash, Equil, Elute, or Clean block by clicking it.
2. Click the Segment **G** button to display the Gradient Segment Setup dialog box (Figure 6-8).

Figure 6-8 Gradient Segment Setup Dialog Box (Percentage Blend Mode)

The default blend settings are the blend settings specified in a previous segment.

3. Select starting and ending **buffer/solvent blends** for the Gradient segment. To change the blend mode (Percent, Eluent, or pH) click **Setup**.

For more information on entering blends in different modes, see Section 5.2.2, Controlling Buffers/Solvents and Blending.

4. Type the **duration** of the segment in min, ml, or CV.

NOTE: Using CV (Column Volume) units makes the method scalable. That is, it operates the same way with different column sizes. Using ml or CV units makes the segment operate the same way independent of flow rate.

CV units are available only if you enter column dimensions in the Configuration dialog box.

5. Type a **Segment Name** to identify the function of the gradient segment. The default name is Gradient Segment. The segment name appears on the Status window when a segment is running.
6. Click **OK** to add the Gradient segment to the block.
7. When you have added all segments to the method, save the method by selecting **Save** from the File menu.

6.2.5.3 Purge Segments

You can add a Purge segment to all separation block types (EQ, L, W, EL, C). A Purge segment quickly flushes the system with a new buffer/solvent blend at a high flow rate while the column is out of line.

A Purge segment:

- Takes the columns out of line
- Changes the buffer/solvent blend to the set blend
- Increases the flow rate to the set rate
- Runs for a set volume duration
- Resets to the General Settings flow rate
- Switches the columns to the specified position

Add a Purge segment to an Equil block to equilibrate the system with your initial buffer/solvent blend.

To add a Purge segment:

1. Select a block by clicking it.
2. Click the Segment **P** button to display the Purge Segment Setup dialog box (Figure 6-9).

**Figure 6-9 Purge Segment Setup
(Eluent Blend Mode)**

3. Select a **buffer/solvent blend** for purging the system. To change the blend mode (Percent, Eluent, or pH) click **Setup**.

For more information on entering blends in different modes, see Section 5.2.2, Controlling Buffers/Solvents and Blending.

4. Type a **Segment Name** to identify the function of the Purge segment. The default name is Purge Segment. The segment name appears in the Status window when a segment is running.
5. Type a **flow rate** for purging. The default is 30 ml/min.

CAUTION

If you are using an analytical flow cell and 0.010-inch ID blue tubing, you may need to reduce the purge flow rate to avoid exceeding the system high-pressure limit.

6. Type a **purge volume** sufficient to purge the system. The default is 10 ml.
7. Select the **Flow Direction at the end of the Purge**. The selections depend on the current plumbing configuration.

NOTE: *If you do not set flow direction, the column is not placed back inline after the Purge segment.*

8. Click **OK** to add the Purge segment to the block.
9. When you have added all segments to the method, save the method by selecting **Save** from the File menu.

6.2.5.4 Inject Segments

You can add an Inject segment to Load or Clean blocks.

Add an Inject segment to a Clean block to expose the column to strong cleaning agents, such as base or acid, without using a buffer/solvent channel.

Add an Inject segment to a Load block to make:

- Autoloader injections
- Direct autoloader injections
- Manual injections

NOTE: *To make pump injections or direct wire autosampler injections, add a Step segment. To make integrated autosampler injections, add an Autosampler Inject segment.*

Hint: *To Inject different samples, or to make injections larger than the maximum for the injection mode you configured, you can add multiple Inject segments to a method.*

To add an Inject segment:

1. Select a Load or Clean block by clicking it.
2. Click the Segment I button to display the Inject Segment Setup dialog box (Figure 6-10).

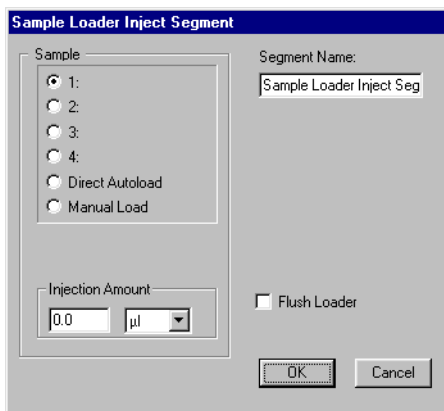


Figure 6-10 Inject Segment Setup Dialog Box

3. Specify the injection mode by clicking an option button:
 - **Autoloader Channel 1 through 4**—Injects through the autoloader, from one of four vials, placed on top of the workstation
 - **Direct Autoload**—Injects through the autoloader, from a vial to the right of the injection valve, bypassing the sample selection valve
 - **Manual Load**—You inject directly into the injection valve using a syringe

NOTE: When you run a method containing a *Manual Load* injection, the method pauses at the point of injection and prompts you to make the injection. After you make the injection, click **OK** or press the **Event Mark** button on the front panel, and the method resumes.

4. If you specify Direct Autoload or Manual Load, specify a **sample name** by double-clicking the Sample text box, then selecting a name from the Sample Library. For information on adding names to the Sample Library, see Section 4.5.2, Customizing the Sample Library.
5. Type the **injection amount** in ml or μl . Valid range depends on the syringe size and sample loop size.
6. Type a **Segment Name** to identify the segment. The default name is Inject Segment. The segment name appears in the Status window when a segment is running.
7. Enable or disable **Flush Loader**.

Flush Loader flushes the sample line, between the sample selection valve and the injection valve. The flush occurs before the injection each time the method runs. The default flush volume is 100 μl . You can change the flush volume in the Configuration dialog box.

Hint: *If you run a method multiple times to make autoloader injections from different samples, enable Flush Loader to prevent sample carryover.*

Hint: *If you run a method multiple times without switching between samples, enabling Flush Loader consumes sample unnecessarily. If you do not switch between samples, disable Flush Loader and flush the loader from the Control Panel Sample menu before starting the first run.*

8. Click **OK** to add the Inject segment to the block.
9. When you have added all segments to the method, save the method by selecting **Save** from the File menu.

6.2.5.5 Autosampler Inject Segments

You can add an Autosampler Inject segment to a Load block.

Add an Autosampler Inject segment to a Load block to make an integrated autosampler injection.

NOTE: To make pump injections or direct wire autosampler injections, add a Step segment. To make autoloader, direct autoloader, or manual injections, add an Inject segment.

To add an Autosampler Inject segment:

1. Select a Load block by clicking it.
2. Click the Segment **AI** button to display the Autosampler Inject Segment dialog box (Figure 6-11).

NOTE: If the Segment **AI** button is dimmed, enter sample vials in the Sample Table. See Section 4.7.3, Entering Vials in Sample Table.

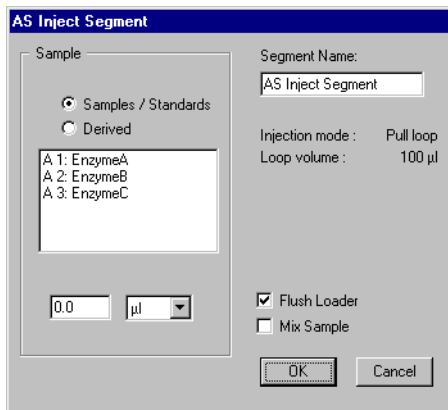


Figure 6-11 Autosampler Inject Segment Dialog Box (Integrated Autosampler)

3. Select the type of samples to display in the Sample list box:

- **Sample/Standards**—Displays a list of all sample vials entered in the Sample Table as Sample, Standard, Control, or Reagent. See Section 4.7.3, Entering Vials in Sample Table.
- **Derived**—Displays a list of all Derived vials created by preceding Prep blocks in this method. This option is available only if your autosampler supports the sample preparation option.

4. Select a **sample** from the Sample list box.

The maximum injection volume and available sample volume for the sample vial are displayed in the dialog box.

5. Type the **injection volume** in ml or μl .

Valid range for autosampler injections depends on the type of injection you specify in the Configuration dialog box (Pull Loop, PushLoop, or Full Loop), the size of the installed sample loop, and the available volume in the sample vials.

NOTE: To calculate the available volume, the software looks at all sample vials with the selected name, finds the vial with the largest volume, and subtracts the dead volume for that vial shape.

NOTE: If your autosampler supports sample preparation, you can make injections larger than 200 μl . The autosampler uses the Prep syringe (2,500 μl) instead of the Sample syringe (250 μl). Both syringes are located at the rear of the autosampler.

6. Type a **Segment Name** to identify the function of the Autosampler Inject segment. The default name is AS Inject Segment. The segment name appears in the Status window when a segment is running.
7. To flush the autosampler with flush solvent after each injection, click the **Flush Loader** check box.
Flush Loader flushes between the needle tip and the autosampler injection syringe. Flushing is recommended to minimize carryover. The default flush volume is 430 μl . You can change the flush volume in the Configuration dialog box.
8. Click the **Mix Sample** check box to mix for 2 seconds before injecting. This option is available only if your autosampler supports the sample preparation option.

CAUTION

Mixing samples may denature proteins.

9. Click **OK** to add the Autosampler Inject segment to the block.

Hint: Autosampler injections can take several minutes to complete. Include a Zero Detector event at the start of the Inject segment.

Insufficient injection volume

When you run a method containing an Autosampler Inject segment, the autosampler injects from the sample vial you select in the Sample list box. If the sample volume in the vial is insufficient to make the injection, a warning is displayed before the method runs. The system:

- Finds the vial with the same sample name and smallest adequate volume (if any) and asks if you want to inject from this vial. Click **OK** to inject from this vial, or click **Cancel** to cancel the method.
- If there is no vial with sufficient volume, the software asks if you want to reduce the injection volume. Click **OK** to reduce the injection volume, or click **Cancel** to cancel the method.

6.2.6 Adding Events

Automatic events versus Added events

There are four types of events in a method:

- **Automatic Events**—Predefined events that are part of a segment. They are automatically added to a method when you add a segment to a method.
- **Added Events**—Miscellaneous events you can add to a method segment individually, to meet your application needs. Added events are flagged with an asterisk (*).
- **Control Panel Added Events**—Events added to a method as a result of an action you make from the Control Panel, such as stopping the pump. These added events are flagged with an asterisk (*) and a plus sign (+) and appear only in methods contained in data files.
- **Documentation Added Events**—Events added to a method during the method run. These events document the result of other actions and are not executable. Examples include Method Cancelled, Peak End, and Threshold Expired. These added events are flagged with an asterisk (*) and a minus sign (-) and appear only in methods contained in data files.

Adding events

You can add events to all separation segment types (S, G, I, AI, and P). You cannot add events to preparation segment types (D, C, and H/M). You can add as many events as you like.

NOTE: *In the Method Editor, events are displayed in Sequence view only. To change to Sequence view, select **Sequence View** from the Display menu.*

To add an event to a segment:

1. Select a **separation segment** in the Method Editor window. You can click on any portion of the segment. The position at which the new event is inserted is determined by the time or volume you enter for the event.

2. Click the **Add Event** button at the top of the Method Editor window.

A list of events is displayed. Scroll through the list to find the event to add. Events are described in the “Method events” on page 6-35.

3. Select the **event** to add.
4. Click **Add**.

A dialog box appears prompting you to type a time (minutes) or volume (ml or CV) for the selected event to occur within the segment.

5. Type a **time**, **volume**, or **CV** value, then click **OK** to insert the event.

NOTE: *The unit (minutes, ml, or CV) that you specify for an event is the same unit that you specified for the segment. For example, if you set the segment in ml, you set the added event in ml.*

Hint: *You can add events to the end of a segment to perform functions such as stopping the pump. If you change the duration units (minutes, ml, or CV) of a segment, the event is automatically set to occur at the end of the segment. You do not need to change the event time to the end of the segment.*

6. When you have added all events to the method, save the method by selecting **Save** from the File menu.

Deleting events

To delete an event that you added, select the event, then select **Cut Event** from the Edit menu.

NOTE: *When you perform an operation from the Control Panel, such as stopping the pump, an event is added to the method in a data file. This event is flagged with an asterisk (*) and a plus sign (+), and you cannot delete it using Cut Event.*

*To delete this type of event, first save the file as a method file (.MET) by selecting **Save As** from the File menu.*

Method events The following tables describe events that you can add to a method.

NOTE: The events displayed are determined by the method configuration (not the system configuration). For more information, see Chapter 4, Configuring and Preparing the BioCAD Software.

Pump Events	
Event	Description
Pump Off	Turns off pump. NOTE: If you add a Pump Off event to a segment specified in CV or ml, the event is automatically added to the end of the segment. NOTE: The pump automatically turns off at the end of a method or multi-method.
Pump On	Turns on pump.
Set Flow Rate	Starts a new flow rate and uses this flow rate until next flow rate event is encountered. This added flow rate event is not changed when using the flow rate template. Valid ranges are 0.20 to 60.00 ml/min NOTE: If you add a Set Flow Rate=0.00 event to a segment specified in CV or ml, the event is automatically added to the end of the segment.

NOTE: Plumbing events displayed are determined by the column plumbing option in the method configuration.

Plumbing Events	
Event	Description
Set Column Offline	Bypasses columns and directs flow from injection valve directly to detectors. Available only with Single and Tandem column plumbing configurations. NOTE: If you add a Set Column Offline event to a segment specified in CV or ml, the event is automatically added to the end of the segment.
Set Column 1 Inline	Places column 1 inline in Single, Tandem, and Three column plumbing configurations.
Set Column 2 Inline	Places column 2 inline in Tandem and Three column plumbing configurations.
Set Column 3 Inline	Places column 3 inline in Three column plumbing configuration.
Set Column 1 Reversed	Reverses flow through the column. Available only in Single column plumbing configuration.
Set Column 1 and 2 Inline	Directs flow through column 1 and column 2 prior to the detection module. Available only in Tandem column plumbing configuration.

pH/Conductivity Detector Events	
Event	Description
Set Detectors Inline	Places pH and conductivity monitors in the flow path.
Set Detectors Offline	Removes pH and conductivity monitors from the flow path.

UV/Vis Detector Events	
Event	Description
Set UV Detector Wavelength	<p>Sets a new wavelength for the internal UV/Vis detector and automatically zeroes the detector.</p> <p>Valid ranges are:</p> <p>Single wavelength—190 to 800 nm. Dual-wavelength—190 to 450 nm OR 366 to 700 nm, because the detector cannot switch between the deuterium and tungsten lamps.</p> <p>NOTE: <i>Wavelength change is not instantaneous, especially when switching wavelengths between the UV and visible regions (365 nm). In these cases, one lamp turns off while the other turns on. The deuterium lamp (UV) takes several minutes to turn on. During the warm-up period, no meaningful data is collected.</i></p> <p>NOTE: <i>If you enable an external fluorescence detector in hardware setup, dual wavelength mode is not available for the UV/Vis detector. If you enable an external UV/Vis detector in hardware setup, only dual wavelength mode is available for the UV/Vis detector.</i></p> <p>NOTE: <i>When you run a method with dual wavelength enabled, the setting changes to single wavelength at the end of a method to prevent wear of the detector grating.</i></p>

Continued

UV/Vis Detector Events (Continued)	
Event	Description
Turn UV Detector Lamp On	Turns on: <ul style="list-style-type: none"> • Deuterium lamp (UV) for wavelengths up to 365 nm • Tungsten lamp (Vis) for wavelengths above 365 nm
Turn UV Detector Lamp Off	Turns off the internal and external UV/Vis detector lamp. <i>Hint: Include this event in the last run of the day.</i>
Zero UV Detector	Zeroes the absorbance detector output.
UV Detector Event Mark	Marks the UV/Vis data channel in the Strip Chart Recorder and data files with an event mark (red MRK).

Fluorescence Detector Events	
Event	Description
Turn Fluorescence Detector Lamp On	Turns on the lamp in the fluorescence detector.
Turn Fluorescence Detector Lamp Off	Turns off the lamp in the fluorescence detector lamp. <i>Hint: Include this event in the last run of the day.</i>
Set Fluorescence Detector Wavelength	Sets new Emission and Excitation wavelengths for the fluorescence detector and automatically zeroes the detector . Valid entries are (even values only): <ul style="list-style-type: none"> • Excitation—200 to 650 nm • Emission—200 to 800 nm
Zero Fluorescence Detector	Zeroes the fluorescence detector output.
Fluorescence Detector Event Mark	Marks the fluorescence data channel in the Strip Chart Recorder and data files with an event mark (red MRK).

Fraction Collector Events	
Event	Description
Collect Fraction	If a fraction collector is not configured, places the fraction/waste valve in the Fraction position. If a fraction collector is configured, controls the diverter valve on the external fraction collector to divert flow to fraction collection tubes.
Divert to Waste	If a fraction collector is not configured, places the fraction/waste valve in the Waste position. If a fraction collector is configured, controls the diverter valve on the external fraction collector to divert flow to waste.
Start Fraction Collection	Starts fraction collection according to parameters you set. For information on setting fraction collection parameters, see “Setting fraction collection parameters” on page 6-47.
Stop Fraction Collection	Stops fraction collection, diverts flow to waste, and advances one tube.
Reset Fraction Collector	Resets the fraction collector diverter valve to the home position (tube 1) and diverts flow to waste. NOTE: Use this event to pool fractions when you run the same method multiple times.
Advance to Next Tube	Advances the fraction collector diverter valve to the next tube in the rack sequence and collects flow. Hint: If you make multiple runs and do not pool fractions, you can add an Advance event after a Stop event to leave an empty “marker” tube between fractions from different runs.

Sample Loader Events	
Event	Description
Load Sample into Loop	Places injection valve and sample loop in the Load position. Loads loop with volume you specify.
Flush Sample Loader	Places injection valve and sample loop in the Inject position and flushes sample line with indicated sample channel (1, 2, 3, or 4). Flushing clears the sample line from the sample selection valve to the injection valve. NOTE: The volume used is the flush volume set in the Configuration dialog box. Default flush volume is 100 μ l.
Inject Sample	Places injection valve and sample loop in the Inject position. Injects contents of sample loop onto the column.
Prime Sample Loader	Places injection valve and sample loop in the Inject position and primes sample line with indicated sample channel (1, 2, 3, or 4). Priming fills the entire selected sample line, up to the injection valve. NOTE: The volume used is the prime volume set in the Configuration dialog box. Default prime volume is 350 μ l.
Set Sample Loop to Load Position	Places injection valve and sample loop in the Load position.
Set Sample Loop to Inject Position	Places injection valve and sample loop in the Inject position.

NOTE: If Integrated Autosampler events are not listed, configure an AS 3500 as an integrated autosampler and enter samples in the Sample Table.

NOTE: If you add an Autosampler Inject segment to a method, you do not need to add events. If you want more control over the integrated autosampler, add the following events to a Step segment.

Autosampler Events (Integrated AS 3500 Configured)	
Event	Description
Load Sample through Autosampler	Places autosampler injector valve and loop in the Load position. Loads loop with sample and volume you specify.
Inject Sample through Autosampler	Places autosampler injector valve and loop in the Inject position. Injects contents of autosampler loop onto the column.
Flush Autosampler	Flushes autosampler with the volume of flush solvent you specify.
Set Sample Loop to Load Position	Places autosampler injector valve and loop in the Load position.
Set Sample Loop to Inject Position	Places autosampler injector valve and loop in the Inject position.

NOTE: If Direct Wire Autosampler events are not listed, configure a direct wire autosampler.

Autosampler Events (Direct Wire AS 3500 Configured)	
Event	Description
Autosampler Stop	Sends a stop signal to a direct wire autosampler. The autosampler pauses at the point it receives the stop signal and waits for an inject signal from the BioCAD 700E Workstation.
AutoSampler Inject	Sends an inject signal to a direct wire autosampler. The autosampler makes an injection from the next vial in the sample set.
Set Vial Number	Associates a vial number with an Autosampler Inject event. NOTE: This event is for documentation purposes only. This event does not control the sample vial the autosampler injects from.

Special Events	
Event	Description
Display Message Event	<p>Allows you to customize a message to display on screen at the specified point in the method.</p> <p>Type the time at which to display the message, the caption for the message box, the message, and the label to display on the command button in the message box. You can also set the event to beep and to hold the method.</p>
Data Collection Start	<p>Sets starting point for data collection. The default is to start data collection at the start of an Inject or Autosampler Inject segment, or at the beginning of a method, if there is no Inject segment.</p> <p>CAUTION: <i>Add only one Data Collection Start event to a the method. If you add a Data Collection Start event after an Inject segment, data acquisition does not begin until after the Inject segment.</i></p> <p>Hint: <i>If you make a direct wire autosampler injection or a pump injection, add a Data Collection Start event in the Step segment when the sample is introduced. If you do not, data collection starts at the beginning of the method.</i></p>

Continued

Special Events (Continued)	
Data Collection End	<p>Sets end point for data collection. The default is to end data collection at the end of the method.</p> <p>CAUTION: <i>Add only one Data Collection End event to a method. Do not add a Data Collection End event before an Inject or Autosampler Inject segment.</i></p>
Auxiliary Out Event	<p>Sets two TTL output signals and one low and one high level current driver. For information on connecting terminals, see Section 5.2.10, Programming Event Outputs.</p>
Auxiliary In Event	<p>Waits for specified conditions on two TTL input signals. see “Setting Auxiliary In Event conditions” on page 6-50 for instructions on connecting terminals and setting conditions.</p>

Setting fraction collection parameters

When you add a Start Fraction Collection event, the Fraction Collector Setup dialog box is displayed (Figure 6-12).

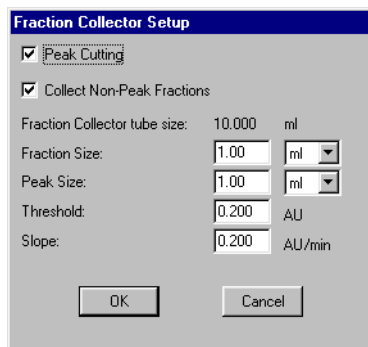


Figure 6-12 Fraction Collector Setup Dialog Box

The Fraction Collector Setup dialog box lets you specify:

- Only Collect Non-Peak Fractions
- Only Peak Cutting
- Both Peak Cutting and Collect Non-Peak Fractions

Only Collect Non-Peak Fractions

If you do not select Peak Cutting, Collect Non-Peak Fractions is enabled by default. The fraction collector collects fractions based on time or volume, whether or not peaks are detected (Figure 6-13).

Type a **Fraction Size**, in ml, min, or CV (column volume).

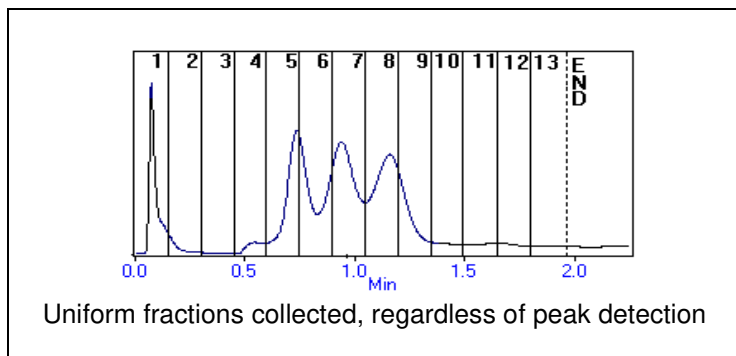


Figure 6-13 Collect Non-Peak Fractions

Only Peak Cutting If you select only Peak Cutting, the fraction collector collects fractions only when a peak is detected, as specified by threshold and slope settings (Figure 6-14).

Type the following:

- **Peak Size**—Size of peak fractions to collect in min, ml, or CV (column volume). If actual peak exceeds the peak size setting, fractions are collected in multiple tubes.
- **Threshold**—Level in AU (absorbance units) that signals a peak.
- **Slope**—Rate of change in AU/minute that signals a peak.

NOTE: Both threshold and slope values must be exceeded to trigger a peak. These values are based on UV/Vis Channel #1 in dual-wavelength systems. When peaks are not detected, the diverter valve on the fraction collector is set to waste.

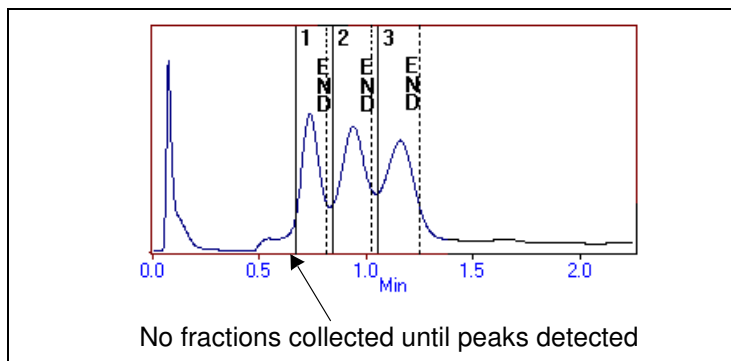


Figure 6-14 Peak Cutting

**Both Peak Cutting
and Collect
Non-Peak
Fractions**

If you select both Peak Cutting and Collect Non-Peak Fractions, the fraction collector collects fractions based on time or volume until a peak is detected (Figure 6-15).

Type the following:

- **Fraction Size**—Size of non-peak fractions to collect in ml, min, or CV (column volume)
- **Peak Size**—Size of peak fractions to collect in ml, min, or CV (column volume)
- **Threshold**—Level in AU (absorbance units) that signals a peak
- **Slope**—Rate of change in AU/min that signals a peak

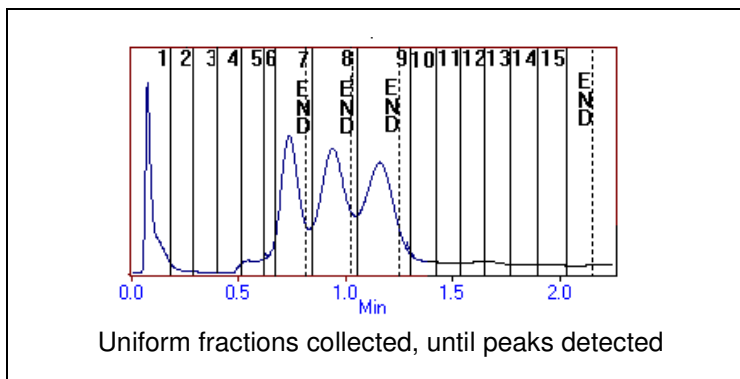


Figure 6-15 Collect Non-Peak Fractions and Peak Cutting

Setting Auxiliary In Event conditions

When you add an Auxiliary In Event to a method, the Auxiliary In Event dialog box is displayed (Figure 6-16).

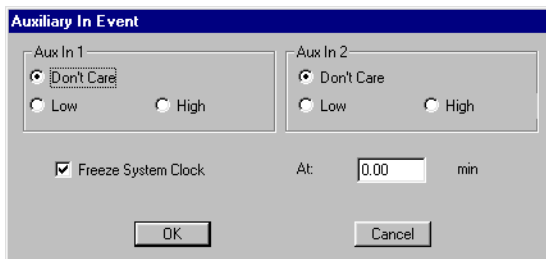


Figure 6-16 Auxiliary In Event Dialog Box

The In Event signal is useful if you need to synchronize the method with the operation of an auxiliary device.

With this event, you can set the method to wait until the BioCAD 700E Workstation receives a low or high signal on one or both input terminals. If you select Freeze System Clock, the clock stops when the method reaches this event. Pump flow and current conditions continue. When the BioCAD 700E Workstation receives the specified signal, the method continues and the clock starts again.

To use the Auxiliary In Event method event, make the following connections to the auxiliary connector on the left side of the BioCAD 700E Workstation.

Signal	BioCAD Connector Pin
Input #1—TTL	Auxiliary Pins 7 (ground) and 8
Input #2—TTL	Auxiliary Pins 7 (ground) and 9

6.2.7 Using the Blend Graph

The Blend Graph is a pictorial representation of any buffer/solvent blends that you program in a method. Check the Blend Graph after creating a method to verify any buffer/solvent blends or gradients in your method.

To display the Blend Graph:

1. Select **Blend Graph** from the Display menu in the Method Editor.

The Blend Graph is displayed at the bottom of the Method Editor window (Figure 6-17).

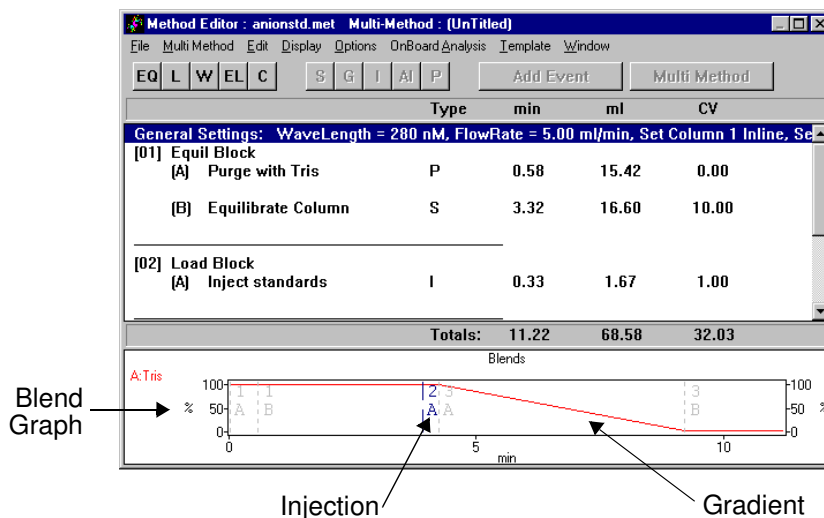


Figure 6-17 Blend Graph (Percent Blend Mode)

2. To customize the Blend Graph, select **Blend Graph Setup** from the Display menu.

The Blend Graph Setup dialog box is displayed (Figure 6-18).

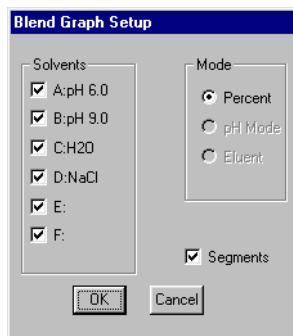


Figure 6-18 Blend Graph Setup Dialog Box

3. Select the **buffer/solvents** to display.
4. Select the **blend mode** (Percent, pH, or Eluent) to display.
5. To mark the Blend Graph when a new segment begins, click the **Segments** box.
6. Click **OK**.

Blend graph annotations

The Blend Graph includes the following annotations:

- **Segment marks**—Each segment is designated by a number and letter. For example, the first segment in the first block is marked 1A, the second 1B, and so on.
- **Injection marks**—Injections are designated by a vertical blue line.

6.2.8 Creating Prep Blocks

If you configure an integrated autosampler that supports sample preparation, you can add Prep blocks to a method.

You typically add a Prep block to a method after entering the General Settings and adding an Equil block. You can add additional Prep blocks anywhere in the method, depending on your application.

To add a Prep block:

1. Add sample vials to the Sample Table as described in Section 4.7.3, Entering Vials in Sample Table.

NOTE: You can access the Sample Table from the Method Editor by selecting **Edit Sample Table** from the Options menu. The changes you make are added to the method configuration, but not the system configuration.

NOTE: You cannot alter the Sample Table when a method is running. However, you can make an injection from the Sample Table when a method is running.

2. Click a divider line below a block in the Method Editor window.
The block buttons are now active on the Method Editor toolbar.
3. Click the **PR** block button at the top of the Method Editor window.

The Prep Block Setup dialog box is displayed (Figure 6-19).

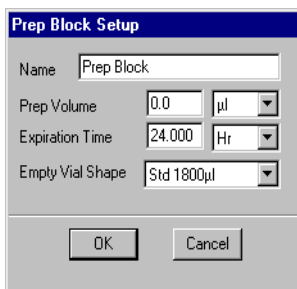


Figure 6-19 Prep Block Setup Dialog Box

4. Enter the following information:

Parameter	Description
Name	<p>Name assigned to the final vial. If you do not enter a name, the default name assigned to the final vial will be "Prep Block #" where # represents the number of the Prep block in the method.</p> <p>NOTE: After the Prep block runs, the state of the final vial is set to Derived. You can select this Derived vial by its name in subsequent Prep blocks or Autosampler Inject segments within the method.</p> <p>After the entire method is complete, the state of all Derived vials is set to Sample, so that you can use the vial in subsequent methods.</p>
Prep Volume	<p>Total volume to prepare for the contents of the final vial. Valid range is greater than 0 and less than the maximum volume for the vial shape.</p> <p>The total volume you enter in the Prep Volume text box determines the component volumes used to prepare the sample.</p>

Continued

Parameter	Description
Expiration Time	Not available at this time.
Empty Vial Shape	Vial shape (Std 1800 µl, Std 2000 µl, Micro, or Unspecified) of the final vial and all intermediate vials that the Prep block creates. NOTE: Make sure you enter Empty vials in the Sample Table with this same vial shape. See “Entering single vials or batches of vials” on page 4-47.

5. Click **OK**.

A Prep block is added to the method, and the Prep block segment buttons (D, C, H/M) are now active on the Method Editor toolbar.

Prep block final vials Each Prep block creates one final vial. The final vial is the last vial the Prep block creates.

The final vial name is the Prep block name. You can select the final vial by this name later in the method. You can select the final vial in any segments where you select Derived vials (Autosampler Inject, Dilute, Combine, or Heat/Mix segments).

After a Prep block runs, the final vial is changed to a Derived vial. After the method runs, all Derived vials are changed to Sample vials.

NOTE: You cannot use a Prep block to create more than one final vial. Do not add more than one Dilute segment or one Combine segment to a Prep block.

Prep block intermediate vials Prep block segments (Dilute, Combine, or Heat/Mix) create intermediate vials. Intermediate vials are temporary vials created to prepare the one final vial (for example, when making serial dilutions or combining samples).

The intermediate vial name is the Prep block segment name plus the label "Product". For example, if you name a Dilute segment "Diluted BSA", the intermediate vial name is "Diluted BSA Product". You can select intermediate vials later in the Prep block in any segments where you select Derived vials. You cannot select an intermediate vial later in the method.

After a Prep block runs, intermediate vials are changed to Waste vials.

NOTE: You must include the same shape of empty vials (Std 1800 μ l, Std 2000 μ l, Micro, or Unspecified) for the final vial and all intermediate vials. If a Prep block creates a final vial that is a Micro vial, you must include empty Micro vials for the intermediate vials.

6.2.9 Creating Prep Block Segments

Overview Prep block segment buttons, at the top of the Method Editor window, are dimmed until you select a Prep block. Click a Prep block in the method, and the appropriate segment buttons are activated.

- **D**—Dilute
- **C**—Combine
- **H/M**—Heat/Mix

NOTE: Segment buttons are dimmed until you select a block. by default, the line below the block is selected, instead of the block. Display the method in Sequence view to get a more detailed look at the method.

6.2.9.1 Dilute Segments

You can add a Dilute segment to a Prep block only. A Dilute segment adds a diluent and sample in a specified ratio to the first Empty vial found in the Sample Table.

NOTE: Do not add more than one Dilute segment to a Prep block. Do not add a Dilute segment to a Prep block that contains a Combine segment. Because a Prep block can create only one final vial, the method would ignore the first segment.

If you need two Dilute segments or a Dilute segment and a Combine segment, add them to separate Prep blocks.

To add a Dilute segment to a Prep block:

1. Click the Prep block and click the Segment **D** button to display the Dilute Segment Setup dialog box (Figure 6-20).

NOTE: If the Segment D button is dimmed, enter vials in the Sample Table. Select the same vial shape for Empty vial types in the Sample Table and for the Empty Vial Shape in the Prep Block Setup dialog box. Configure autosampler solvents; and update the method configuration.

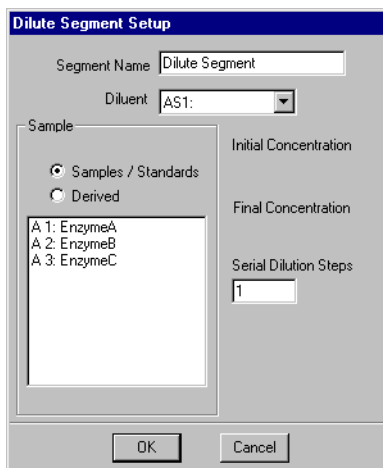


Figure 6-20 Dilute Segment Setup Dialog Box

2. Select a **diluent** from the Diluent drop-down list box. The list contains the solvents you configured (AS1 through AS4) for the autosampler in “Autosampler solvents” on page 4-43. Click **OK**.

3. Select the type of samples to display in the Sample list box:
 - **Sample/Standards**—Displays a list of all sample vials entered in the Sample Table as Sample, Standard, Control, or Reagent. See Section 4.7.3, Entering Vials in Sample Table.
 - **Derived**—Displays a list of all Derived vials created by preceding Prep blocks in this method, and preceding segments in this Prep block.
4. Select a **sample** from the Sample list box.

The initial concentration for the selected sample is displayed:

If you select...	Initial concentration is...
Sample/Standard	Concentration of 1st component entered in Sample Editor for sample.
Derived	Concentration of 1st component listed in the Dilute or Combine segment that created the Derived sample.

5. Type **final concentration** for the dilution. The BioCAD software uses the final concentration you enter and the initial concentration to determine the ratio of volumes to use in the dilution.
6. Type the number of steps over which to make the dilution in the **Serial Dilution Steps** text box. The number must be between 1 and 20. For example, if you want to make a 1:1,000,000 dilution, and you type 3 for Serial Dilution Steps, the Dilute segment makes 3 dilutions of 1:100.

NOTE: Before running the method, add one empty vial to the autosampler tray and to the corresponding location in the Sample Table, for each Serial Dilution Step in each Dilute segment. Location of empty vials in the autosampler tray is not important. The autosampler finds the first available empty vial of the required shape.

7. Type a **Segment Name** to identify the purpose of the Dilute segment. The default name is Dilute segment. The segment name appears in the Status window when a segment is running. Intermediate vials created by this segment are given the segment name and the label “Product”.
8. Click **OK**.

A Dilute segment is added to the Prep block.

Hint: Add a Heat/Mix segment after a Dilute segment to produce a uniform mixture.

6.2.9.2 Combine Segments

You can add a Combine segment to a Prep block only. A Combine segment combines multiple samples and reagents in the first Empty vial found in the Sample Table.

NOTE: Do not add more than one Combine segment to a Prep block. Do not add a Combine segment to a Prep block that contains a Dilute segment. Because a Prep block can create only one final vial, the method would ignore the first segment.

If you need two Combine segments or a Combine segment and a Dilute segment, add them to separate Prep blocks.

To add a Combine segment to a Prep block:

1. Click the Prep block and click the Segment **C** button to display the Combine Segment Setup dialog box (Figure 6-21).

NOTE: If the Segment C button is dimmed, enter vials in the Sample Table. Select the same vial shape for Empty vial types in the Sample Table and for the Empty Vial Shape in the Prep Block Setup dialog box. Update the method configuration.

Sample	Primary Component	Initial Conc	Final Conc	Ratio
FITC stock	FITC	100.000 ug/ml	50.000 ug/ml	50.00 %

Add Sample

Diluent: AS4: MEQH 50.00 %

Total 100.00 %

OK Cancel

Figure 6-21 Combine Segment Setup Dialog Box

2. To select a sample, click the **Add Sample** button to display the Select Sample dialog box (Figure 6-22).

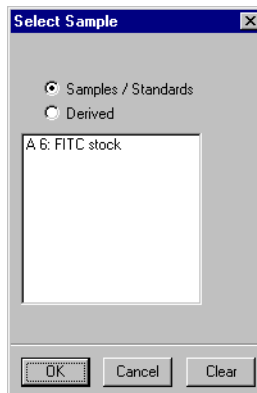


Figure 6-22 Select Sample Dialog Box

3. Select the type of samples to display in the Sample list:
 - **Sample/Standards**—Displays a list of all sample vials entered in the Sample Table as Sample, Standard, Control, or Reagent. See Section 4.7.3, Entering Vials in Sample Table.
 - **Derived**—Displays a list of all Derived vials created by preceding Prep blocks in this method, and preceding segments in this Prep block.
4. Select a **sample** then click **OK**.
The sample is added to the list in the Combine Segment Setup dialog box. The component name and initial concentration for the first component entered for the sample in the Sample Library are also displayed.
5. Type the **final concentration** for the selected sample.
This is the concentration of the component in the final combination.
6. To select additional samples, click the **Add Sample** button and repeat step 3 through step 5.

7. Select a **diluent** from the Diluent drop-down list box. The list contains the solvents you configured (AS1 through AS4) for the autosampler in “Autosampler solvents” on page 4-43. Click **OK**.

The diluent is added if the combined percentages of samples do not equal 100%. When all samples and diluent are selected, the Ratio column displays the percentage of each used in the final combination.

NOTE: *If the concentration for samples yields a combined ratio of 100%, it is not necessary to select a diluent. However, for best sample preparation accuracy, add at least 25% diluent to ensure all samples are flushed into the empty vial. If the concentration for samples yields a combined ratio of more than 100%, you must reduce sample concentrations.*

8. Type a **Segment Name** to identify the function of the Combine segment. The default name is Combine Segment. The segment name appears in the Status window when a segment is running. Intermediate vials created by this segment are given the segment name and the label “Product”.
9. Click **OK**.

A Combine segment is added to the Prep block.

NOTE: *Before running the method, add one empty vial to the autosampler tray and to the corresponding location in the Sample Table for each Combine segment. Location of empty vials in the autosampler tray is not important. The autosampler finds the first available empty vial of the required shape.*

Hint: *Add a Heat/Mix segment after a Combine segment to produce a uniform mixture.*

6.2.9.3 Heat/Mix Segments

You can add a Heat/Mix segment to a Prep block only. A Heat/Mix segment heats or mixes a sample vial. When a method includes a Heat/Mix segment, the autosampler places the specified sample vial in the heat chamber, and optionally mixes by spinning the tube using the compressed air source connected to the autosampler.

NOTE: Do not use plastic vials if you are using Heat/Mix segments in a method. Plastic vials do not conduct heat well.

Hint: Add a Heat/Mix segment after a Dilute segment or a Combine segment to produce a uniform mixture.

To add a Heat/Mix segment to a Prep block:

1. Set the temperature for the autosampler heat chamber in the Sample Preparation Configuration dialog box or from the Sample Table. For more information see “Sample heating (heat chamber)” on page 4-42 or “Controlling sample heating and cooling” on page 5-50.
2. Click the Prep block and click the Segment **HM** button to display the Heat/Mix Segment Setup dialog box (Figure 6-23).



Figure 6-23 Heat/Mix Segment Setup Dialog Box

NOTE: If the Segment HM button is dimmed, update the method configuration after you enter sample vials in the Sample Table. See Section 4.7.3, Entering Vials in Sample Table.

3. Select the type of samples to display in the Sample list box:
 - **Sample/Standards**—Displays a list of all sample vials entered in the Sample Table as Sample, Standard, Control, or Reagent. See Section 4.7.3, Entering Vials in Sample Table.
 - **Derived**—Displays a list of all Derived vials created by preceding Prep blocks in this method, and preceding segments in this Prep block.
4. Select a **sample** to heat or mix from the Sample list box.
5. Type the **duration** for the segment.
6. To specify mixing, click the **Mix** check box.

CAUTION

Mixing samples may denature proteins.

Hint: *To mix without heating, turn off the autosampler heat chamber.*

7. Type a **Segment Name** to identify the function of the Heat/Mix segment. The default name is Heat/Mix Segment. The segment name appears in the Status window when a segment is running. Intermediate vials created by this segment are given the segment name and the label "Product".
8. Click **OK**.

A Heat/Mix segment is added to the Prep block.
9. Before running the method, check that the temperature has reached the set point. The Status window displays the heat chamber temperature.

6.2.10 Specifying Data Storage Options

After you create a method, specify data storage options for storing the data files that the method creates.

To specify data storage options:

1. Select **Data Storage** from the Options menu in the Method Editor.

The Set Data File Name and Directory dialog box is displayed (Figure 6-24).

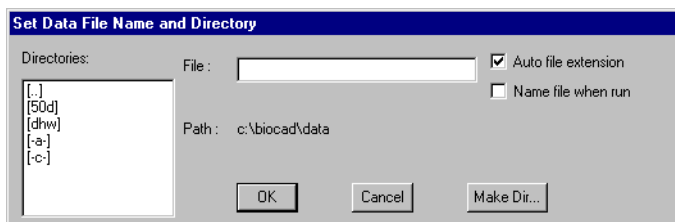


Figure 6-24 Set Data File Name and Directory

2. Enable or disable **Name file when run**.

Hint: If you want to name the data files individually each time you run the method, enable **Name file when run** and click **OK**.

3. If you disable Name file when run, type a **file name** in the File text box. For more information, see “File naming conventions” on page 1-43.
4. Enable or disable **Auto file extension**.

If you enable Auto file extension, you can run the method multiple times using a single data file name. The data files are differentiated by their extension, starting with .B00 and incrementing one number for each method run.

NOTE: The maximum number of files you can generate using the auto file extension is 100 (00 through 99). If you attempt to run more than 100 files, an error message is displayed.

If you disable Auto file extension, the data file is given a .BIO extension.

CAUTION

If you disable Auto file extension and run a method multiple times, all data files are given the same file name with a .BIO extension and will overwrite each other.

5. Specify a **directory** and **path** in which to store the data files. You can use the default directory (C:\BIOCAD\DATA), specify another directory, or create a new directory. The path is the location of the file within the directory tree, including the file name.

Hint: To easily find data files related to specific users and projects, you can create subdirectories in the C:\BIOCAD\DATA directory.

6. To create a new directory from the Method Editor, click the **Make Dir** button to display the Set Data File and Directory dialog box. Type the name of a **subdirectory** to add to the C:\BIOCAD\DATA directory, then click **OK**.

NOTE: You can also create directories using Windows Explorer. For information on creating directories, refer to the Microsoft Windows User's Guide.

7. Click **OK**.
The data file name and directory settings are saved with the method.

NOTE: If you do not specify a file name for the data file, the data file name is DEFAULT.BIO.

6.2.11 Specifying Print Settings

After you create a method, you can specify for the method to print various information after a data file is collected. This option allows you to automatically print information without setting up a custom report.

To specify print settings:

1. Select **Print After Run** from the Options menu.
A check mark appears next to the command.
2. Select **Print Settings** from the Options menu to display the Print Options dialog box (Figure 6-25).

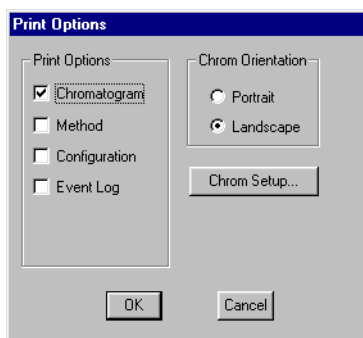


Figure 6-25 Print Options Dialog Box

3. Select any of the following **Print Options**:
 - **Chromatograms**—Prints the data file chromatograms without baselines
 - **Method**—Prints the method as it appeared when the data was collected
 - **Configuration**—Prints the method configuration
 - **Event Log**—Prints the Event Log (.EVL) for the data file

4. Select the **Chromatogram Orientation**:
 - **Portrait**—Page is vertical when you view the chromatogram upright
 - **Landscape**—Page is horizontal when you view the chromatogram upright
5. Click the **Chrom Setup** button to display the Display Settings dialog box.

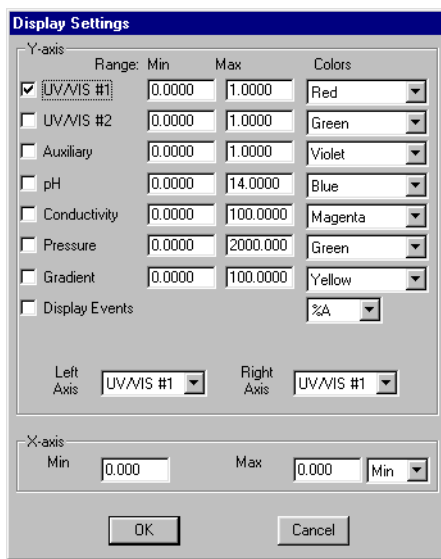


Figure 6-26 Display Settings Dialog Box

6. Set the data channels displays, axes, ranges, and colors as desired, then click **OK**.
7. Click **OK**.

The print settings are saved with the method. The options you selected will print each time the method runs.

6.2.12 Specifying Analysis and Printing After Run

After you create a method, you can specify automatic analyzing and printing after the method run. For information on setting analysis parameters, see the *Data Analysis User's Guide*.

NOTE: The data file will be analyzed with the analysis parameters embedded within the method (.MET) file.

To automatically analyze data files created by the method run:

1. Select **Analyze and Print Report After Run** from the Options menu in the Method Editor window.

A check mark appears next to the command.

2. Select **Analysis Setup** from the Options menu.

The Analysis Setup dialog box is displayed (Figure 6-27).

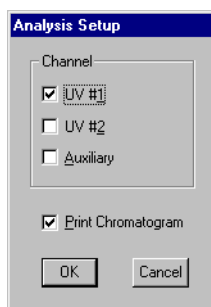


Figure 6-27 Analysis Setup Dialog Box

3. Select up to three **data channels** (UV #1, UV #2, or Auxiliary) to analyze.

4. To print a chromatogram in addition to a report, select **Print Chromatogram**.
5. Click **OK** and save the method.

After you run the method, the following occur:

- The generated data file is automatically analyzed, using the analysis parameters (.MTH) embedded in the method file.
- A report (and optionally a chromatogram) prints, using the report format parameters (.RPT) embedded in the method file.

6.2.13 Saving Methods

To save a method, select **Save** or **Save As** from the File menu.

Methods are saved as separate files with an .MET extension. Methods are also saved in the data files (.B*) created by the method.

6.3 Creating Typical Methods

This section describes the following:

- Creating a simple method
- Making autoloader injections in a method
- Making direct autoloader injections in a method
- Making manual injections in a method
- Making pump injections in a method
- Making integrated autosampler injections in a method
- Making direct wire autosampler injections in a method

6.3.1 Creating a Simple Method

This section provides an example of the General Settings, blocks, segments, and events in a simple, typical method. For detailed descriptions of creating each method part, see Section 6.2, Creating a New Method.

To create a simple, typical method:

Method Editor and General Settings

1. Display the Method Editor by selecting **Method Editor** from the Window menu.
2. For a detailed view of the method, select **Sequence View** from the Display menu.
3. Double-click the **General Settings** line to display the General Settings dialog box (see Figure 6-5 on page 6-15).
4. Type **flow rate** and **wavelengths**. Select **column state** (selections depend on column configuration). Click **Inline** to place the pH and conductivity monitors inline. Click **OK**.

CAUTION

*If you are using organic solvents, click **Bypass** to take pH and conductivity monitors out of line before running.*

Equil block

1. Click the **EQ** button to add an Equil block.
2. Click the Equil block line and click the **P** button to add a Purge segment to the Equil block.

The Purge Segment Setup dialog box is displayed (see Figure 6-9 on page 6-25).

3. Specify **purge blend**, **flow rate**, and **volume**. Select a **column** under Flow Direction at end of Purge. Click **OK**.

The Purge segment is added to the Equil block.

4. Click the **S** button to add a Step segment to the Equil block.

The Step Segment Setup dialog box is displayed (see Figure 6-7 on page 6-20). The blend settings are the same as those you entered in the Purge segment.

5. Enter a **buffer/solvent blend** for equilibrating the column and type a **duration** (minutes, ml, or CV).

Hint: Change the Segment Name to **Equilibrate Column**. If you do not change the segment name, it is labeled Step Segment in the method. Click **OK**.

The Step segment is added to the Equil block.

NOTE: Step 6 through step 8 are optional, but recommended.

6. Click the Equilibrate Column Step segment and click the **Add Event** button to display the Add Event dialog box.
7. Scroll down to the Detector Event Types, select **Zero UV Detector**, and click **Add**.

The Zero UV Detector dialog box is displayed.

8. Type a **duration** (minutes, ml, or CV) that corresponds to the end of the Equilibrate Column Step segment. Click **OK**.

This event zeroes the detector after the column is equilibrated, but before you make an injection.

- Load block**
1. Click the line below the Equilibrate Column Step segment and click the **L** button to add a Load block.
 2. Click the Load block line and click the **I** button to add an Inject segment.

The Inject Segment Setup dialog box is displayed (see Figure 6-10 on page 6-28).

3. Select an **injection mode** and type an **injection amount**. Click **OK**.

The Inject segment is added to the Load block.

NOTE: You can use Inject segments to make autoloader, direct autoloader, and manual injections. If you make a pump or direct wire autosampler injection, use a Step segment. If you make an integrated autosampler injection, use an Autosampler Inject segment. For more information, see the following sections on making injections in a method.

- Wash block**
1. Click the line below the Inject segment and click the **W** button to add a Wash block.
 2. Click the Wash block line and click the **S** button to add an Step segment.

The Step Segment Setup dialog box is displayed (see Figure 6-7 on page 6-20).

3. Leave the blend conditions you entered in the Equil block. Type a **duration** (minutes, ml, or CV) for the wash. Click **OK**.

The Step segment is added to the Wash block.

Elute block

1. Click the line below the Step segment and click the **EL** button to add an Elute block.
2. Click the Elute Block line and click the **G** button to add a Gradient segment.

The Gradient Segment Setup dialog box is displayed (see Figure 6-8 on page 6-23).

3. Select starting and ending **buffer/solvent blends** for the gradient and type a **duration** (minutes, ml, or CV). Click **OK**.
4. Click the **S** button to add a Step segment.

The Step Segment Setup dialog box is displayed (see Figure 6-7 on page 6-20). The blend settings are the same as those you entered in the Gradient segment.

5. Enter the ending **buffer/solvent blend** used in the gradient and type a **duration** (minutes, ml, or CV) to hold the blend. Change the Segment Name to **Hold Gradient**. Click **OK**.

NOTE: When the method runs, the Hold Gradient Step segment holds the ending gradient conditions for the specified time.

6.3.2 Making Autoloader Injections in a Method

- Before you begin** Before you run a method that makes an autoloader injection:
- Prepare up to four samples. For more information, see Section 3.3.1, Preparing Samples for Autoloader Injections (Small Vessels) or Section 3.3.2, Preparing Samples for Autoloader Injections (Large Vessels).
 - Configure the autoloader. For more information, see Section 4.2.3, Configuring the Autoloader.

Creating the method The following table outlines the General Settings, blocks, segments, and events to add to a method to make an autoloader injection.

General Settings		
Specify a flow rate, wavelength, initial column state, and initial detector state appropriate for your application.		
Block	Segment	Settings and Events
Equil	Purge	Specify an appropriate purge blend , flow rate , and volume .
	Step	<ul style="list-style-type: none"> • Specify a buffer/solvent blend and duration to equilibrate the column. • Add a Zero UV Detector event at the end of the segment.
Load	Inject	<ul style="list-style-type: none"> • Select sample channel 1, 2, 3, or 4 and type an injection amount. • Add a Flush Sample Loader or Prime Sample Loader event at the start of the segment.

6.3.3 Making Direct Autoloader Injections in a Method

Before you begin Before you run a method that makes a direct autoloader injection:

- Prepare your sample. For more information, see Section 3.3.3, Preparing Samples for Direct Autoloader Injections.
- Configure the autoloader. For more information, see Section 4.2.3, Configuring the Autoloader.

Creating the method The following table outlines the General Settings, blocks, segments, and events to add to a method to make a direct autoloader injection.

General Settings		
Specify a flow rate, wavelength, initial column state, and initial detector state appropriate for your application.		
Block	Segment	Settings and Events
Equil	Purge	Specify an appropriate purge blend , flow rate , and volume .
	Step	<ul style="list-style-type: none"> • Specify a buffer/solvent blend and duration to equilibrate the column. • Add a Zero UV Detector event at the end of the segment.
Load	Inject	<ul style="list-style-type: none"> • Select Direct Autoload and select a sample from the Sample Library by double-clicking the Sample text box. Type an injection amount. • Add a Prime Sample Loader event at the start of the segment. <p>NOTE: To conserve sample, you can also prime from the Sample menu.</p>

6.3.4 Making Manual Injections in a Method

Before you begin Before you run a method that makes a manual injection, prepare your sample. For more information, see Section 3.3.4, Preparing Samples for Manual Injections.

NOTE: When making manual injections, Autoloader configuration does not matter.

Creating the method The following table outlines the General Settings, blocks, segments, and events to add to a method to make a manual injection.

General Settings		
Specify a flow rate, wavelength, initial column state, and initial detector state appropriate for your application.		
Block	Segment	Settings and Events
Equil	Purge	Specify an appropriate purge blend , flow rate , and volume .
	Step	<ul style="list-style-type: none"> Specify a buffer/solvent blend and duration to equilibrate the column. Add a Zero UV Detector event at the end of the segment.
Load	Inject	Select Manual Load and select a sample from the Sample Library by double-clicking the Sample text box. Type an injection amount. NOTE: When making manual injections, there are no sample lines to prime.
NOTE: When you run a method containing a Manual Load injection, the method pauses at the point of injection and prompts you to make the injection. After you make the injection, click OK or press the Event Mark button on the front panel, and the method resumes.		

6.3.5 Making Pump Injections in a Method

Before you begin

Before you run a method that makes a pump injection:

- Prepare your sample. For more information, see Section 3.3.5, Preparing Samples for Pump Injections.
- Prime the buffer/solvent sample line. For more information, see Section 3.4.4, Priming Buffer/Solvent Sample Lines for a Pump Injection.
- Configure the buffer/solvent channel that you are using for your sample as type Sample. For more information, see Section 4.2.2, Configuring Buffers/Solvents, and Section 4.5.1, Customizing the Buffer/Solvent Library.

NOTE: When making pump injections, Autoloader configuration does not matter.

Creating the method

The following table outlines the General Settings, blocks, segments, and events to make a pump injection.

General Settings		
Specify a flow rate, wavelength, initial column state, and initial detector state appropriate for your application.		
Block	Segment	Settings and Events
Equil	Purge	Specify an appropriate purge blend , flow rate , and volume .
	Step	<ul style="list-style-type: none"> • Specify a blend and duration to equilibrate the column. • Add a Zero UV Detector event at the end of the segment.

Continued

Block	Segment	Settings and Events
Load	Step	<ul style="list-style-type: none"> <li data-bbox="705 265 1174 326">• Select the buffer/solvent channel and set to 100%. <i>Hint: Use the blending feature to make dilutions of your sample. For example, type 80% for the sample channel and 20% for a channel containing water or equilibration buffer.</i> <li data-bbox="705 565 1174 626">• Type a duration (ml, min, or CV) for injection volume. <i>NOTE: If you type duration in minutes, the injection volume is equal to flow rate (ml/min) x duration of segment (min).</i> <li data-bbox="705 796 1174 857">• Add a Data Collection Start Event at the start of the segment.

6.3.6 Making Integrated Autosampler Injections in a Method

Before you begin Before you run a method that makes an integrated autosampler injection:

- Prepare your samples and the integrated autosampler. For more information, see Section 3.6, Preparing an Autosampler.
- Configure an integrated autosampler and enter samples in the Sample Table. For more information, see Section 4.7, Configuring and Preparing the BioCAD Software for an Integrated Autosampler.

Creating the method The following table outlines the General Settings, blocks, segments, and events to add to a method to make an integrated autosampler injection.

General Settings		
Specify a flow rate, wavelength, initial column state, and initial detector state appropriate for your application.		
Block	Segment	Settings and Events
Equil	Purge	Specify an appropriate purge blend , flow rate , and volume .
	Step	<ul style="list-style-type: none"> • Specify a buffer/solvent blend and duration to equilibrate the column. • Add a Zero UV Detector event at the end of the segment.
Load	Auto-sampler Inject	<ul style="list-style-type: none"> • Select a sample from the Sample list box and type an injection amount. • Enable Flush Loader if appropriate for your application.

6.3.7 Making Direct Wire Autosampler Injections in a Method

Before you begin Before you run a method that makes a direct wire autosampler injection:

- Prepare your samples, the autosampler method, and the direct wire autosampler. For more information, see Section 3.6, Preparing an Autosampler.
- Configure a direct wire autosampler. For more information, see Section 4.8, Configuring and Preparing the BioCAD Software for a Direct Wire Autosampler.

Creating the method The following table outlines the General Settings, blocks, segments, and events to add to a method to make a direct wire autosampler injection.

General Settings		
Specify a flow rate, wavelength, initial column state, and initial detector state appropriate for your application.		
Block	Segment	Settings and Events
Equil	Purge	Specify an appropriate purge blend , flow rate , and volume .
	Step	<ul style="list-style-type: none"> • Specify a buffer/solvent blend and duration to equilibrate the column. • Add a Zero UV Detector event at the end of the segment.
Load	Step	<ul style="list-style-type: none"> • Add an Autosampler Inject event at 0.0 minutes. • Add a Data Collection Start Event at 0.01 minutes.

6.4 Opening an Existing Method

This section describes:

- Opening a method from a method file
- Opening a method from a data file

6.4.1 Opening a Method From a Method File

To open a method from a method file:

1. Display the Method Editor by selecting **Method Editor** from the Window menu.
2. Select **Open** from the File menu to display the Open File dialog box.
3. Select a **method file (.MET)** to open and click **OK**.

NOTE: To open a method file created in a multi-method, type **.G** in the File Name text box to display *.G##* files.

Configuration Mismatch

When you open an existing method, the Configuration Mismatch dialog box (Figure 6-28) is displayed if the method configuration is different from the current system configuration.

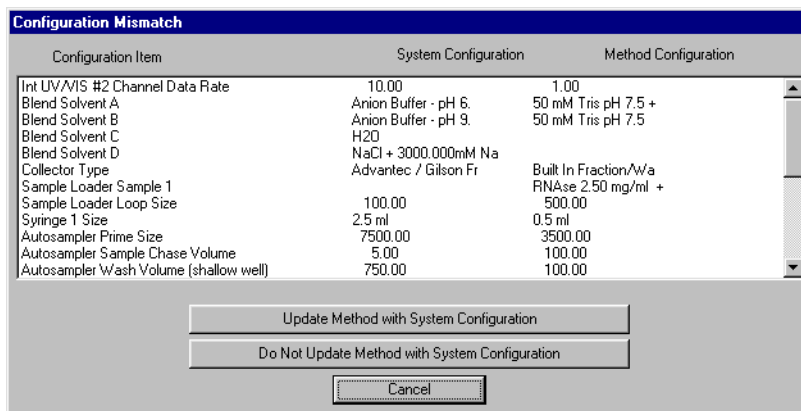


Figure 6-28 Configuration Mismatch Dialog Box

The Configuration Mismatch dialog box displays the settings for each configuration item in the system configuration and the method configuration. Use this dialog box to compare the two configurations. For more information on system and method configurations, see “System versus method configuration” on page 4-3.

You can:

- Click **Update Method Configuration** to change the method configuration to match the current system configuration
- Click **Do Not Update Method Configuration** to ignore the system configuration and leave the method configuration unchanged

NOTE: *If you select Do Not Update Method Configuration, the method configuration does not match the system configuration. The system configuration will be used when you run the method.*

Do not run the method unless you change the system configuration to match the method configuration. You can edit the system configuration or load the method configuration as the system configuration. For more information, see Section 4.4, Loading an Existing Configuration.

Event warnings

You will also see warning messages if:

- The method contains events that do not apply to the current system configuration.
- The Buffer Duration for the Strip Chart Recorder (set in the Configuration dialog box) is shorter than the method run time.
- Files names created by the method will overwrite existing files on your hard disk.
- The method requires a sample name or a sample volume that is not available in the system configuration.

If an error message occurs, you can accept or edit the events.

6.4.2 Opening a Method From a Data File

Data files (.B*) that were created by a method run always contain the method used to create them.

NOTE: Data files that you save from the Strip Chart Recorder do not contain a method.

To open the method file associated with a data file:

1. Display the Method Editor by selecting **Method Editor** from the Window menu.
2. Select **Open** from the File menu to display the Open File dialog box.
3. Select **Data Files (*.B*)** from the List Files of Type drop-down list box.

No files are listed because you are looking at the methods directory instead of the data directory.

4. To change the directory, double-click the **biocad** folder in the Directories list box.

All subdirectories in the biocad directory are now displayed.

5. Double-click the **data** folder.

All data files are listed.

6. Select a **data file**, then click **OK**.

If the configuration stored with the method is different from the current system configuration, the Configuration Mismatch dialog box is displayed. For more information, see "Configuration Mismatch" on page 6-84.

7. Click **Do Not Update Method Configuration**.

The Method Editor displays the method associated with the selected data file.

Editing and saving

You can edit a method associated with a data file, but you cannot save it to the same file name. You must save it under a new file name.

6.5 Editing a Method

After you open a method, you can edit, delete, copy, or move method components. After you make changes you can save the method.

Editing To edit a method:

1. Double-click **blocks**, **segments**, or **events** to display the original dialog box used to create the block, segment, or event.

NOTE: *If you adjust the duration of a segment that contains an event at the end of the segment, the software automatically adjusts the timing of the event to the end of the segment. You do not have to manually change the event time to the end of the segment.*

2. Make changes in the dialog box and click **OK**.

Deleting To delete a block, segment, or event:

1. Select the item to delete.
2. Select **Cut Block**, **Cut Segment**, or **Cut Event** from the Edit menu.

NOTE: *When you perform an operation from the Control Panel, such as turning off the pump, an event is added to the method in a data file. This event is flagged with an asterisk (*) and a plus sign (+), and you cannot delete it using Cut Event.*

*To delete this type of event, first save the file as a method file (.MET) by selecting **Save As** from the File menu.*

Copying To copy a block, segment, or event to another point in the method:

1. Select the item to copy.
2. Select **Copy Block, Copy Segment, or Copy Event** from the Edit menu.
3. Click the location in the method where you want to copy the item.
4. Select **Paste Block, Paste Segment, or Paste Event** from the Edit menu.

Moving To move a block, segment, or event from one point to another in the method:

1. Select the item to move.
2. Select **Cut Block, Cut Segment, or Cut Event** from the Edit menu.
3. Click the location in the method where you want to move the item.
4. Select **Paste Block, Paste Segment, or Paste Event** from the Edit menu.

CAUTION

Do not cut or copy another item until you paste the current item into its new location. If you do so, the original item is permanently deleted.

Saving When you have made all changes, save the method:

1. Select **Save As** from the File menu.
The Save File dialog box is displayed.
2. Type a **file name** and click **OK**.
3. To close the Method Editor, double-click the Control Menu box in the top left corner of the window.

6.6 Running a Method

Overview To run a method:

- Load a configuration (optional)
- Open a method
- Specify settings (optional)
- Run the method
- Stop and resume a method (optional)

6.6.1 Loading a Configuration

When to load a configuration The BioCAD 700E Workstation uses the system configuration when you run a method.

To run a method using a configuration other than the current system configuration, load a configuration from a configuration file (.CFG), method file (.MET), or data file (.B*).

NOTE: *If you load a configuration or change the current system configuration while a method is running, the running method and all methods in the Method Queue abort.*

Loading a configuration To load a configuration from a configuration file, method file, or data file:

1. Select **Open Configuration** from the from the Control Panel File menu.

The Open File dialog box is displayed.
2. Select **Config Files (*.CFG)**, **Method Files (*.MET)**, or **Data Files (*.B*)** from the List Files of Type drop-down list box.
3. Double-click the **biocad** folder in the Directories list box to display configuration files (.CFG). Double-click the **methods folder** to display method files (.MET) or double-click the **data folder** to display data files (.B*).

4. Select a **configuration file**, **method file**, or **data file**, then click **OK**.

The configuration settings associated with the selected file are loaded as the system configuration.

5. Check the physical setup of the BioCAD 700E Workstation and make sure it matches the system configuration. If necessary, change the physical configuration or the system configuration as appropriate for the method.

6.6.2 Opening a Method

For information on opening a method, refer to one of the following sections:

- Section 6.4.1, Opening a Method From a Method File
- Section 6.4.2, Opening a Method From a Data File

6.6.3 Specifying Settings

Each method has the following settings saved with it. To change these settings, see the appropriate section:

- **Data Storage Options**—See Section 6.2.10, Specifying Data Storage Options
- **Print Settings**—See Section 6.2.11, Specifying Print Settings
- **Analysis Parameters**—See the *Data Analysis User's Guide*

6.6.4 Running a Method

This section describes:

- Running a method
- Running a method STAT
- Making manual injections
- Viewing the run status

Running a method

You can run a method from two windows:

- **Control Panel**—Select **Run Method** from the File menu, then select the method file (.MET) to run.

NOTE: *If the configuration stored with the method is different from the current system configuration, the Configuration Mismatch dialog box is displayed. For more information, see “Configuration Mismatch” on page 6-84.*

- **Method Editor**—Select **Run Method** from the File menu to run the method currently displayed in the Method Editor window.

Hint: *This is the easiest way to test a new method, because you do not have to save the method.*

NOTE: *When you run a method from the Method Editor, the method uses the settings displayed in the Method Editor window, even if you have not saved the changes.*

Running a method STAT

You can send a method to run as soon as possible by selecting **Run Method Stat** from the File menu in the Control Panel. The method you select is added to the beginning of the Method Queue, and runs as soon as the currently running method is complete.

NOTE: A STAT method runs using the system configuration. If your STAT method requires a different configuration, stop the currently running method, load the appropriate configuration, then run the STAT method.

If a multi-method is in process when you add a method to the beginning of the queue, the method runs as soon as the current method in the multi-method is complete.

Making manual injections

If you are making manual injections, the method pauses at the Inject segment and prompts you to make an injection.

To make the manual injection:

1. Insert the needle port adaptor in port 6 of the injection valve.
2. Load a syringe with your sample.
3. Insert the syringe in the needle port adaptor and inject the sample (see Figure 5-22 on page 5-41).
4. Click **OK** or press the **Event Mark** button on the front panel.

The method resumes.

Viewing the run status

You can view the status of a method run from three windows:

- **Method Queue**—Displays all methods that are waiting to run. For more information, see Section 8.1, Using the Method Queue.
- **Status Window**—Displays information about the pump, detector, fraction collector, and the running method. For more information, see Section 8.2, Using the Status Window.
- **Strip Chart Recorder**—Displays data as it is acquired. Can also display events. For more information, see Section 8.3, Using the Strip Chart Recorder.

6.6.5 Stopping and Resuming a Method

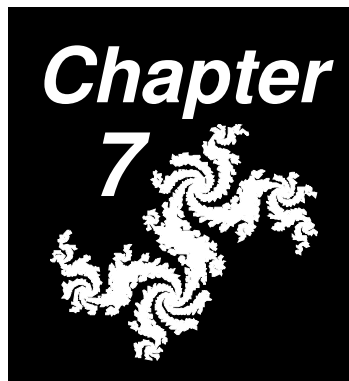
There are three ways to stop a method:

- Hold
- Stop pump
- Abort

To stop a method ...	Description	To resume the method ...
Click the Hold button in the Method section of the Control Panel	Suspends the method and freezes the workstation in its present state, with the pump running. If a gradient is running, conditions do not change. You can perform manual actions from the Control Panel, such as replenishing buffers and priming lines.	Click the Resume button in the Method section of the Control Panel.
Stop the pump by clicking the Stop button in the Pump section of the Control Panel or by pressing the Pump button on the front panel of the workstation	Suspends the method and freezes the workstation in its present state, with the pump off.	Start the pump to resume the method. Click the Start button in the Pump section of the Control Panel or press the Pump button on the front panel of the workstation.
Click the Abort button in the Method section of the Control Panel or click the Abort button in the Method Queue	Clears the method and shuts down the pump. All methods are cleared from the Method Queue. Data files contain data acquired up to the time you click Abort. NOTE: To delete unwanted data files after clicking Abort, use Windows Explorer. For more information, refer to the Microsoft Windows User's Guide.	You cannot resume a method after aborting it. You must run the method again.

6

7 Creating and Running Templates and Multi-Methods



This chapter includes the following sections:

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7.1.3	Linear Gradient Template	7-16
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7.1.5	Auto Cycling Template	7-19
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7.1.7	Analysis Template (Direct Wire Autosampler)	7-28
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7.2.1	Opening an Existing Multi-Method File	7-40
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7.1 Creating and Running Templates

7

This section includes:

- Overview
- Using templates and systematic method development
- Viewing the Method Queue
- Viewing the Status window
- Viewing data files and group files
- Stopping a template

Overview A BioCAD template is a predefined program that you set up to perform a sequence of method runs in which one or more parameters change from run to run.

BioCAD templates include:

- **pH Map**—Changes pH
- **Loading Study**—Changes injection volume
- **Linear Gradient**—Changes gradient slope
- **Flow Rate Study**—Changes flow rate
- **Auto Cycling**—Runs the method multiple times
- **Column Switching**—Changes columns
- **Analysis (Integrated Autosampler)**—Changes the sample that is injected and the number of injections
- **Analysis (Direct Wire Autosampler)**—Documents the autosampler vial number and injection number in the method and data files
- **Step Optimization**—Changes blending conditions for column equilibration and step elution
- **Combinatorial Optimization**—Changes up to six parameters

For more information on the Column Switching template, see the *SCOUT Column Selector User's Guide*. For more information on remaining templates, see the following sections.

Using templates for systematic method development

The following procedure is a general description of how to use templates for systematic method development. For information on creating and running specific templates, see the following sections.

1. Open or create a method as described in Chapter 6, Creating and Running Methods.
2. Select a **template** from the Templates menu in the Method Editor window.

The template dialog box is displayed.

3. Enter **values** for the parameter you wish to vary.
4. Click **Run**.

The method runs multiple times, changing the specified parameter each time.

5. When the template is complete, use the Group Analysis window to examine the data files from the template.
6. Select the data file with the optimized separation, then select **Open Method** from the File menu.

The method that generated the optimized separation is displayed. It contains the optimized run conditions.

7. Set up the next template of interest, run the template, and examine the data files in the Group Analysis window. Select the optimized separation, open the method, and continue with the next template.

Templates and autosampler injections

NOTE: *The following does not apply to the Analysis template.*

When you run a template on a method containing an Autosampler Inject segment, the autosampler injects from the sample vial you select in the Sample list box. If the sample volume in the vial is insufficient to make the injection, the system:

- Automatically injects from another vial with the same sample name and sufficient volume (if one exists)
- If there is no vial with sufficient volume, the software displays a warning. Click **OK** to reduce the injection volume for the current method run of the template. Click **Cancel** to cancel the template.

Viewing the Method Queue

After you start a template run, view the Method Queue to see a listing of all the methods in the template. To view the Method Queue, select **View Method Queue** from the File menu in the Control Panel.

For more information, see Section 8.1, Using the Method Queue.

Viewing the Status window

After you start a template run, view the Status window to see the status of the template run. The Status window indicates that a method is running. It displays the method file name, data file name, and sequence (##) of the currently running method.

For more information, see Section 8.2, Using the Status Window.

Viewing data files and group files

After the template run is complete, use the Group Analysis window to view the data files (.B##) and group file (.GRO) that the template creates.

A *data file* (chromatogram) is created for each method run in the template. All data file names are the same. The data file name is the name you specified for data files before running the template method, with a .B## extension, beginning with B00 and incrementing one digit for each run.

NOTE: *The maximum number of data files you can generate using a template is 100 (.B00 through .B99).*

One *group file* is created for the template run. It contains a list of all data files that the template generates. The group file name is the same as the data file names, but with a .GRO extension.

If you run more than one template with the same data file name, all data files are added to the same group file. New files are added to the end of the group file.

For more information, see the *Data Analysis User's Guide*.

Stopping a template

You can stop a template the same way you stop a method. You can hold the method, stop the pump, or abort the method.

For more information, see Section 6.6.5, Stopping and Resuming a Method.

7.1.1 pH Map Template

Overview A pH Map template allows you to make a sequence of runs, each containing the same eluent gradient. The buffer/solvent pH changes from run to run.

NOTE: The buffer/solvent pH does not change within a run.

The pH Map Template dialog box is shown in Figure 7-1.

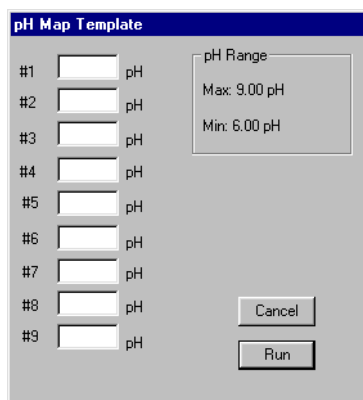


Figure 7-1 pH Map Template

The following sections describe:

- Buffer/Solvent requirements
- Configuration requirements
- Method requirements
- Creating and running a pH Map template

7.1.1.1 Buffer/Solvent Requirements

Before running a pH Map template, prepare four buffers/solvents:

- Two pH blend buffers (anion or cation)
- A salt solution
- Water

There are three types of pH blend mode buffers you can prepare:

- **pH Blend Buffers**—Special anion or cation pH buffers for which the workstation is already calibrated. These buffers exist in the Buffer/Solvent Library. Recipes for these buffers appear on page 3-21. You specify a *pH* value in the pH Map template. The BioCAD software then calculates the percent of each buffer needed to achieve the desired pH.
- **Custom pH Blend Buffers (Calibrated)**—Any other pair of pH buffers can be used for pH blending. You must calibrate custom buffers using the Calibrate pH Mode Buffers feature. (For more information, see Section 5.2.9, Calibrating Custom pH Blend Buffers.) You must add these buffers to the Buffer/Solvent Library. You specify a *pH* value in the pH Map template. The BioCAD software then calculates the percent of each buffer needed to achieve the desired pH.
- **Custom pH Blend Buffers (Uncalibrated)**—Any other pair of pH buffers can be used for pH blending. You must add these buffers to the Buffer/Solvent Library. You specify a *percentage* value in the pH Map template.

7.1.1.2 Configuration Requirements

Before running a pH Map template, configure the four buffers/solvents as follows:

If you are using ...	Then configure ...	As Type ...
pH Blend Buffers	Anion Buffer - pH 6.0 + 100.0 mM Tris/bis-Tris propane OR Cation Buffer - pH 4.5 + 100.0 mM MES/HEPES/Acetate	Buffer
	Anion Buffer - pH 9.0 + 100.0 mM Tris/bis-Tris propane OR Cation Buffer - pH 7.5 + 100.0 mM MES/HEPES/Acetate	Buffer
	NaCl + 3000.0 mM NaCl	Eluent
	H2O	H2O
Calibrated Custom pH Blend Buffers OR Uncalibrated Custom pH Blend Buffers	pH Buffer 1 (anion or cation)	Buffer
	pH Buffer 2 (anion or cation)	Buffer
	Salt solution	Eluent
	Water	H2O
NOTE: For information on configuring buffers/solvents as different types, see Section 4.2.2, Configuring Buffers/Solvents, and Section 4.5.1, Customizing the Buffer/Solvent Library.		



7.1.1.3 Method Requirements

The method requirements for a pH Map template depend on the buffers/solvents you use. The following sections describe pH Map template method requirements for:

- Calibrated pH buffers
- Uncalibrated pH buffers

Using calibrated pH buffers

To run a pH Map template, your method must include at least one segment programmed in pH blend mode. The Table 7-1 lists guidelines for creating a method for use with a pH Map template, using either of the following calibrated pH buffers:

- pH blend buffers
- Calibrated Custom pH buffers

NOTE: *If you use either of the above calibrated pH buffers, you can run one of the sample methods, ANIONPH.MET or CATPH.MET, to run a pH Map template. The sample methods are provided in Appendix D, BioCAD 700E Sample Methods.*

Table 7-1 Method Guidelines for a pH Map Template Using Calibrated pH Buffers

Block	Segment	Settings
General Settings		Appropriate for your application
Equil	Purge	<ol style="list-style-type: none"> 1. Click Setup and select pH blend mode. 2. Set the blend concentration for your application. 3. Set pH within the range of the buffers used. 4. Set flow rate and volume for your application. 5. Set column inline after purge.
	Step (equilibrates)	<ol style="list-style-type: none"> 1. Leave blend settings as is. 2. Set the duration to 10 CV.
	Add Event	Zero UV Detector at 10 CV .
Load	Inject	<ol style="list-style-type: none"> 1. Select a sample channel. 2. Set the injection volume.
Wash	Step	Set a duration appropriate for your application.
Elute	Gradient	<ol style="list-style-type: none"> 1. Leave Blend settings as is. 2. Enter the starting and ending eluent concentrations. For information on valid eluent concentration range, see “pH mode” on page 5-11. 3. Enter a gradient duration. <p>NOTE: <i>The Gradient segment within each method runs an eluent gradient, not a pH gradient.</i></p>
	Step (holds gradient at final conditions)	<ol style="list-style-type: none"> 1. Leave Blend settings as is. 2. Set the duration to 5 CV.

Using uncalibrated pH buffers

To run a pH Map template, your method must include at least one segment programmed in pH blend mode. Table 7-2 lists guidelines for creating a method for use with a pH Map template, using uncalibrated Custom pH buffers.

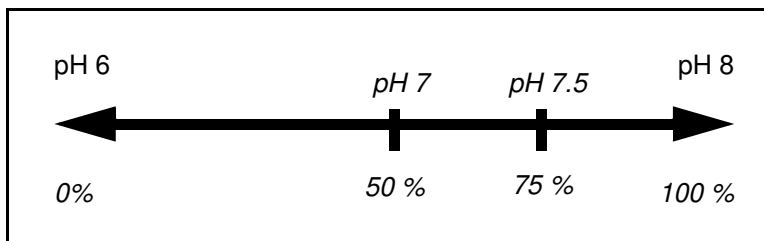
Table 7-2 Method Guidelines for a pH Map Template Using Uncalibrated pH Buffers

Block	Segment	Settings
General Settings		Appropriate for your application
Equil	Purge	<ol style="list-style-type: none"> 1. Click Setup and select pH blend mode. 2. Set the blend concentration for your application. 3. Set a percent (%) value that represents the desired pH value. See “Understanding percent value in pH blending” on page 7-11 for an example of how percent value relates to pH value. 4. Set flow rate and volume for your application. 5. Set column inline after purge.
	Step (equilibrates)	<ol style="list-style-type: none"> 1. Leave blend settings as is. 2. Set the duration to 10 CV.
	Add Event	Zero UV Detector at 10 CV .
Load	Inject	<ol style="list-style-type: none"> 1. Select a sample channel. 2. Set the injection volume.
Wash	Step	Set a duration appropriate for your application.
Elute	Gradient	<ol style="list-style-type: none"> 1. Leave Blend settings as is. 2. Enter the starting and ending eluent concentrations. For information on valid eluent concentration range, see “pH mode” on page 5-11. 3. Enter a gradient duration. <p>NOTE: The Gradient segment within each method runs an eluent gradient, not a pH gradient.</p>

Block	Segment	Settings
Elute (cont'd)	Step (holds gradient at final conditions)	<ol style="list-style-type: none"> 1. Leave Blend settings as is. 2. Set the duration to 5 CV.

Understanding percent value in pH blending

The percent (%) value you enter in a method segment or in the pH Map template corresponds to the pH of the two buffers. For example, you use two buffers of pH 6 and pH 8. To set the pH blend map for pH 7, enter 50% (50% of pH 6 and pH 8 is approximately pH 7). The titration curve of the buffers affects the actual pH achieved.



Below is another example using buffers from pH 4 to pH 5:

Buffers	Enter % ...	To achieve pH ...
pH 4 and pH 5	10%	4.1
	20%	4.2
	30%	4.3

NOTE: Because the software assumes a linear correlation between the two pH buffers, pH values achieved using this method are approximate. The pH Map template is designed to illustrate different chromatographic results at varying pH values, not to deliver precise pH conditions.

7.1.1.4 Creating and Running a pH Map Template

To create and run a pH Map template:

1. Prepare and configure your buffers.

For more information, see Section 7.1.1.1, Buffer/Solvent Requirements, and Section 7.1.1.2, Configuration Requirements.

2. Open the Method Editor by selecting **Method Editor** from any Window menu.
3. Create or open a method by selecting **Open** from the File menu.

For more information, see Section 7.1.1.3, Method Requirements.

NOTE: *If you are using pH blend buffers or calibrated custom pH buffers, you can use one of the sample methods, ANIONPH.MET or CATPH.MET, to run your pH Map template. If you use calibrated custom pH buffers, a Configuration Mismatch dialog box appears, because the buffers in the method do not match the buffers configured. If you click **Yes** to update the method with current system configuration, you can use the sample method to run a pH Map template.*

4. Select **pH Map** from the Template menu.

The pH Map Template dialog box is displayed. The dialog box displayed depends on the type of pH buffers you configured (Figure 7-2).

The screenshot shows a dialog box titled "pH Map Template". On the left, there are nine rows labeled #1 through #9, each with a text input field followed by "pH". On the right, there is a "pH Range" section with "Max: 9.00 pH" and "Min: 6.00 pH". At the bottom right, there are "Cancel" and "Run" buttons.

Using pH Blend Buffers or Calibrated Custom pH Buffers

The screenshot shows a dialog box titled "pH Map Template". On the left, there are nine rows labeled #1 through #9, each with a text input field followed by "%". On the right, there is a "pH Range" section with "Max: 100.00 %" and "Min: 0.00 %". At the bottom right, there are "Cancel" and "Run" buttons.

Using Uncalibrated Custom pH Buffers

Figure 7-2 pH Map Template

5. Type **pH** or **percent (%) values** in the text boxes for each buffer pH value at which you want to run your method.

If you are using ...	Enter ...
pH Blend Buffers	pH values within the range of the buffers used.
Calibrated Custom pH Blend Buffers	pH values within the range of the buffers used.
Uncalibrated Custom pH Blend Buffers	Percent (%) values that represents the desired pH value. See "Understanding percent value in pH blending" on page 7-11 for examples of how percent value relates to pH value.

6. Click **Run** to run the template.

NOTE: When you run a pH Map template and enter a percent instead of a pH value, the actual pH is still measured and plotted in the Strip Chart Recorder and data file.



7.1.2 Loading Study Template

Method requirements

The Loading Study template allows you to change the sample injection volume during multiple runs of the same method.

Your method must contain a Load block with either an Inject (I), Autosampler Inject (AI), or Step (S) segment. The Loading Study template runs on only the first segment in the first Load block in a method.

The following table describes the segment to use with each injection mode.

Injection mode	Method Segment
Autoloader or direct autoloader	Inject (I)
Integrated autosampler	Autosampler Inject (AI)
Pump	Step (S)

Creating and running

To create and run a Loading Study template:

1. Open a method or create a new method as described in Chapter 6, Creating and Running Methods. Make sure your method meets the requirements listed in above.
2. Select **Loading Study** from the Templates menu in the Method Editor window.

The Loading Study Template dialog box is displayed (Figure 7-3). The minimum and maximum loading range is displayed.

NOTE: *If you use a Step segment to make a pump injection, the loading range is unlimited. For other injection modes, the loading range is a function of syringe size, and the injection volume is a function of sample loop size. For example, using a 5 ml syringe and a 2 ml loop allows you to load up to 5 ml, but inject only 2 ml. You can overfill the sample loop. For full loop injections, overfill the sample loop by a factor of three.*

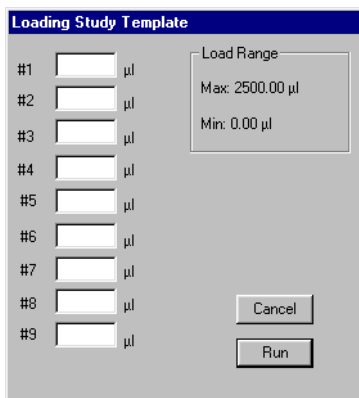


Figure 7-3 Loading Study Template

3. Type up to nine **loading values** in the text boxes.

NOTE: The units used for the loading values depend on the duration units (minutes, ml, μl , or CV) you select in the method segment. For example, if you make a pump injection, and you add a Step segment to a method programmed in minutes, the Loading Study dialog box allows you to enter a loading time (minutes) instead of an injection volume.

4. Click **Run**.
5. If you chose to name files when run in the Data Storage options, type a **data file name** in the Set Data File Name and Directory dialog box and click **OK**.

Each method in the template is executed.

7.1.3 Linear Gradient Template



Method requirements

The Linear Gradient template allows you to change the slope of a gradient elution during multiple runs of the same method.

Your method must contain an Elute block with a Gradient segment. The Linear Gradient template runs on only the first Gradient segment in the first Elute block in a method.

The Linear Gradient template allows you to change the gradient slope during elution by changing time or volume.

Creating and running

To create and run a Linear Gradient template:

1. Open a method or create a new method as described in Chapter 6, Creating and Running Methods. Make sure your method meets the requirements listed in above.
2. Select **Linear Gradient** from the Templates menu in the Method Editor window.

The Linear Gradient Template dialog box is displayed (Figure 7-4). The gradient range is also displayed.

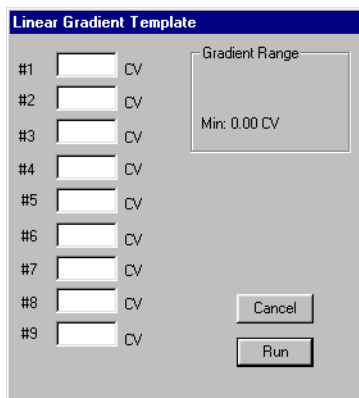


Figure 7-4 Linear Gradient Template

3. Type up to nine **gradient durations** (minutes, ml, or CV) in the text boxes.

NOTE: *The duration units (minutes, ml, or CV) are the same as the duration units you select for the Gradient segment in the method.*

Shorter durations produce gradients with steeper slopes, and longer durations produce more shallow gradients.

4. Click **Run**.
5. If you chose to name files when run in the Data Storage options, type a **data file name** in the Set Data File Name and Directory dialog box and click **OK**.

Each method in the template is executed.

7.1.4 Flow Rate Study Template

The Flow Rate Study template allow you to change the flow rate during multiple runs of the same method.

Creating and running

To create and run a Flow Rate Study template:

1. Open a method or create a new method as described in Chapter 6, *Creating and Running Methods*.
2. Select **Flow Rate Study** from the Templates menu in the Method Editor window.

The Flow Rate Study Template dialog box is displayed (Figure 7-5). The flow rate range is also displayed.

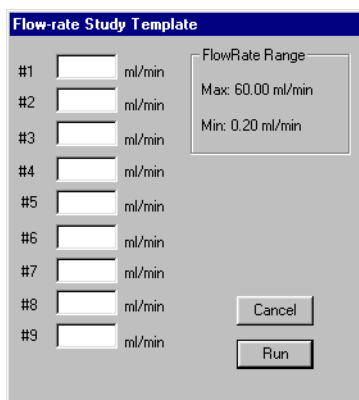


Figure 7-5 Flow Rate Study Template

3. Type up to nine **flow rates** (ml/min) in the text boxes.
4. Click **Run**.
5. If you chose to name files when run in the Data Storage options, type a **data file name** in the Set Data File Name and Directory dialog box and click **OK**.

Each method in the template is executed.

NOTE: The flow rates in the template are substituted for the flow rate in the General Settings. The Flow Rate Study template does not affect flow rate changes triggered by added events in a method.

NOTE: The effect of a change in global flow rate on the method is a function of the units you use to program each segment in the method:
Segments programmed in volume (ml) or column volume (CV) execute more quickly as the flow rate increases.
Segments programmed in time (minutes) require a greater volume of liquid as flow rate increases.

7.1.5 Auto Cycling Template

The Auto Cycling template allows you to run the same method a number of times. It is useful in preparative chromatography to cycle a method repetitively and pool the product fractions.

Creating and running

To create and run an Auto Cycling template:

1. Open a method or create a new method as described in Chapter 6, Creating and Running Methods.
2. Select **Auto Cycling** from the Templates menu in the Method Editor window.

The Auto Cycling Template dialog box is displayed (Figure 7-6).

7

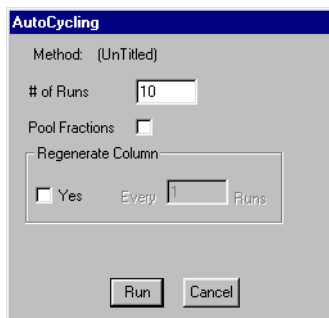


Figure 7-6 Auto Cycling Template

3. In the **# of Runs** text box, type the number of times to run your method.
4. If you want to use the template to pool the product fractions, click the **Pool Fractions** check box to reset the fraction collector after each run.

NOTE: *If the Pool Fractions check box is dimmed, add a Start Fraction Collection event to your method.*

5. If you want to override the Clean block in the method, click **Yes** under Regenerate Column and enter the **number of runs** between cleanings. If you do not select Yes, the Clean block runs every time the method runs.

NOTE: *If the Regenerate Column section is dimmed, add a Clean block and segment to your method.*

6. Click **Run**.
7. If you chose to name files when run in the Data Storage options, type a **data file name** in the Set Data File Name and Directory dialog box and click **OK**.

Each method in the template is executed.

7.1.6 Analysis Template (Integrated Autosampler)

This section describes:

- Overview
- System and method requirements
- Creating and running
- Example method for Analysis Template

7.1.6.1 Overview

The Analysis template (when used with an integrated autosampler) allows you to change the sample injected and the number of injections during multiple runs of the same method.

The Analysis template automatically substitutes and injects from different sample vials in Autosampler Inject segments. If your integrated autosampler supports sample preparation, the template can also substitute sample vials in Prep blocks.

Applications

Use the Analysis template for the following applications:

- **Analysis**—Inject multiple samples using the same method (Figure 7-7).
- **Preparation**—Inject multiple samples that require sample preparation using the same method. Your autosampler must support sample preparation (Figure 7-8). See example on page 7-26.
- **Analysis and Preparation**—Prepare multiple samples for incubation using one method, and then inject these samples using another method. Your autosampler must support sample preparation (Figure 7-9).

How the template works

You specify which samples to substitute (maximum of 100) in the Analysis template. When you run your method, the system substitutes these samples for the first sample specified in your method, either in an Autosampler Inject segment or Prep block.

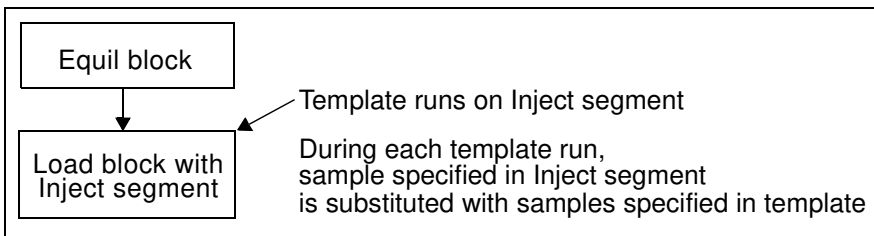


Figure 7-7 Using Analysis Template for Analysis

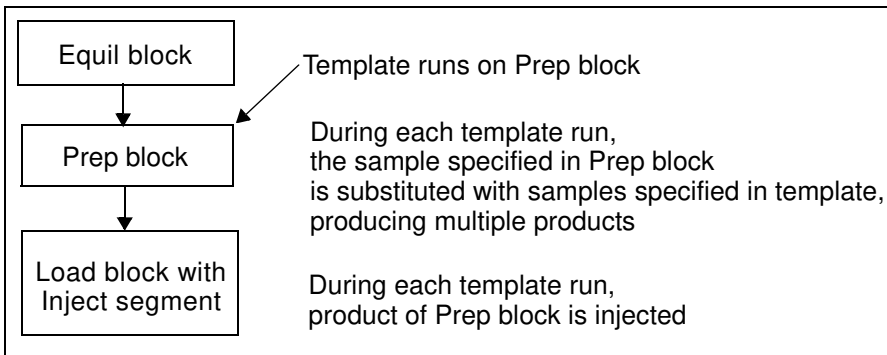


Figure 7-8 Using Analysis Template for Preparation

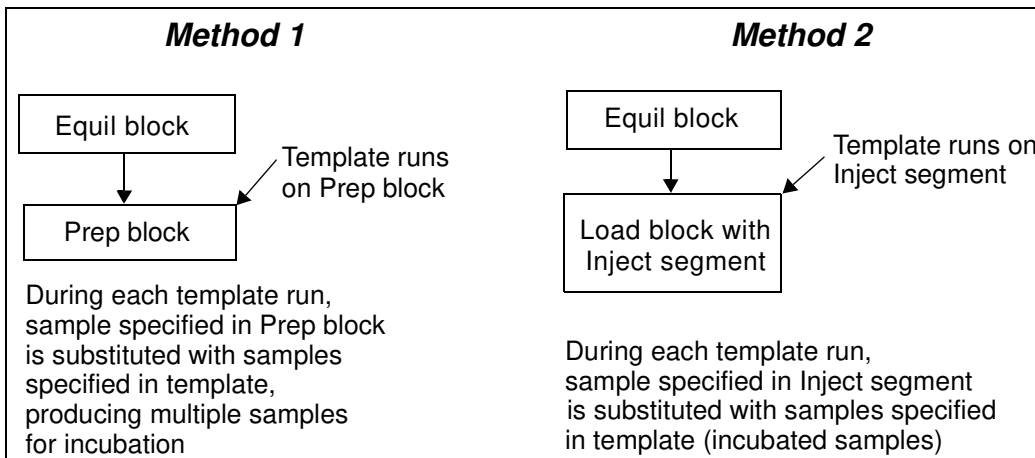


Figure 7-9 Using Analysis Template for Analysis and Preparation (Requires Two Methods)

7.1.6.2 System and Method Requirements

Your system and method must meet the following requirements for each application.

Application	System and Method Requirements
<p>Analysis—Inject multiple samples using the same method (Figure 7-7)</p>	<ul style="list-style-type: none"> • An AS 3500 integrated autosampler is configured • Sample vials are loaded in the AS 3500 autosampler and entered in the Sample Table • Method contains a Load block with an Autosampler Inject segment
<p>Preparation—Inject multiple samples that require sample preparation using the same method (Figure 7-8)</p> <p><i>NOTE: An example of using the Analysis template for this application is shown on page 7-26.</i></p>	<ul style="list-style-type: none"> • An AS 3500 integrated autosampler that supports the sample preparation option is configured • Sample vials are loaded in the AS 3500 autosampler and entered in the Sample Table • Method contains a Prep block followed by a Load block with an Autosampler Inject segment • Autosampler Inject segment injects the product (Derived vial) of the Prep block <p><i>NOTE: If you add a Combine segment to the Prep block, the first sample you add to the Combine Segment Setup dialog box is the sample that is substituted during each template run.</i></p>
<p>Analysis and Preparation— Prepare multiple samples for incubation using one method, and then inject these samples using another method (Figure 7-9)</p>	<ul style="list-style-type: none"> • An AS 3500 integrated autosampler that supports the sample preparation option is configured • Sample vials are loaded in the AS 3500 autosampler and entered in the Sample Table • First method contains a Prep block that prepares sample for incubation • Second method contains a Load block with an Autosampler Inject segment that injects the product (Derived vial) of the Prep block



7.1.6.3 Creating and Running

To create and run an Analysis template:

1. Prepare your system according to the requirements listed in Section 7.1.6.2, System and Method Requirements.
2. Open a method or create a new method as described in Chapter 6, Creating and Running Methods. Make sure your method meets the requirements listed in Section 7.1.6.2, System and Method Requirements.
3. Select **Analysis** from the Templates menu in the Method Editor.

The Analysis template dialog box is displayed (Figure 7-10). The Samples list includes all sample vials you entered in the Sample Table as Sample, Standard, Control, or Reagent. See Section 4.7.3, Entering Vials in Sample Table.

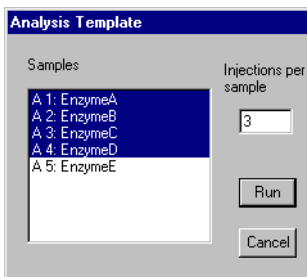


Figure 7-10 Analysis Template Dialog Box

4. Select the **sample vials** to inject or prepare.

If your method contains an Autosampler Inject segment first, it *injects* the samples you select. If your method contains a Prep block first, it *prepares* the samples you select.

To select multiple sample vials:

- Press and hold the Shift key, then click samples to select a range of contiguous samples
 - Press and hold the Control key, then click samples to select noncontiguous samples.
5. Enter the **number of injections** or **number of sample preparations** for the selected sample vials.
 6. Click **Run**.
 7. If you chose to name files when run in the Data Storage options, type a **data file name** in the Set Data File Name and Directory dialog box and click **OK**.

The method runs repeatedly to inject or prepare the samples you specified, the number of times you specified. The maximum number of runs is 100.

For example, the template illustrated in Figure 7-10 runs the method 12 times. The first three runs inject or prepare sample A1, the next three runs sample A2, the next three runs sample A3, and the last three runs sample A4.

NOTE: When you run an Analysis template, the autosampler injects from the vial you select in the Analysis Template dialog box. If the sample volume in the vial is insufficient to make the injection, a warning is displayed. Click **OK** to cancel the current method and continue the template.

NOTE: If your method has a Prep block, include enough empty vials in the autosampler tray and in the Sample Table to allow for all the Derived vials the template creates.

7.1.6.4 Example Method for Analysis Template

Figure 7-11 illustrates an example method for using the Analysis template to inject multiple samples that require sample preparation. The method contains a Prep block followed by an Inject segment.

Prep block

The Prep block contains a Combine segment that combines two samples and places the product in the first empty vial entered in the Sample Table (in this example, vial A7). Vial A7 becomes a Derived vial and contains the product of the Prep block. The vial that receives the product of the Prep block changes with each template run.

Autosampler Inject segment

The Autosampler Inject segment injects the product of the Prep block.

NOTE: To instruct an Autosampler Inject segment to inject the product of a Prep block, click the Derived option button in the Autosampler Inject Segment Setup dialog box. Select the sample vial that has the name of the Prep block. For more information, see “Autosampler Inject Segments” on page 6-30.

In first template run, Prep block combines sample A1 and sample B1, creating sample A7

In next template run, first sample specified in Analysis Template (sample A2) is substituted for sample A1

Elapsed	Block	Event
		General Settings: Set Column 1 Inline
		General Settings: UV Detector Wavelength = 254 nM
		General Settings: Flow Rate = 1.00 ml/min
		Pump On
0.00 min		Method Start
0.00 min	[01]	Equil Block
0.00 min	(A)	Step Segment
0.00 min		0.00 min Set Solvent Blend 100% A
1.00 min		1.00 min End Solvent Blend 100% A
1.00 min		
1.00 min	[02]	Prep Block : 300.0 µl : Expire after 24.0 Hr
	(A)	Combine Segment
		Pickup Sample: 60.0 µl of A 1: Sample
		Pickup Sample: 240.0 µl of B 1: Antibody/Enzyme Conjugate
		Dispense Sample: 300.0 µl to Enzyme 20.00 % v/v + Conjugate 80.00 % v/v
1.00 min	[03]	Load Block
1.00 min	(A)	Inject Segment
1.00 min		0.00 min Load Sample through AutoSampler: 50.0 µl of A7 : Prep Block 2
2.25 min		1.25 min Inject Sample through AutoSampler
2.25 min		1.25 min Flush AutoSampler with 430 µl of Flush solvent
2.25 min		
2.25 min	[04]	Wash Block

Figure 7-11 Example Method for Analysis Template

If you create and run the Analysis template shown in Figure 7-10, the next sample specified by the template is substituted for the first sample listed in the Prep block. The Autosampler Inject segment injects the product of the Prep block:

Template Run	Prep block with Combine segment combines samples	Autosampler Inject segment injects product of Prep block
First	A1 + B1	A7
Second	A1 + B1	A8
Third	A1 + B1	A9
Fourth	A2 + B1	A10
Fifth	A2 + B1	A11
Sixth	A2 + B1	A12
Seventh	A3 + B1	A13
Eighth	A3 + B1	A14
Ninth	A3 + B1	A15
Tenth	A4 + B1	A16
Eleventh	A4 + B1	A17
Twelfth	A4 + B1	A18

NOTE: Only the first sample that you select in the Prep block dialog box is substituted with each run. This sample is not necessarily the first sample listed in the method Prep block.

You can use this combination of Prep block and Analysis template to incubate the same antibody/enzyme conjugate (B1) with each sample (A1, A2, A3).



7.1.7 Analysis Template (Direct Wire Autosampler)

The Analysis template (when used with a direct wire autosampler) allows you to document the vial number and injection number during multiple runs of the same method.

NOTE: *The Analysis template does not change the vial number or number of injections. You specify these parameters using the controls on the direct wire autosampler.*

Hardware requirements

Connect and prepare a direct wire autosampler, including programming the autosampler method and sample set using the autosampler controls. For more information, see “Connecting direct wire autosampler” on page 2-22, and Section 3.6, Preparing an Autosampler.

Configuration requirements

Configure and prepare the BioCAD software for a direct wire autosampler before turning on the autosampler. For information, see Section 4.8, Configuring and Preparing the BioCAD Software for a Direct Wire Autosampler.

Method requirements

Your method must contain a Load block with a Step segment and an Autosampler Inject and Data Collection Start event. For more information, see Section 6.2.6, Adding Events, and Section 6.3.7, Making Direct Wire Autosampler Injections in a Method.

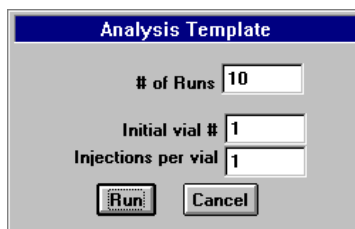
Creating and running

To create and run an Analysis template with a direct wire autosampler:

1. Verify that the BioCAD 700E Workstation and the direct wire autosampler meet the hardware and configuration requirements described above. Start the autosampler. (If you are using an AS 3500 autosampler from Applied Biosystems, press **Run** on the front panel to start it.)
2. Open a method or create a new method as described in Chapter 6, Creating and Running Methods. Verify that your method meets the requirements listed in above.

3. Select **Analysis** from the Templates menu in the Method Editor window.

The Analysis Template dialog box is displayed (Figure 7-12).



**Figure 7-12 Analysis Template
(Direct Wire Autosampler)**

4. Enter the **Number of runs** (number of vials x injections per vial), the **Initial vial number**, and the **Injections per vial**. Use the same initial vial number and injections per vial that you specified in the autosampler method and sample set (programmed using the autosampler controls).

NOTE: *The Analysis template does not control the initial vial number or the injections per vial. You must specify these parameters using the controls on the direct wire autosampler.*

5. Click **Run**.
6. If you chose to name files when run in the Data Storage options, type a **data file name** in the Set Data File Name and Directory dialog box and click **OK**.

Each method in the template runs. The vial number and injection number information from the Analysis template is documented in the method stored with each data file (chromatogram) and in the title bar of each data file.

7.1.8 Step Optimization Template

The Step Optimization template allows you to change the eluent concentration of the buffer/solvent blend during multiple runs of the same method.

You use the Step Optimization template to optimize blending conditions for:

- Column equilibration (loading conditions)
- Step elution

This template is useful when developing preparative methods and for process validation.

Method requirements

Your method must contain one or both of the following:

- Equil block with a Step segment programmed in pH or Eluent blend mode
- Elute block with a Step segment programmed in pH or Eluent blend mode

NOTE: The Step Optimization template affects only the first Equil and the first Elute block in a method. However, you can change multiple Step segments within the Elute block.

Creating and running

To create and run a Step Optimization template:

1. Open a method or create a new method as described in Chapter 6, Creating and Running Methods. Make sure your method meets the requirements listed in above.
2. Select **Step Optimization** from the Templates menu in the Method Editor window.

The Step Optimization Template dialog box is displayed (Figure 7-13).

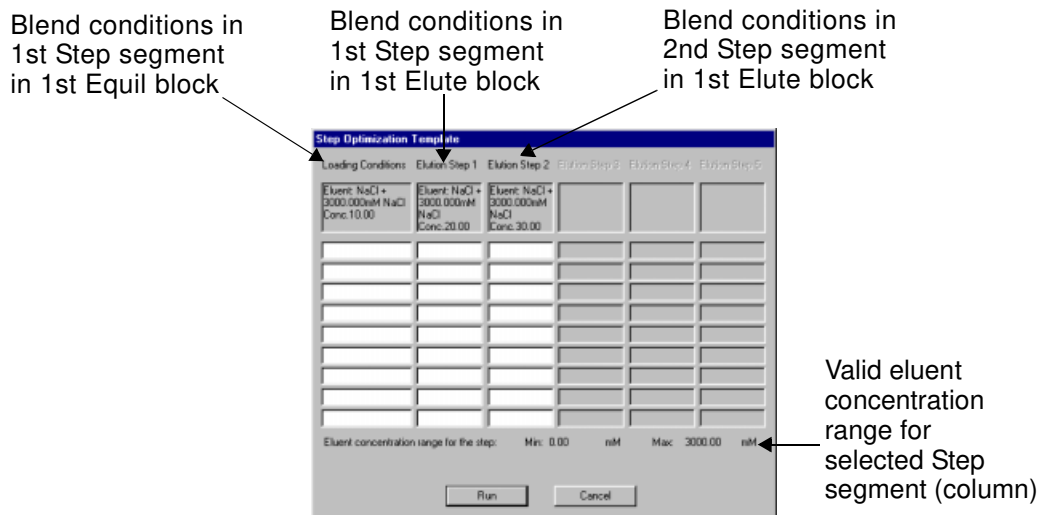


Figure 7-13 Step Optimization Template

The Step Optimization Template dialog box contains six columns. Each column represents a Step segment:

- **Loading Conditions**—Blend conditions in the first Step segment programmed in pH or Eluent blend mode in the first Equil block. This column is blank if the method does not contain a Step segment programmed in pH or Eluent blend mode in an Equil block.
- **Elution Steps**—Blend conditions in the first five Step segments programmed in pH or Eluent blend mode in the first Elute block. These columns are blank if the method does not contain any Step segments programmed in pH or Eluent blend mode in an Elute block.

3. In each row, enter an **eluent concentration** (mM) for each Step segment (column) represented in the template.

For information on valid eluent range, see “Valid eluent concentration” on page 7-32.

Valid eluent concentration

Any fields that you leave blank will use the initial eluent concentration displayed at the top of the column.

4. Click **Run**.
5. If you chose to name files when run in the Data Storage options, type a **data file name** in the Set Data File Name and Directory dialog box and click **OK**.

A method is created and executed for each row in the template.

The minimum and maximum eluent concentration that you can enter for each Step segment (column) is displayed at the bottom of the dialog box when you click on each column.

Valid eluent concentration range for each Step segment is determined by:

- Requested concentration for the blend entered in the Step segment
- Initial concentration of the buffers entered in the Buffer/Solvent Library
- Initial concentration of the eluent entered in the Buffer/Solvent Library

For example, assume the initial concentration of each buffer is 200 mM. If you set the blend concentration to 100 mM of buffer, the system uses 50 percent of the buffer to achieve this concentration ($100 \text{ mM} / 200 \text{ mM} = 50 \text{ percent}$).

Assume the initial concentration of the eluent is 3,000 mM. If 50 percent of the final solution is made up of buffer, the remaining 50 percent is made up of eluent and water. The valid range for the eluent in this example is 0 to 1,500 mM ($50 \text{ percent of } 3,000 \text{ mM} = 1,500 \text{ mM}$).

Blank fields

If you leave any fields in a row blank, the initial eluent concentration displayed at the top of the column is used when you run the template. The next time you open the Step Optimization template, fields that you left blank display the initial eluent concentration in parentheses (Figure 7-14).

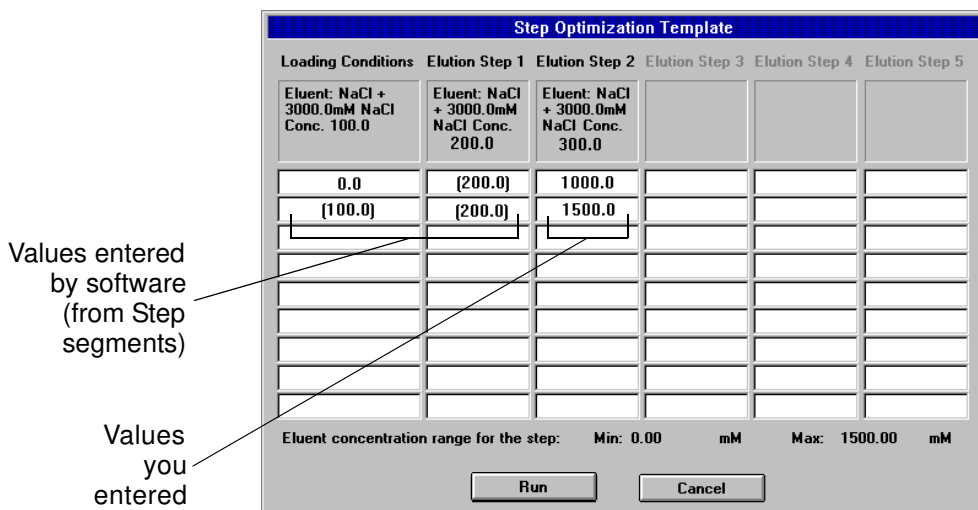


Figure 7-14 Blank Fields in Step Optimization Template

Gradient segments in the method

If a gradient segment programmed in pH or Eluent blend mode follows any of the Step segments listed in the template, the software automatically adjusts the initial gradient conditions to match the conditions of the preceding Step segment.

If a gradient segment programmed in pH or Eluent blend mode precedes any of the Step segments listed in the template, the software automatically adjusts the final gradient conditions to match the conditions of the following Step segment.

If a gradient segment programmed in pH or eluent mode is between two Step segments listed in the template, the software automatically adjusts initial and final conditions to match the conditions of the preceding and following Step segments.

Viewing results

After you start a Step Optimization template, you can view the status of the template methods in the Method Queue. For each method, the first eluent concentration that you entered in each row in the template, is displayed in the Label column in the Method Queue.

NOTE: Eluent concentrations automatically entered by the software are not displayed in the Label column in the Method Queue.



7.1.9 Combinatorial Optimization Template

The Combinatorial Optimization template combines the functionality of six templates in one template.

The six parameters you can systematically vary with the Combinatorial Optimization template include:

- **pH**—Changes pH, comparable to pH Map template
- **Sample Load**—Changes injection volume, comparable to Loading Study template
- **Flow Rate**—Changes flow rate, comparable to Flow Rate template
- **Gradient Duration**—Changes gradient slope, comparable to Linear Gradient template
- **Column**—Changes column position on the SCOUT Column Selector, comparable to Column Switching template
- **Cycling**—Changes number of runs, comparable to Auto Cycling template

The Combinatorial Optimization template allows you to enter values for any or all of the above six parameters, then generates methods based on these values. You can create a sequence of methods, and vary one or more parameters from method to method.

The following sections describe:

- Method and system requirements
- Creating and running
- Viewing results

7.1.9.1 System and Method Requirements

Your system and method must meet the following requirements if you want to change the corresponding parameter in the Combinatorial Optimization template:

Parameter	Method and System Requirements
pH	Method must have at least one Step segment or Gradient segment programmed in pH mode
Sample Load	Method must have a Load block containing an Inject segment or Step segment
Flow Rate	None
Gradient Duration	Method must have an Elute block containing a Gradient segment
Column	System must have a SCOUT Column Selector configured
Cycle	None

NOTE: For information on method blocks and segments, see Chapter 6, Creating and Running Methods.

7.1.9.2 Creating and Running

To create and run a Combinatorial Optimization template:

1. Open a method or create a new method as described in Chapter 6, Creating and Running Methods. Make sure your method meets the requirements listed in Section 7.1.9.1, System and Method Requirements.
2. Select **Combinatorial Optimization** from the Templates menu in the Method Editor window.

The Combinatorial Optimization dialog box is displayed, with a table containing a column for each of the six parameters (Figure 7-15).

NOTE: If minimum, maximum, and default values appear at the top of a parameter column, the method and system requirements are met for the parameter, and you can control the parameter.

If no values appear at the top of a parameter column, then the method and system requirements are not met for the parameter, and you cannot control the parameter.

Parameter is not available if no values appear here

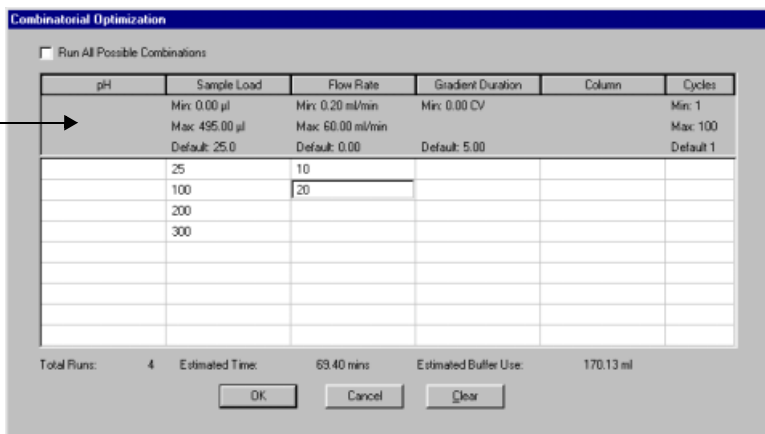


Figure 7-15 Combinatorial Optimization Dialog Box

3. Select or deselect **Run All Possible Combinations**.

Hint: *Select Run All Possible Combinations if you want to enter parameters and let the software create all possible methods.*

If you *select* Run All Possible Combinations:

- One method is generated for each possible combination of the values entered in the table.
- The maximum number of methods and runs is 100.
- The Cycles parameter is not available.

For example, if you select Run All Possible Combinations, as in Figure 7-15, the template generates eight methods, four combining each of the four sample load volumes with the 5 ml/min flow rate, and four combining each of the four sample load volumes with the 10 ml/min flow rate.

Hint: *Deselect Run All Possible Combinations if you want to specify parameters for each method individually.*

If you *deselect* Run All Possible Combinations:

- One method is generated for each row of the table that contains any values.
- The maximum number of different methods is nine.
- The maximum total number of runs is 100. (Each method runs the number of times specified by the Cycles parameter.)
- More than one value can change from method to method.
- Default values are used when table cells have no values entered in them.

For example, if you deselect Run All Possible Combinations in Figure 7-15, the template generates four methods, one for each row containing values.

4. Type **values** in the table under the appropriate parameter.

NOTE: The units and default values for each parameter, displayed at the top of each column, are based on the current method.

To navigate through the table, click on a cell to select it, press **Tab** to move right, press **Enter** to move down, or use the arrow keys.

The following table describes the impact of each parameter on the method:

Parameter	Units	Impact on Method
pH	pH or %	The blend conditions of any segments programmed in pH mode will change to the table value.
Sample Load	<ul style="list-style-type: none"> • μl or ml (Inject segment) • minutes, ml, CV (Step segment) 	The sample volume of the first Inject or Step segment in the first Load block will change to the table value.
Flow Rate	<ul style="list-style-type: none"> • ml/min • cm/hr • CV/min 	The General Settings flow rate will change to the table value.
Gradient Duration	minutes, ml, or CV	The duration of the first Gradient step in the first Elute block will change to the table value.
Column	column position on SCOUT (A–F)	The column position in General Settings and in any Purge blocks will change to the table value.
Cycle	number	The method runs the number of times you specify.

NOTE: As you enter values, the *Total Runs*, *Estimated Time*, and *Estimated Buffer Use* are updated and displayed at the bottom of the dialog box. Check this information and verify that you are prepared for the time and buffer volumes required to run the template methods.

5. Click **OK**.
6. If you chose to name files when run in the Data Storage options, type a **data file name** in the Set Data File Name and Directory dialog box and click **OK**.

Each method in the template runs.

Saving template values

The values you enter in the Combinatorial Optimization table are saved after the template runs. These values appear when you re-open the Combinatorial Optimization template.

Clearing template values

To clear all values from the Combinatorial Optimization table, click **Clear**.

7.2 Creating and Running Multi-Methods

7

The multi-method feature in the Method Editor allows you to create your own custom templates by:

- Making sequential modifications to a method and saving the methods in a multi-method file
- Saving a group of different methods together in a multi-method file

When you run a multi-method, all methods in the multi-method file run. The multi-method allows you to run methods sequentially without attending the workstation.

The following sections describe:

- Opening an existing multi-method file
- Creating a new multi-method file
- Adding method files to a multi-method file
- Running a multi-method

7.2.1 Opening an Existing Multi-Method File

To open an existing multi-method file (.GMM):

1. Select **Method Editor** from the Window menu to display the Method Editor window.
2. Select **Open Multi Method** from the Multi-Method menu.

The Open File dialog box is displayed.

3. Select a **multi-method file** (.GMM), then click **OK**.

The multi-method file name is displayed in the title bar of the Method Editor.

4. To view the method files in the multi-method, select **Edit Multi Method** from the Multi Method menu.

7.2.2 Creating a New Multi-Method File

To create a multi-method file (.GMM):

1. Select **Method Editor** from the Window menu to display the Method Editor window.
2. Select **New Multi Method** from the Multi-Method menu.

The Save File dialog box is displayed.

3. Type a **file name** for the multi-method, then click **OK**.

The Method Editor window is displayed with the Multi-method file name and a .GMM extension listed on the title bar. The Multi-Method button is now available at the top of the Method Editor window.

4. Add methods to your multi-method file. See Section 7.2.3, Adding Methods to a Multi-Method.

7.2.3 Adding Methods to a Multi-Method

You can add methods to the multi-method in two ways:

- Click the Multi-Method button
- Select Edit Multi Method from the Multi Method menu

Hint: *If you want to make sequential modifications to a method and save the methods in a multi-method, use the Multi-Method button to add methods to a multi-method file.*

If you want to save a group of different methods together in a Multi-method file, use the Edit Multi Method command.

7.2.3.1 Using the Multi-Method Button to Add Methods to a Multi-Method

Use the Multi-Method button to add methods to a multi-method if you want to make sequential modifications to a method and save the resulting methods.

To add methods to a multi-method:

1. Create a new Multi-method, as described in Section 7.2.2, Creating a New Multi-Method File.
2. Open a method by selecting **Open** from the File menu.
3. Click the **Multi Method** button at the top of the Method Editor to add the current method to the multi-method.
4. Modify the current method, or open a new method and add to the multi-method by clicking the **Multi Method** button.

Each method is added to the multi-method under its original file name and a .G## extension, starting with .G00 and incrementing one digit for each method with the same file name.

5. To view the method files you added to the multi-method, select **Edit Multi Method** from the Multi Method menu.

Example For example:

1. In the Method Editor window, select **New Multi Method** from the Multi Method menu to display the Save File dialog box.
2. Type **Test** in the File Name text box and click **OK**.
3. Open METHOD0A. Click the **Multi Method** button.
METHOD0A.G00 is added to TEST.GMM.
4. Change the **flow rate** in METHOD0A. Click the **Multi Method** button.
METHOD0A.G01 is added to TEST.GMM.

5. Change the **wavelength** in METHODDA. Click the **Multi Method** button.
METHODDA.G02 is added to TEST.GMM.
6. Open METHODDB. Click the **Multi Method** button.
METHODDB.G00 is added to TEST.GMM.
7. Change the **flow rate** in METHODDB. Click the **Multi Method** button.
METHODDB.G01 is added to TEST.GMM.
8. Change the **wavelength** in METHODDB. Click the **Multi Method** button.
METHODDB.G02 is added to TEST.GMM.
9. Select **Edit Multi Method** from the Multi Method menu to view the methods you added to the new multi-method.
The Multi-Method dialog box is displayed (Figure 7-16).

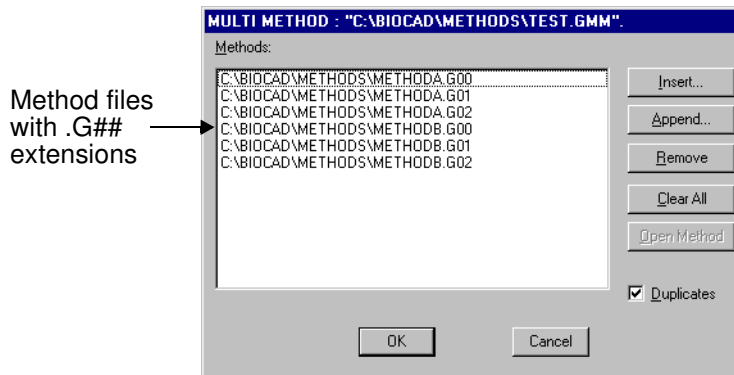


Figure 7-16 Methods Added to a Multi-Method

NOTE: A multi-method is automatically saved every time you make changes to it.

7.2.3.2 Using Edit Multi-Method to Add Methods to a Multi-Method

Use the Edit Multi Method command to add methods to a multi-method if you want to save a group of different methods together in a multi-method file.

To add methods to a multi-method:

1. Open or create a multi-method as described in Section 7.2.1, Opening an Existing Multi-Method File, or Section 7.2.2, Creating a New Multi-Method File.
2. Select **Edit Multi Method** from the Multi Method menu.

The Edit Multi Method dialog box is displayed (Figure 7-17).

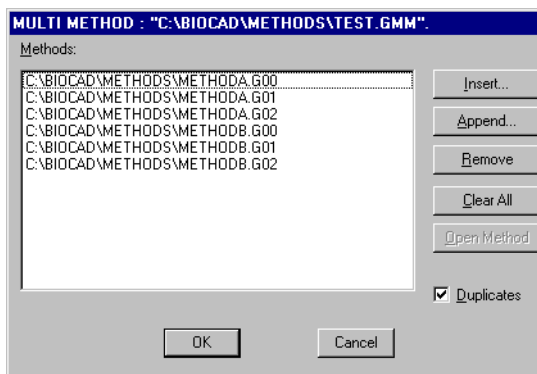


Figure 7-17 Edit Multi Method Dialog Box

3. Click the following buttons to add, arrange, and remove methods in the multi-method:
 - **Insert**—Displays the Open File dialog box. Select a **method** and click **OK**. Inserts the method you select above the current line, using an .MET extension.
 - **Append**—Displays the Open File dialog box. Select a **method** and click **OK**. Adds the method you select to the end of the method list, using an .MET extension.
 - **Remove**—Removes the selected method from the multi-method.
 - **Clear All**—Removes all methods from the multi-method.

NOTE: *When you use Insert or Append to add methods to a multi-method, you do not get a Configuration Mismatch warning until you run the multi-method.*

4. To view the contents of a method listed in the multi-method, select the method, then click the **Open Method** button.
5. To load the same method twice, enable the **Duplicates** check box before using the Insert or Append commands.
6. Click **OK** when you have added all methods to the multi-method.
7. To close the Multi-Method window, double-click the Control menu at the top left corner of the Method Editor window.

NOTE: *A multi-method is automatically saved every time you make changes to it.*

7.2.4 Running a Multi-Method

To run a multi-method:

1. Select **Method Editor** from the Window menu to display the Method Editor window.
2. Open or create a multi-method as described in Section 7.2.1, Opening an Existing Multi-Method File, or Section 7.2.2, Creating a New Multi-Method File.
3. Select **Run Multi Method** from the Multi Method menu.

The Set Data File Name and Directory dialog box is displayed.

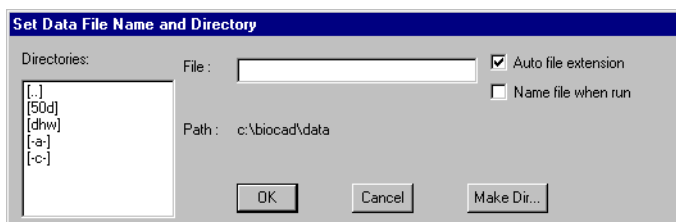


Figure 7-18 Set Data File Name and Directory

4. Type the **file name** for the data files the multi-method will create. The maximum number of characters in the file name is eight, not including the extension.
5. Click **OK**.

Each method in the multi-method runs.

Viewing the Method Queue

After you start a multi-method run, view the Method Queue to see a listing of all the methods in the multi-method. To view the Method Queue, select **Method Queue** from the File menu in the Control Panel.

For more information, see Section 8.1, Using the Method Queue.

Viewing the Status window

After you start a multi-method run, view the Status window to see the status of the multi-method run. The Status window indicates that a method is running. It displays the method file name, data file name, and sequence (##) of the currently running method.

For more information, see Section 8.2, Using the Status Window.

Viewing data files and group files

After the multi-method run is complete, use the Group Analysis window to view the data files (.B##) and group file (.GRO) that the multi-method creates.

A *data file* (chromatogram) is created for each method run in the multi-method. All data file names are the same. The data file name is the name you specified for data files before running the multi-method, with a .B## extension, beginning with B00 and incrementing one digit for each run.

NOTE: *The maximum number of data files you can generate using a multi-method is 100 (.B00 through .B99).*

One *group file* is created for the multi-method run. It contains a list of all data files that the multi-method generates. The group file name is the same as the data file names, but with a .GRO extension.

For more information, see the *Data Analysis User's Guide*.

Stopping a multi-method

You can stop a multi-method the same way you stop a method. You can hold the method, stop the pump, or abort the method.

For more information, see Section 6.6.5, Stopping and Resuming a Method.

7

8 Viewing Results and Checking Status



This chapter includes the following sections:

8.1	Using the Method Queue	8-2
8.2	Using the Status Window	8-4
8.3	Using the Strip Chart Recorder	8-7
8.4	Using the Timer.....	8-14
8.5	Using the Event Log.....	8-15

8.1 Using the Method Queue

After you start a method, template, or multi-method, each method is loaded into the Method Queue (Figure 8-1). To display the Method Queue, select **View Method Queue** from the File menu in the Control Panel.

Click-drag
divider line
to widen
Label column

Parameters that
define each run

Click and hold
right scroll arrow
to scroll right

Queued Methods			
Method	Group File	Data File	Label
ANIONST	test1	c:\biocad\data\test1.B01	PL30.00 ml, FR 5.00 ml/min
ANIONST	test1	c:\biocad\data\test1.B02	PL30.00 ml, FR 10.00 ml/min
ANIONST	test1	c:\biocad\data\test1.B03	PL50.00 ml, FR 5.00 ml/min
ANIONST	test1	c:\biocad\data\test1.B04	PL50.00 ml, FR 10.00 ml/min
ANIONST	test1	c:\biocad\data\test1.B05	PL100.00 ml, FR 5.00 ml/min
ANIONST	test1	c:\biocad\data\test1.B06	PL100.00 ml, FR 10.00 ml/min
ANIONST	test1	c:\biocad\data\test1.B07	PL200.00 ml, FR 5.00 ml/min
ANIONST	test1	c:\biocad\data\test1.B08	PL200.00 ml, FR 10.00 ml/min

Figure 8-1 Method Queue

The Method Queue contains the following column headings:

- **Method**— Displays the method file name.
- **Group File**— Displays the group file name (.GRO) if you run methods using a template or multi-method. This column is blank if you run a single method.
- **Data File**— Displays the data file name each method creates.
- **Label**— If running a single method, displays the injection volume and the sample name. If running a template or a multi-method, displays the parameters that define each run.

Label column The following table lists the abbreviations used for each template parameter in the Label column of the Method Queue:

Template Parameter	Abbreviation in Label Column of Method Queue
pH	pH
Sample Load	SL (autoloader or autosampler injection) PL (pump injection)
Flow Rate	FR
Gradient Duration	GD
Column	CS
Cycle	#
Step Optimization	ST

If the parameter information in the Label column of the Method Queue is not all visible, you can:

- Scroll right by clicking and holding the right scroll arrow at the bottom of the Method Queue to display all the information.
- Widen the Label column by positioning the pointer on the divider line between the Data File column and the Label column. Click-drag the divider line to the left.

Printing the Method Queue

Print the Method Queue by clicking the **Print** button at the bottom of the dialog box. Printing the Method Queue provides a record of which parameters correspond to each data file. The parameters are also displayed in the title bar of each data file.

Removing methods from the Method Queue

To remove a method from the Method Queue, select the method, then click **Delete**. To remove all methods, click **Abort**.

The Abort button has the same function as the Abort button in the Control Panel. Clicking Abort deletes all methods in the Method Queue and stops the pump.

8.2 Using the Status Window

The Status window (Figure 8-2) initially appears to the right of the Control Panel. You can position it anywhere on the screen. Sometimes it is useful to keep the Status window in the corner of the screen so you can see it while you are performing other software operations.

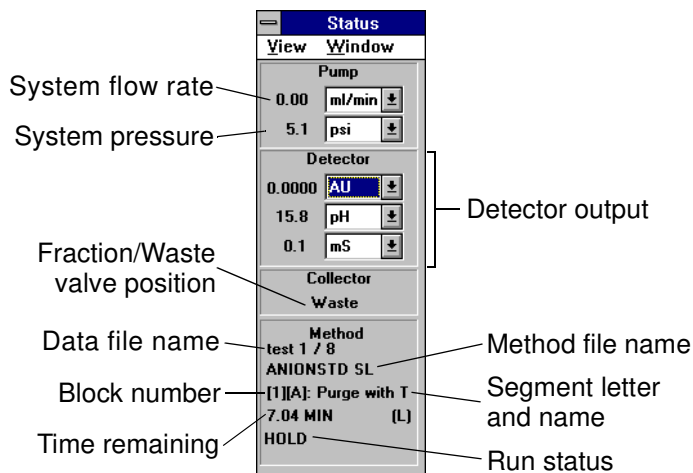


Figure 8-2 Status Window

Status information

The Status window provides the following real-time status information during runs:

- Pump
- Detector
- Collector
- Method

NOTE: You can change units for values displayed in the Status window by clicking the down arrow to the right of the item.

Pump The Pump section of the Status window displays:

- **System flow rate**—You can select ml/min, cm/hr or CV/min flow rates. See “Flow rate units” on page 5-14.
- **System pressure**—You can select psi (pounds per square inch), bar, or MPa (megapascal) pressure units. (1 bar = approximately 15 psi = 0.1 MPa)

Detector The Detector section of the Status window displays any three of the following five detector outputs:

- **UV/Vis**—Displays absorbance values for one or both UV/Vis wavelengths in AU (absorbance units).
- **Fluor**—Displays the fluorescence emission values in FU (fluorescence units).
- **pH**—Displays the pH measured in the pH flow cell.
- **Conductivity**—Displays conductivity in conventional mS (millisiemen) units or custom calibrated units. See Section 9.5.4, Calibrating the Conductivity Monitor.
- **Temperature**—Displays the temperature in the conductivity flow cell.
- **Auxiliary Detector Output**—Displays auxiliary detector output in V (volts) or custom units. See “Configuring the auxiliary detector” on page 4-18.

NOTE: To change the detection display, select a different option from the View menu in the Status window.

Collector The Collector section of the Status window displays the valve position of the Fraction/Waste valve. If a fraction collector is configured, it displays the current fraction collector tube.

Method The Method section of the Status window displays the following information about a method:

- Currently running data file name and number in sequence (if template or Multi-method run)
- Currently running method file name
- Currently running block number and segment name
- Time elapsed or remaining in run
- Run status

NOTE: To change the time display, select **Time Elapsed** or **Time Left** from the View menu.

8.3 Using the Strip Chart Recorder

The Strip Chart Recorder window (Figure 8-3) appears below the Control Panel and the Status window. You can resize the strip chart recorder or click-drag the window anywhere on the screen.

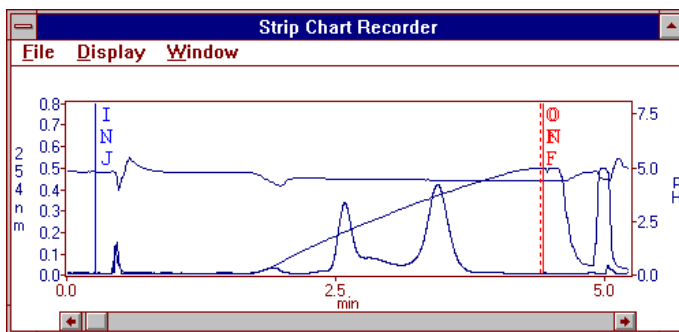


Figure 8-3 Strip Chart Recorder Window

When the pump is running, the electronic Strip Chart Recorder continuously stores and displays data for all signals you selected in the Configuration dialog box. For more information, see “Configuring data channels” on page 4-16.

You can use the Strip Chart Recorder to:

- View data in real time as it is being acquired (Real-time mode)
- View previously acquired data by scrolling through the window (History mode)
- Save Strip Chart Recorder data in a data file. This is useful when you make an injection from the Control Panel without creating a method.

Real-time and History mode

The strip chart recorder can run in two modes:

- **Real-time**—As data is acquired, it is displayed in the Strip Chart Recorder. When the Strip Chart Recorder is in Real-time mode, the Real-Time command on the Display menu is checked, and there is no scroll bar displayed at the bottom of the window.
- **History**—Data acquired up until the time you switched to History mode is displayed. When the Strip Chart Recorder is in History mode, the Real-Time command on the Display menu is not checked, and there is a scroll bar displayed at the bottom of the window.

To place the Strip Chart Recorder in Real-time mode, select (check) **Real-Time** from the Display menu.

To place the Strip Chart Recorder in History mode, deselect (uncheck) **Real-Time** from the Display menu, or click anywhere on the Strip Chart Recorder.

Strip Chart Recorder annotations

The Strip Chart Recorder can display the following annotations:

- **MRK** (dark red)—Appears any time you add a chart mark, by pressing the Event Mark button on the front panel of the workstation, by clicking the Chart Mark button in the Control Panel, or by adding a detector event mark in a method.
- **ON/OFF** (red)—Appears any time you turn the pump on or off, from the front panel of the workstation, from the Control Panel, or by using a method event.
- **INJ** (blue)—Appears any time you make an autoloader, manual, or autosampler injection from a method.

NOTE: No INJ mark appears if you make a pump injection from a method. No INJ mark appears if you make an injection from the Control Panel.

- **1, 2, 3** and so on (green)—Fraction numbers. A solid line indicates the start of a fraction. A dotted line indicates the end of a fraction, if triggered by a threshold. If the fraction end is not triggered by a threshold, no fraction end is indicated.

Customizing the Strip Chart Recorder

You can customize the Strip Chart Recorder to display various data channels, ranges, units, and colors.

To customize the Strip Chart Recorder:

1. Select **White Background** or **Black Background** from the Display menu in the Strip Chart Recorder window.
2. Select **Options** from the Display menu in the Strip Chart Recorder window.

The Strip Chart Settings dialog box is displayed (Figure 8-4).

Y-axis	Range: Min:	Max:	Units:	Colors:
<input checked="" type="checkbox"/> Int UV/VIS #1	-8.0000	8.0000	AU	Blue
<input type="checkbox"/> UV/VIS #2				
<input checked="" type="checkbox"/> pH	0.0	7.0	pH	Dark Red
<input type="checkbox"/> Conductivity	0.0	1.3	mS	Dark Red
<input type="checkbox"/> Pressure	0.0	997.6	psi	Dark Green
<input type="checkbox"/> Auxiliary				
<input type="checkbox"/> Gradient	-10.00	110.00	% A	Dark Green

Display Events
 Left Axis: Right Axis:

X-axis
 Scroll Position: Chart Range:

Figure 8-4 Strip Chart Settings

3. Select the Y-axis **data channels** for display and select a range, unit, and color for each. Choices are:
 - Int UV/Vis #1
 - Int UV/Vis #2, Ext UV/Vis, or Ext Fluor
 - pH
 - Conductivity
 - Pressure
 - Auxiliary Detector Output

NOTE: If Y-axis variables are dimmed, it indicates that you did not select these variables in the Data Recorder Configuration dialog box. For more information, see “Configuring data channels” on page 4-16.

4. Click the **Gradient check box** to display the percentage of one of the six buffer/solvent channels that you programmed in the Control Panel or in a Gradient segment.

NOTE: In the Strip Chart Recorder, the actual percent of the buffer/solvent detected by the conductivity monitor is offset from the programmed percent due to system delay volume.

5. Check the **Display Events** box to display events on the Strip Chart Recorder. Events include:
 - Injection marks (displayed in blue)
 - Pump on/off (displayed in red)
 - UV/Vis chart marks (displayed in dark red)
 - Fraction start and end and fraction tube changes (displayed in green)
6. Specify the **labels** to display on the left and right axes.
7. Select the following for the X-axis:
 - **X-Axis Scroll Position**—Available only in History mode. Determines the number of previous minutes, ml, or CV displayed. For more information, see “X-Axis Scroll Position and Chart Range” on page 8-11.
 - **Chart Range**—Sets the total number of minutes, ml, or CV displayed at one time on the Strip Chart Recorder. As new data is acquired, older data scrolls to the left out of the window. For example, if you set the range to 15 minutes, 15 minutes worth of data is displayed. For more information, see “X-Axis Scroll Position and Chart Range” on page 8-11.
 - **Units**—Minutes, ml, or CV (column volumes).

X-Axis Scroll Position and Chart Range

X-Axis Scroll Position and Chart Range settings work together to determine how data is displayed in the Strip Chart Recorder. Figure 8-5 shows examples of the portion of the data displayed in the Strip Chart Recorder window using different settings.

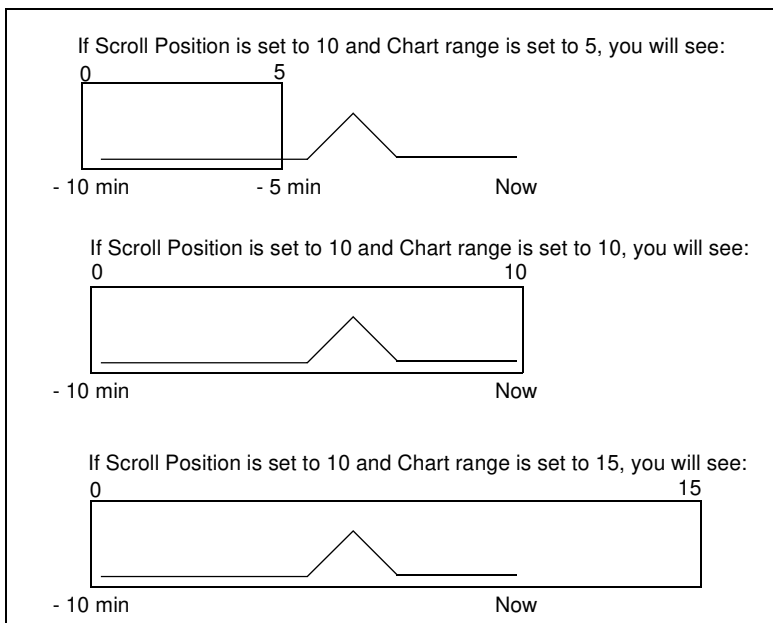


Figure 8-5 X-Axis Scroll Position and Chart Range

Moving around the Strip Chart Recorder

To move around the Strip Chart Recorder:

1. To zoom in on any area of the Strip Chart Recorder:
 - Move the pointer into the Strip Chart Recorder.
The cursor arrow changes to a crosshair.
 - Hold down the left mouse button.
 - Click-drag a box around the area you want to zoom on.
 - Release the mouse button.

The selected area is expanded to full screen.

The data channel you selected as the Left Axis label in Strip Chart Settings is zoomed vertically and horizontally. All other data channels are zoomed horizontally only.

NOTE: *Zooming on the Strip Chart Recorder during Real-Time mode automatically places the Strip Chart Recorder to History mode.*

2. To unzoom, select one of the following commands from the Display menu:
 - **Unzoom**—Undoes the previous zoom. You can also double-click on the window to unzoom.
 - **Reset**—Returns to the default settings for the Strip Chart Recorder (-8 to 8 AU).
3. You can scroll through the Strip Chart Recorder when it is in History mode only. To change from Real-time mode to History mode, do one of the following:
 - Deselect the Real-time command on the Display menu
 - Zoom in on an area of or click on the Strip Chart Recorder

Saving Strip Chart Recorder data to a data file

You can save all data currently displayed in the Strip Chart Recorder to a data file. You can analyze the data file in the Group Analysis window. This is useful when you make an injection from the Control Panel without creating a method.

To save Strip Chart Recorder data to a data file:

1. Zoom in on or expand the Strip Chart Recorder to display the data you want to save. Only the segment of data shown on the X-axis is saved. All signals collected are saved, even if they are not displayed in the Strip Chart Recorder.
2. Select **Save Data As** from the Strip Chart Recorder File menu.
3. Enter a **file name**. The default file extension is .BIO. Specify a directory, if desired. Click **OK**.

NOTE: *Method and configuration information is not stored in data files created from the Strip Chart Recorder. Data files created from the Strip Chart Recorder cannot be exported.*

8.4 Using the Timer

The Timer window (Figure 8-6) does not appear on the screen when you start the BioCAD software. To display the Timer window, select **Timer** from the Window menu.

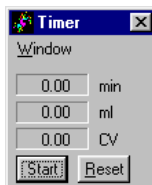


Figure 8-6 Timer Window

The Timer is an electronic stopwatch. It gives you an independent measurement of both elapsed time and volume.

To use the Timer:

1. Click the **Start** button to start timing in minutes, ml, and CV (column volume).
2. Click the **Stop** button to stop the Timer.
3. Click the **Reset** button to reset the values to zero.

Note the following when using the Timer:

- The ml counter does not start if the pump is off
- The CV counter does not start if the pump is off, a column is not configured, or a column is not inline

8.5 Using the Event Log

As the system runs, all events and errors that occur are stored in the Event Log. If errors occur while a method is running, these errors are also stored in the data file.

The Event Log is stored in the SYSTEM.EVL file, which is located in the C:\BIOCAD directory.

This section describes:

- Displaying the Event Log window
- Types of errors and events
- Filtering events
- List of error codes
- Displaying Event Log detail
- Searching
- Displaying the Event Log for data files
- Printing
- Clearing

Displaying the Event Log window

To display the Event Log window, do one of the following:

- Select **Event Log** from the Window menu in the Control Panel to display events and errors that occurred since the last time the Event Log was cleared. This is the *system* Event Log.
- Select **Open Event Log** from the File menu in the Group Analysis window to display events and errors that occurred while the selected data file was acquired. This is a *data file* Event Log.

NOTE: You can also display the event log for a data file by selecting *Open* from the File menu in the Event Log window.

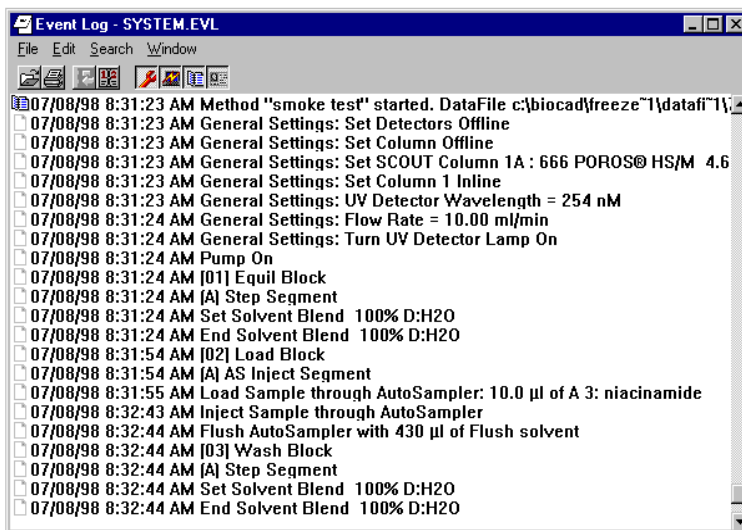






Figure 8-7 Event Log

Types of errors and events

The Event Log stores four types of errors and events. One of the following icons appears to the left of each item in the Event Log:


- **Hardware** ( **wrench icon**)—Problem with hardware, such as an instrument malfunction, valve problem, hardware board failure
- **System** ( **folder icon**)—Error related to a system problem, such as math, serial communications, file I/O errors
- **Resource** ( **hand icon**)—Not enough memory or disk space to complete the requested action
- **Method** ( **book icon**)—Start and end of a method run

Filtering events

There are two ways to filter errors and events displayed in the Event Log:

- **Select Filter Records from the Edit menu**—A cascade menu is displayed listing Hardware Errors, System and Resource Errors, Method Events, and GMP Events. A check mark indicates the error or event is displayed in the Event Log.

To suppress an event, click the checked event type. To show an event, click the unchecked event type. You can show or suppress multiple event types.

- **Click the filter buttons at the top of the Event Log**—  If a filter button is active, it has a white background. If a filter button is inactive, it has a gray background.

To suppress an event type, click an active filter button. To show an event type, click an inactive filter button. You can show or suppress multiple event types.

List of error codes

The Event Log also lists error codes. For an explanation of individual error codes, see Section 10.2, Error Codes.

**Displaying
Event Log detail**

Double-click on a line in the Event Log to display the Event Log Detail dialog box (Figure 8-8).

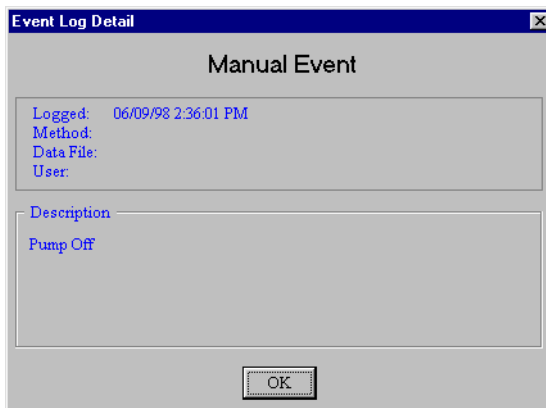


Figure 8-8 Event Log Detail Dialog Box


The error is displayed in the Description box. The date, time, method file name, and data file name that were running at the time the error occurred are displayed at the top of the dialog box.

If Method and Data File fields are blank, no method was running at the time the error occurred.

Searching

You can search for a specific event or error by date.

To search for a specific date:

1. Select **Date** from the Search menu or click the **Calendar** icon .

The Find Date dialog box is displayed.

2. Type a **date** in MM/DD/YY format and click **OK**.

The first event that matches the date you typed is selected and scrolled into view.

Displaying the Event Log for data files

To display events and errors that occurred while a specific data file was being acquired:

1. Select **Open** from the File menu in the Event Log window.

The Open File dialog box is displayed.

2. Select **Data Files (*.B*)** from the List Files of Type drop-down list box.
3. Double-click the **data folder** or the folder where the desired data file is stored.
4. Select the **data file** and click **OK**.

The data file name is displayed in the menu bar of the Event Log window, and events and errors for the data file are displayed in the Event Log window.

If no events and errors are stored with the data file, or if the data file was collected on an earlier version of BioCAD software, a message is displayed.

5. To display the complete Event Log again, select **System Log** from the File menu, or click the **folder** icon. The name of the event and error log file (SYSTEM.EVL) is displayed in the menu bar of the window.

NOTE: You can also display the Event Log for a specific data file from the Group Analysis window.

Printing You can manually print the contents of the Event Log by selecting Print commands from the File menu in the Event Log window:

- **Print All**—Prints all errors
- **Print Item**—Prints only the error you have selected (clicking the printer icon also prints the selected item)

You can automatically print the contents of the Event Log for a data file at the end of each run by setting the method to Print after Run. For more information, see Section 6.2.11, Specifying Print Settings.

Clearing Clear the Event Log when the information is no longer needed, or if you need to free some disk space.

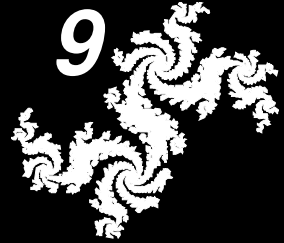
NOTE: *Clearing the system Event Log does not affect the Event Log stored in individual data files.*

Before clearing the Event Log, do one of the following:

- Select **Print All** from the Print menu and save the list of error codes.
- Rename the SYSTEM.EVL file and save it on a floppy diskette.

To clear the Event Log, select **Clear** from the Edit menu.

Chapter 9



9 Maintenance

This chapter includes the following sections:

9.1	Maintenance Overview	9-2
9.2	Maintenance Schedule	9-3
9.3	General Maintenance	9-5
9.4	UV/Vis Detector Maintenance.....	9-8
9.5	pH and Conductivity Monitor Maintenance.....	9-16
9.6	Pump Maintenance.....	9-23
9.7	Autoloader Maintenance.....	9-34
9.8	Valve Maintenance	9-37
9.9	Autosampler Maintenance	9-38
9.10	Hard Disk Maintenance	9-47
9.11	Long Term Shutdown.....	9-52

9.1 Maintenance Overview

The following sections describe:

- Maintenance schedule
- General maintenance
- UV/Vis detector maintenance
- pH/conductivity monitor maintenance
- Pump maintenance
- Autoloader maintenance
- Valve maintenance
- Hard disk maintenance
- Long term shutdown

CAUTION

Only the parts listed in this chapter are recommended for replacement by customers. If you attempt to service any parts not listed in this chapter, you may invalidate your warranty.

NOTE: *You can purchase a Scheduled Maintenance Visit from Applied Biosystems. For more information, contact Technical Support. See the back cover of this guide for Technical Support contact information.*

A maintenance log for use with the BioCAD 700E Workstation is provided in Appendix G, Maintenance Log.

9.2 Maintenance Schedule

Regular preventative maintenance will help keep your BioCAD 700E Workstation functioning properly. Perform the following procedures as indicated:

When to perform	Task	Page
Daily	Purge system and run SYSCLEAN.MET	page 2-38
	Calibrate pH probe (GLP)	page 9-19
	Calibrate conductivity monitor (GLP)	page 9-21
Weekly	Change pump seal wash solution	page 3-22
	Calibrate pH probe	page 9-19
	Calibrate conductivity monitor	page 9-21
	Back up data	page 9-48
	Test hard disk integrity (run SCANDISK)	page 9-50
Monthly	Clean fan filter	page 9-7
	Clean pH probe and cell	page 9-16
	Clean pump filter/mixer frit	page 9-33
	Optimize disk performance (run DEFRAG)	page 9-51

Continued

When to perform	Task	Page
Every 6 months	Clean UV/Vis flow cell	page 9-12
	Replace solvent pump seals, clean piston, clean pump heads	page 9-23
	Clean check valves	page 9-30
	Clean fluorescence flow cell	see <i>Fluorescence Detector Manual</i>
	Inspect autoloader syringe seal	page 9-34
	Clean injection/column switching valve stators	page 9-37

9.3 General Maintenance

General maintenance procedures include:

- Replacing the main power fuse
- Cleaning the fan filter

9.3.1 Replacing the Main Power Fuse

WARNING

For continued protection against fire hazard, replace fuses with those of the same type and rating.

AVERTISSEMENT

Remplacez les fusibles par ceux de même type et puissance pour éviter les risques d'incendie.

To replace the main power fuse:

1. Power down the workstation.
2. Remove the power cord from the workstation.
3. Pry out the voltage selector cover using a small flat-blade screwdriver (Figure 9-1).
4. Rotate the selection wheel until the proper voltage is displayed.
5. Pull out on the white tab to remove the fuse block (Figure 9-1).

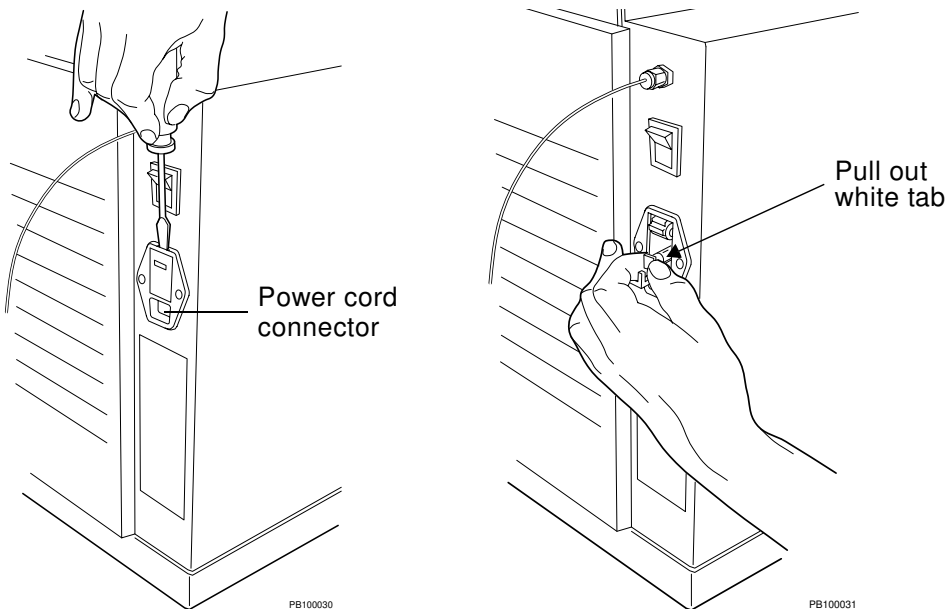


Figure 9-1 Removing the Voltage Selector Cover and Removing the Fuse Block

6. Remove the fuse and replace with a fuse of the proper rating.

Volts/Amps	Fuse
100V~6A	T6A 250V
120V~5A	T5A 250V
220V~3A	T3A 250V
240V~2.5A	T2.5A 250V

7. Insert the fuse block into the receptacle.
8. Replace the voltage selector cover.
9. Plug in and power up the workstation.

9.3.2 Cleaning the Fan Filter

Clean the fan filter monthly to allow proper ventilation of the unit:

1. Pry off the filter holder on the rear panel of the workstation.
2. Remove the filter.
3. Vacuum or shake dust from the filter.

NOTE: *If you rinse the filter in water, dry thoroughly before replacing.*

4. Replace the filter and the filter holder.

9.4 UV/Vis Detector Maintenance

This section describes:

- Changing the UV/Vis detector lamps
- Cleaning the UV/Vis detector flow cell
- Changing the UV/Vis detector flow cell

9.4.1 Changing the UV/Vis Detector Lamps

Change the UV/Vis detector lamps after 1,000 hours of use.

NOTE: To determine hours of use, check the gauge in the UV/Vis detector compartment (Figure 9-2).

Removing

To remove the UV/Vis detector lamps:

1. Power down the workstation and remove the power cord.

WARNING

UV light can damage eyes and skin. Power down and remove the power cord before replacing the lamp.

AVERTISSEMENT

La lumière UV peut être dangereuse pour les yeux et la peau. Mettez hors tension et débranchez le câble d'alimentation avant de remplacer la lampe.

2. Allow the lamps to cool for ten minutes.
3. Remove the screw or knurled knob from the detector lamp cover and remove the cover.

The tungsten and deuterium lamps are exposed (Figure 9-2).

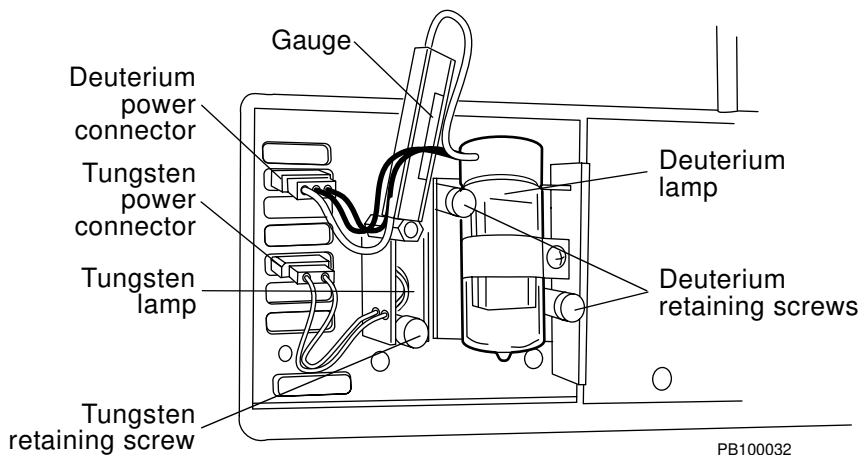


Figure 9-2 UV/Vis Detector Compartment

Tungsten 4. To remove the tungsten lamp:

- Disconnect the bottom power connector.
- Unscrew the retaining screw at the base of the lamp (see Figure 9-2).
- Pull out the lamp.

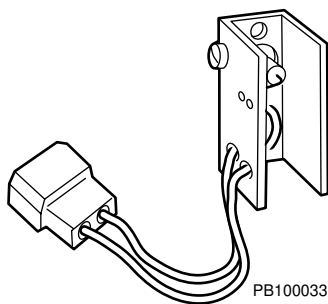


Figure 9-3 Tungsten Lamp

Deuterium 5. To remove the deuterium lamp:

- Disconnect the top power connector.
- Unscrew the two retaining screws (see Figure 9-2).
- Pull out the lamp.

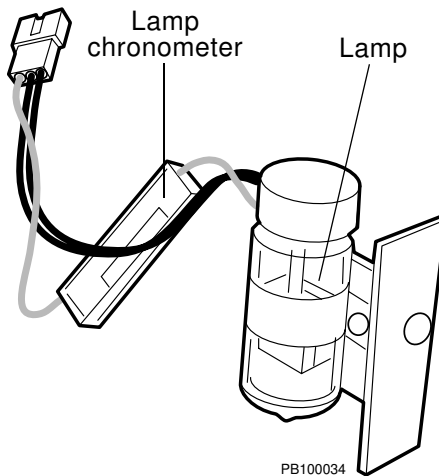


Figure 9-4 Deuterium Lamp

Replacing**CAUTION**

Do not touch lamp glass. Fingerprints can cause poor lamp performance.

Tungsten

To replace the tungsten lamp:

1. Position the lamp mounting bracket on the pins at the left of the lamp compartment. See Figure 9-2 for tungsten lamp position.
2. Replace the retaining screw.
3. Plug in to the bottom power connector.
4. Replace the detector cover and replace the retaining screw or knurled knob.
5. Connect the power cord, and power up. Allow the lamp to warm up for 20 minutes.

Deuterium

To replace the deuterium lamp:

1. Position the lamp mounting bracket on the pin at the right of the lamp compartment. See Figure 9-2 for deuterium lamp position.
2. Replace the two retaining screws.
3. Plug in to the top power connector.
4. Replace the detector cover and replace the retaining screw or knurled knob.
5. Connect the power cord, and power up. Allow the lamp to warm up for 20 minutes.

9.4.2 Cleaning the UV/Vis Detector Flow Cell

Clean the UV/Vis detector flow cell every six months. If you have problems with the UV/Vis detector performance, such as excessive noise or unexpected readings, clean the UV/Vis detector flow cell more often.

WARNING

Wear gloves, safety glasses, and a laboratory coat when performing this procedure.

AVERTISSEMENT

Veillez porter des gants, des lunettes de sécurité, et une blouse de laboratoire lors de cette opération.

To clean the UV/Vis detector flow cell:

1. Bypass the pH and conductivity monitors by clicking the **Bypass** option button in the Detector section of the Control Panel.
2. Flush the system with 1 N NaOH. For more information, see Section 5.2.4, Purging the System.
3. Flush the system completely with deionized water.

NOTE: *Check the pH of the waste stream to determine if the system has been completely flushed with deionized water.*

4. If necessary, flush the system with 1 N HCl, then flush with deionized water.

If cleaning does not correct the problem, change the UV/Vis detector flow cell. See Section 9.4.3, Changing the UV/Vis Detector Flow Cell.

NOTE: *As an alternative to flushing the system you can inject NaOH and HCl into the system through the injection valve.*

9.4.3 Changing the UV/Vis Detector Flow Cell

CAUTION

Do not touch the UV/Vis lens. Fingerprints can cause poor detector performance.

Removing To remove the UV/Vis detector flow cell:

1. Remove the detector flow cell cover (Figure 9-5).

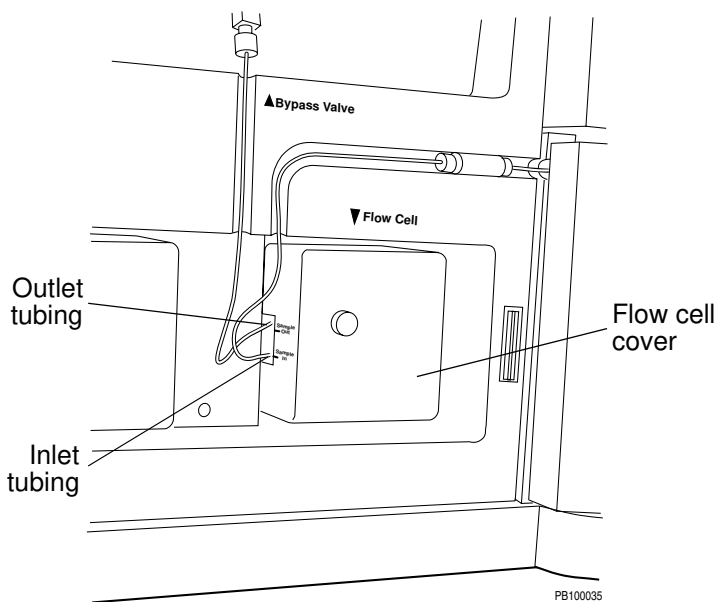
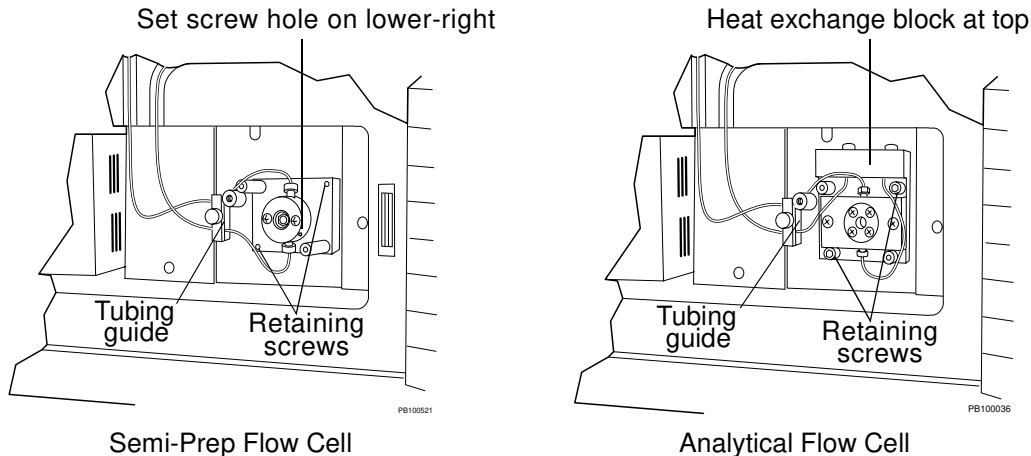


Figure 9-5 Detector Flow Cell Cover

2. Disconnect the inlet (bottom) and outlet (top) tubing from the Column Switching and Detector Bypass valve.
3. Remove the sample diode by removing the knurled knobs.
4. Remove the tubing from the tubing guide (Figure 9-6).



**Figure 9-6 Removing Flow Cell Screws
(Sample Diode Not Shown)**

5. Unscrew the flow cell retaining screws (Figure 9-6). If you are removing an analytical flow cell, you need a 9/64-inch hex wrench (provided in the startup kit).
6. Remove the flow cell from the alignment pins.

Replacing

To replace the UV/Vis detector flow cell:

1. Position the new flow cell over the alignment pins:
 - **Semi-prep flow cell**—Position the set screw hole on the lower-right side of the cell.
 - **Analytical flow cell**—Position the warming block at the top of the cell.
2. Replace the retaining screws. Make sure the cable is not in the way before tightening the screws.
3. Replace the sample diode and retaining screws.

CAUTION

Plumb the UV/Vis flow cell so that fluid flows into the cell at the bottom and out of the cell at the top. If plumbing connections are reversed, air bubbles can be trapped in the flow cell and cause excessively noisy baselines.

4. Connect the inlet (bottom) and outlet (top) tubing. Secure the inlet and outlet tubing in the tubing guide. Do not overtighten.
5. Replace the flow cell cover.
6. Connect the detector inlet tubing (bottom) to the high-pressure connection from the Column Switching valves. Connect the outlet tubing (top) to the green connector (port 5) on the Detector Bypass valve.

9.5 pH and Conductivity Monitor Maintenance

This section describes:

- Cleaning the pH probe
- Calibrating the pH probe
- Storing the pH probe
- Calibrating the conductivity monitor

9.5.1 Cleaning the pH Probe and Cell

Clean the pH probe and cell monthly. To clean the pH probe and cell:

- Remove the pH probe
- Clean the pH probe
- Clean the pH cell
- Replace the pH probe

Removing pH probe

To remove the pH probe:

1. Unscrew the retaining cap holding the probe in place.

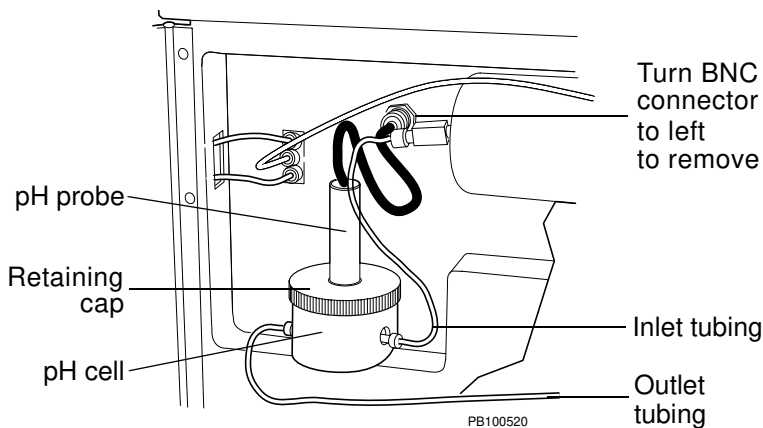


Figure 9-7 Removing the pH Probe

2. Disconnect the round BNC connector by pushing it in and turning to the left.
3. Pull the probe straight up and out of the cell.

Cleaning pH probe

To clean the pH probe:

WARNING

Wear gloves, safety glasses, and a laboratory coat when performing this procedure.

AVERTISSEMENT

Veillez porter des gants, des lunettes de sécurité, et une blouse de laboratoire lors de cette opération.

- If the coating is soft, clean the probe by stirring vigorously in water or 1 N HCl.
- If the coating is hard or caused by an organic chemical, replace the probe.

CAUTION

Do not scrub the probe. Abrasion will damage the probe.

Cleaning pH cell

To clean the pH cell:

1. Disconnect the inlet and outlet tubing from the cell.
2. Lift the cell out of its holder.
3. Place the cell under running water.
4. Replace the cell.

NOTE: *Replace the cell so that the arrow on the bottom of the cell points in the same direction as the flow.*

5. Connect the inlet and outlet tubing to the cell.

**Replacing
pH probe**

To replace the pH probe:

1. Make sure the black O-ring is on the tip of the pH probe.
2. Carefully push the probe into the cell until the probe is all the way to the bottom of the flow cell.
3. Tighten the retaining cap only until it is snug.

CAUTION

Do not overtighten the retaining cap. Overtightening can break the glass membrane of the probe.

9.5.2 Calibrating the pH Probe

pH values *NOTE: The pH probe in the BioCAD 700E Workstation provides a way to monitor pH values during methods development and execution. It is not intended for use as a precision benchtop pH meter.*

When to calibrate Calibrate the pH probe weekly.

NOTE: Calibrate the pH probe daily if you are operating under Good Laboratory Practices.

Calibration solution For accurate performance, calibrate the pH probe with one of the following:

- Standards at pH 4 and 7.
- Standards at pH 7 and 10.
- Two buffers that cover the range of interest. Check the buffers with a calibrated pH meter before calibrating the BioCAD 700E pH probe.

CAUTION

Do not expose the probe to organic solvents.

Calibrating This procedure performs a two-point calibration. To calibrate the pH probe:

1. Select **Calibrate pH** from the Detector menu in the Control Panel.

The pH and conductivity monitors are placed in bypass mode and the pH Calibration dialog box is displayed (Figure 9-8).

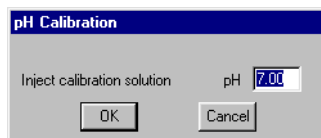


Figure 9-8 pH Calibration Dialog Box

2. Enter the **pH value** of the first calibration standard (or buffer) in the pH text box and click **OK**.
3. Using a syringe, inject the first calibration standard (or buffer) through port 1 (black connector) on the Detector Bypass valve (Figure 9-9). Continue injecting until the pH reading in the Status window stabilizes. Click **OK**.

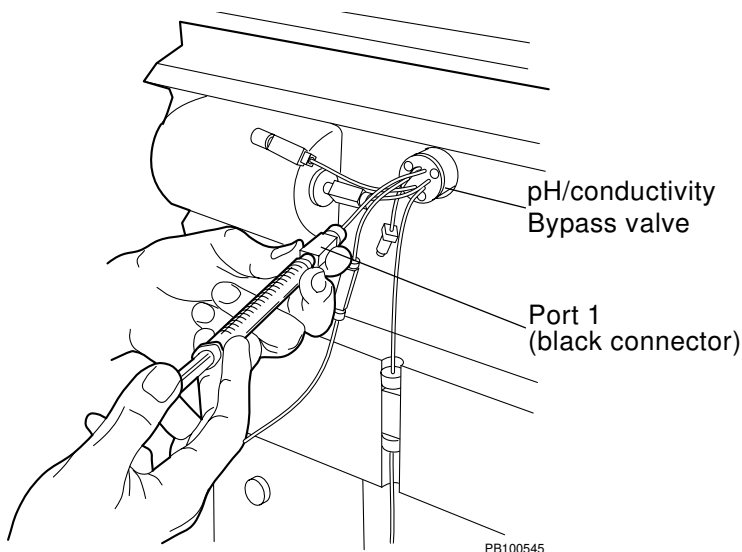


Figure 9-9 Injecting Calibration Solution

A second pH Calibration dialog box is displayed.

4. Enter the **pH value** of the second calibration standard (or buffer) in the pH text box and click **OK**.
5. Using a syringe, inject the second calibration standard (or buffer) through port 1 (black connector) on the Detector Bypass valve (Figure 9-9). Continue injecting until the pH reading in the Status window stabilizes. Click **OK**.

The pH probe is now calibrated.

9.5.3 Storing the pH Probe

Short term shutdown When the pH probe is not in use, flush the system with 1 M salt solution. Then click the **Bypass** option button in the pH/Cond section of the Control Panel to remove the pH and conductivity monitors from the flow path before flushing the system with other solutions.

Long term shutdown If you will not be using the pH probe for longer than one week, or if you will be moving the workstation, remove the probe and store it in the shipping bottle with 4.0 M KCl.

9.5.4 Calibrating the Conductivity Monitor

When to calibrate Calibrate the conductivity monitor weekly.

NOTE: Calibrate the conductivity monitor daily if you are operating under Good Laboratory Practices.

Calibration solutions Use commercially available conductivity standards to calibrate the conductivity monitor. If you do not have standards available, use HPLC-grade water and 1 M NaCl.

Calibrating You can calibrate the conductivity monitor in conventional mS (millisiemen) units or in concentration units of a specific eluent, such as mM for NaCl. The following procedure performs a two-point calibration.

To calibrate the conductivity monitor:

1. Select **Calibrate Conductivity** from the Detector menu on the Control Panel.

The pH and conductivity monitors are placed in bypass mode and the Conductivity Calibration dialog box is displayed (Figure 9-10).

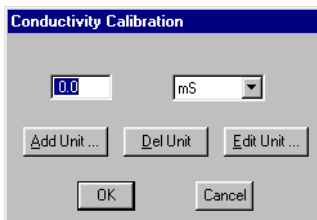


Figure 9-10 Conductivity Calibration Dialog Box

2. Enter the **conductivity value** of the first standard and click **OK**.

NOTE: *The conductivity value for water is 0.0 mS.*

3. Using a syringe, inject a low calibration standard through port 2 (red connector) on the Detector Bypass valve (see Figure 9-9). Continue injecting until the conductivity reading in the Status Window stabilizes. Click **OK**.

A second Conductivity Calibration dialog box is displayed.

4. Enter the **conductivity value** of the second standard and click **OK**.

NOTE: *The conductivity value for 1 M NaCl is 77.8 mS.*

5. Using a syringe, inject a high calibration standard through port 2 (red connector) on the Detector Bypass valve (see Figure 9-9). Continue injecting until the conductivity reading in the Status Window stabilizes. Click **OK**.

The conductivity monitor is now calibrated.

9.6 Pump Maintenance

This section describes:

- Replacing the pump seals and cleaning the pistons and pump heads
- Cleaning the check valves
- Cleaning the filter frit

9.6.1 Replacing the Pump Seals and Cleaning the Pistons and Pump Heads

This section describes:

- When to replace and clean
- Removing pump heads
- Replacing solvent pump seal
- Replacing wash pump seal
- Cleaning the piston
- Cleaning the front pump head
- Replacing pump heads

When to replace and clean

Replace the pump seals and clean the pistons every six months or when the pump is leaking.

NOTE: *When you change pump seals, always change seals in both the primary pump head (right) and the accumulator pump head (left).*

The BioCAD 700E Workstation includes an automatic pump seal wash that significantly extends the pump seal life. For more information, see “Pump seal wash system” on page 1-11. Running the SYSCLEAN.MET daily extends the life of the pump seals. For more information, see “Shutting down” on page 2-38.

Removing pump heads

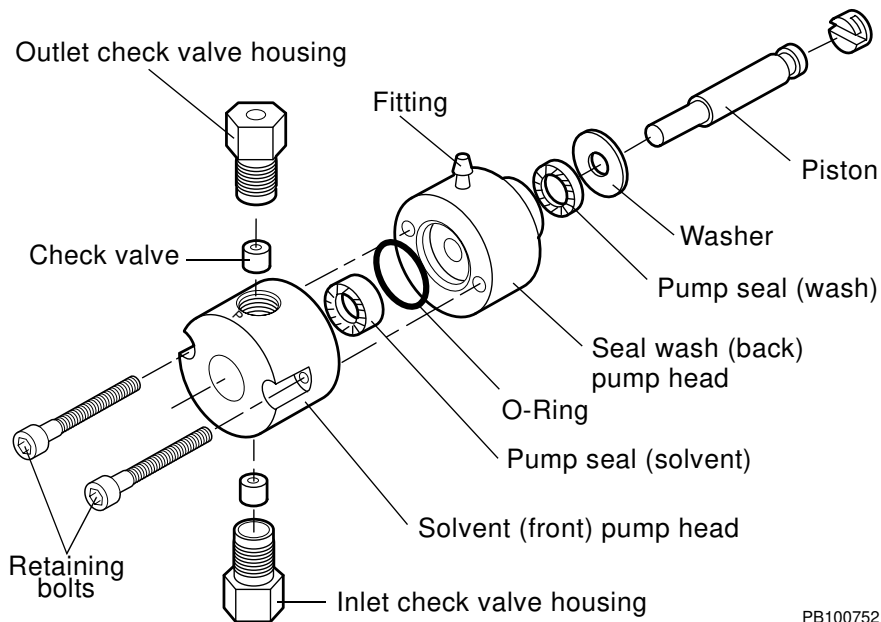
To remove the pump heads:

1. Exit the BioCAD software.

This closes the six solvent proportioning valves in the base of the pump unit and prevents leaking.
2. Remove the pump seal wash lines from the seal wash solution (see Figure 1-4 on page 1-10).
 - **Primary pump head (right)**—Remove the tubing from the outlet (top) check valve housing (Figure 9-11). Remove the barb and tubing from the inlet (bottom) check valve housing. (Do not remove the tubing from the barb.)
 - **Accumulator pump head (left)**—Remove the tubing from the top and bottom of the pump head

NOTE: *It is not necessary to remove the seal wash tubing from the back pump head.*

3. Using a 9/64-inch hex wrench, remove the retaining bolts holding the pump heads in place (Figure 9-11).
4. Remove the pump heads.
5. Separate the front and back pump heads.
 - **Solvent (front) pump head**—Controls *solvent* delivery
 - **Seal wash (back) pump head**—Controls *pump seal wash* delivery



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Figure 9-11 Primary Pump Assembly

NOTE: The Accumulator pump assembly is the same as the Primary pump assembly except that the solvent (front) head on the Accumulator pump has fittings instead of check valve housings. Also, the solvent pump seal and the piston on the Accumulator pump are smaller.

Replacing the solvent pump seal

All parts you need to replace seals are contained in the Pump Head Seal Kit.

To replace the solvent pump seal:

1. Use the flanged end of the removal tool (Figure 9-12) (included in THE Pump Head Seal Kit) to remove the solvent pump seal from the solvent (front) pump head (Figure 9-13).

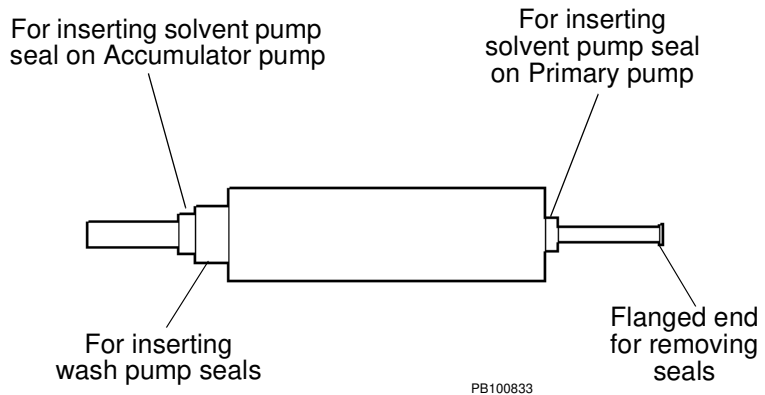


Figure 9-12 Pump Seal Removal and Insertion Tool

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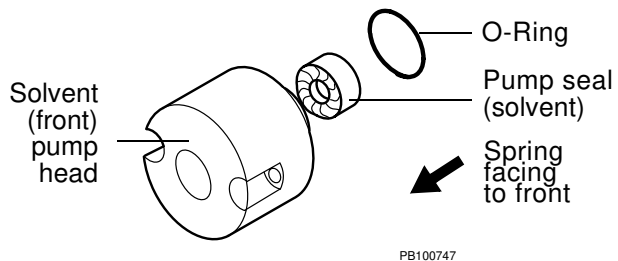


Figure 9-13 Replacing Solvent Pump Seal

2. Use the blunt end of the removal/insertion tool (Figure 9-12) to insert the new solvent pump seal with the spring in the seal facing toward the front of the pump head (Figure 9-13).

NOTE: Be sure to use the larger pump seal for the Primary pump head, and the smaller pump seal for the Accumulator pump head.

3. Use the removal/insertion tool or your finger to seat the new solvent pump seal. The spring is *not* visible when the pump seal is properly installed.

Replacing the wash pump seal

To replace the wash pump seal:

1. Use the flanged end of the removal/insertion tool (Figure 9-12) (in the Pump Head Seal Kit) to remove the wash pump seal from the seal wash (back) pump head (Figure 9-14).

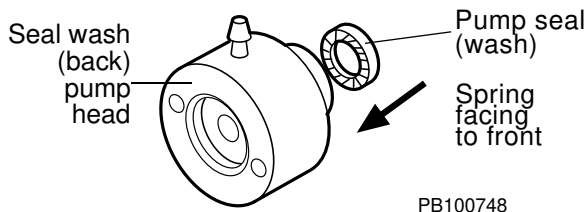


Figure 9-14 Replacing Wash Pump Seal

2. Use the blunt end of the removal/insertion tool to insert the new wash pump seal with the spring in the seal facing toward the front of the pump head (Figure 9-14).

NOTE: The wash pump seals are identical for the Primary pump head and the Accumulator pump head.

3. Use the removal/insertion tool or your finger to seat the new wash pump seal. The spring is *not* visible when the seal is properly installed.

Cleaning the pistons

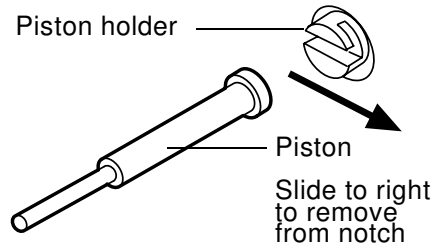
To clean the pistons:

1. Remove the washer from the piston (see Figure 9-11 on page 9-25).

CAUTION

Touch the piston as little as possible. Finger oil can adversely affect pump performance.

2. Remove the piston by grasping the metal base of the piston and sliding it to the right to remove it from the notch in the piston holder (Figure 9-15).



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Figure 9-15 Removing the Piston

3. Using the scrubbing pad (in the Pump Head Seal Kit), scrub the piston using a *rotary motion*.

CAUTION

Do not scrub the piston using a linear motion (back and forth) because this will damage the piston. Touch the piston as little as possible. Finger oil can adversely affect pump performance.

4. Rinse the piston with water.
5. Replace the piston by sliding the metal base into the notch in the piston holder (Figure 9-15).

NOTE: If you replace the piston with a new piston, be sure to use a piston from the correct piston kit (Primary or Accumulator pump head).

6. Reinstall the washer (see Figure 9-11).

Cleaning the solvent pump head

To clean the solvent (front) pump head:

1. Place the solvent pump head (Figure 9-13) under running water and wash off any debris or residue.
2. Thoroughly dry the solvent pump head before reinstalling.

Reinstalling the pump heads

To reinstall the pump heads:

1. Slide the seal wash (back) pump head over the piston (see Figure 9-11). Position the pump head so that the fitting is at the top and the check valve housing is at the bottom.
2. Place the O-ring around the front part of the pump head (see Figure 9-11).
3. Slide the solvent pump head over the piston.

NOTE: Make sure the solvent pump head is oriented so that the opening near the letter "P" faces up.

4. Push the solvent pump head onto the unit, then finger-tighten the retaining bolts. Do not tighten the bolts all the way.

NOTE: If you cannot push the solvent pump head all the way on the unit, do not use excessive force. If pump heads are hard to seat, reach your finger up behind the pump head to verify that the piston is centered.

5. Place the pump seal wash lines in the flush solution and prime the pump seal wash lines. See Section 3.4.2, Priming the Buffer/Solvent Lines, for the priming procedure.

6. Start the BioCAD software, then set the pump flow rate to 5 ml/min.
7. Alternately tighten the retaining bolts as the piston moves. Tightening the bolts as the piston moves aligns the piston and pump seals.
8. Reinstall, then prime the pump lines. See the user's guide provided with your system for the priming procedure.

9.6.2 Cleaning the Check Valves

Clean the check valves on the primary pump head every six months.

To clean the check valves, you must:

- Remove the check valves
- Sonicate the check valves
- Reinstall the check valves

Removing

To remove the check valves from the primary pump head (Figure 9-16):

1. Remove the tubing from the outlet (top) check-valve housing. Remove the barb and tubing from the inlet (bottom) check-valve housing. Do not remove the tubing from the barb.
2. Unscrew the check valve housings from the pump head.

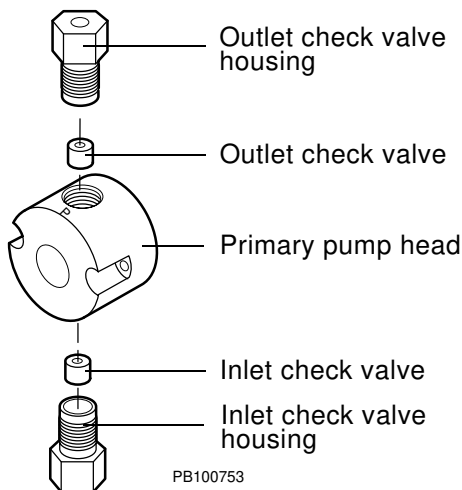


Figure 9-16 Check Valves

3. Remove each check valve from its housing. You may need to force the valves out by injecting water from a syringe (through the housing).

Sonicating

WARNING

Before handling any chemical, refer to its Material Safety Data Sheet provided by the manufacturer, and observe all relevant precautions.

To clean the check valves, sonicate the check valves and housings in 30% isopropanol-water for 10 minutes.

Reinstalling

To reinstall the check valves:

1. Reassemble the check valves and housings as shown in Figure 9-17.

NOTE: The inlet and outlet check valves are interchangeable. However, make sure the three-hole end of each check valve faces up (Figure 9-17).

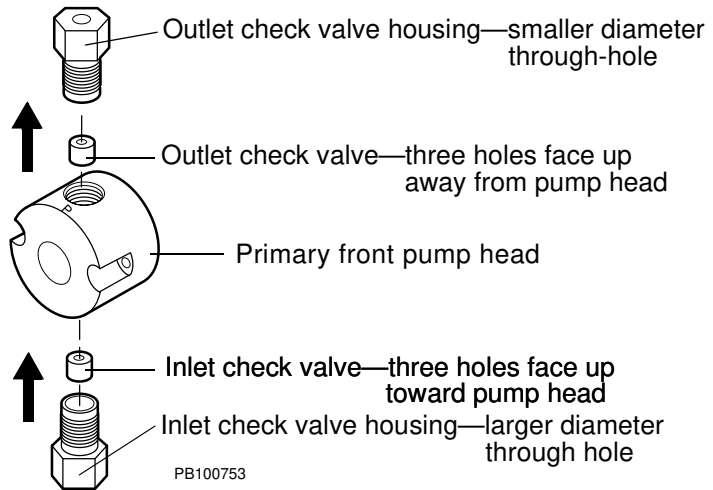


Figure 9-17 Re-assembling Check Valves

2. Install the reassembled check valves and housings on the pump head.

NOTE: Make sure to insert the inlet housing on the bottom of the pump head and the outlet housing on the top of the pump head. The inlet housing has a larger diameter through-hole.

3. Finger-tighten the housings, then tighten an additional 1/8 turn with a small wrench.

NOTE: If the check valve housing does not screw in to the proper depth, the check valve is not seated properly. Unscrew the housing, remove the check valve, reposition it, then reinstall it into the pump head.

9.6.3 Cleaning the Filter Frit

WARNING

Before handling any chemicals, refer to the Material Safety Data Sheet provided by the manufacturer, and observe all relevant precautions.

Clean the filter frit monthly. To clean the filter frit:

Removing

1. Remove the compression ring fitting from end cap at the bottom of the Filter/Mixer (see Figure 1-4 on page 1-10).
2. Using a 1/2-inch wrench, unscrew the end cap from the bottom of the Filter/Mixer.
3. Remove the filter frit (PEEK disk) from the end cap. If the filter frit is not on the end cap, remove it from the Filter/Mixer assembly.

Cleaning

4. Sonicate the filter assembly and filter frit in 30% isopropanol-water for 10 minutes.
5. Rinse the filter assembly and filter frit in deionized water.

NOTE: If the frit is discolored, replace it with a new frit.

Replacing

6. Place the frit on top of the end piece.
7. Screw the end piece into the bottom of the Filter/Mixer, then tighten 1/8 turn with a 1/2-inch wrench.
8. Reinstall the compression ring fitting.

9.7 Autoloader Maintenance

Inspect and change (if necessary) the autoloader syringe seal every six months.

The following sections describe:

- Removing the syringe
- Inspecting the syringe seal
- Changing the syringe seal
- Replacing the syringe

Removing the syringe

To remove the syringe:

1. On the Control Panel, select **Change Syringe** from the Sample menu.

The syringe plunger retracts and the Set Syringe Size dialog box is displayed.

CAUTION

Do not remove the syringe before retracting the plunger. The syringe can break.

2. Unscrew and remove the knurled knob at the base of the plunger.
3. Unscrew the body of the syringe (turn to the left) and remove.

Inspecting the syringe seal

To inspect the syringe seal:

1. Hold the syringe in one hand.
2. Move the plunger through its full range of motion.

You should feel a constant resistance through the entire range. If the plunger slips at any point, the seal is worn and must be replaced. See “Changing the syringe seal” on page 9-35.

Changing the syringe seal

To change the syringe seal:

1. Remove the syringe as described in “Removing the syringe” on page 9-34.
2. Remove the plunger from the barrel of the syringe.
3. Remove the syringe seal from the top of the plunger by gripping it with a pair of pliers approximately one-third of the way down and pulling it off.
4. Slip a new O-ring over the plunger tip.
5. Wet the O-ring and plunger tip with deionized water.
6. Place the new seal on a flat surface with the open end facing up.
7. Press the plunger tip into the hole in the seal until the seal snaps into place.
8. Wet the seal and insert the plunger into the syringe barrel.
9. Replace the syringe as described in “Replacing the syringe” on page 9-36.

Replacing the syringe

To replace the syringe:

1. Place the hole in the base of the plunger over the pin on the syringe piston (Figure 9-18).

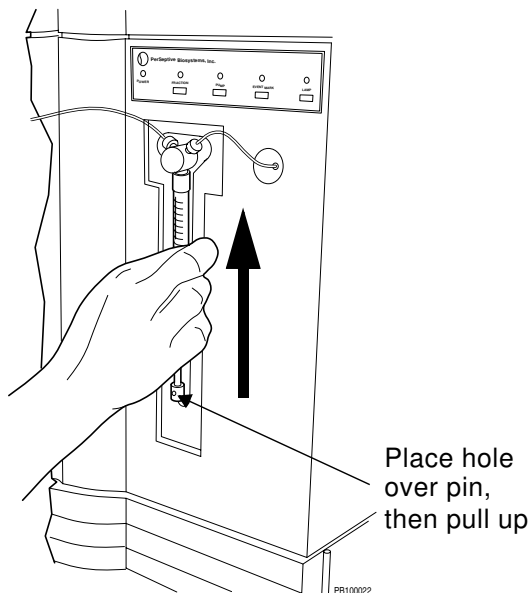


Figure 9-18 Replacing the Syringe

2. Pull up on the syringe body until it reaches the fitting.
3. While pushing up gently, turn the syringe body to the right and screw into place.
4. Replace the knurled knob in the plunger base.
5. Click **OK** in the Set Syringe Size dialog box.

The syringe plunger returns to the home position.

9.8 Valve Maintenance

Clean the injection and column switching valve stators every six months.

Cleaning stators To clean the injection and column switching valve stators:

1. Unscrew all fittings from the injection and column switching valves.
2. Using a 9/64-inch hex wrench, unscrew the three screws holding the metal stator support ring in place. Remove the support ring.
3. Remove the front of the injection and column switching valves. The front piece includes the stator face assembly (PEEK). Separate the two pieces of the valve front.

NOTE: *If the assembly does not include two pieces, the stator face assembly may be stuck in the valve. Remove it from the valve.*

4. Sonicate the two pieces in 30 percent isopropanol for ten minutes.
5. Rinse the two pieces with deionized water. Wipe with a lint-free tissue.
6. Reassemble by aligning the pins on the stator face assembly with the holes on the stator.
7. Place the reassembled pieces in the injection and column switching valves by aligning the pin in the injection and column switching valves with the hole in the stator.
8. Replace the stator support ring. Make sure the numbers on the ring are positioned properly with the number 1 at the top right and the number 2 at the top left.
9. Alternately tighten the three screws until finger-tight. Make sure equal pressure is applied to each screw.
10. Using a 9/64-inch hex wrench, tighten the screws 1/4-turn past finger-tight.

9.9 Autosampler Maintenance

Autosampler maintenance includes:

- Cleaning and replacing the autosampler needle
- Adjusting the needle height
- Cleaning the sample tray area
- Cleaning autosampler rails and rollers

9.9.1 Cleaning and Replacing the Autosampler Needle

Once a month, inspect the autosampler needle to see if it is plugged. If it is plugged, clean or sonicate the needle module. If cleaning does not unplug the needle, replace the needle.

This section describes:

- Removing and inspecting the needle
- Cleaning the needle module
- Installing the needle module

Removing and inspecting the needle

To remove and inspect the needle:

1. Power down the autosampler.
2. Open the autosampler door.
3. Remove the needle module from the holder by carefully unclipping it from the tower and pulling toward the rear of the autosampler (Figure 9-19).

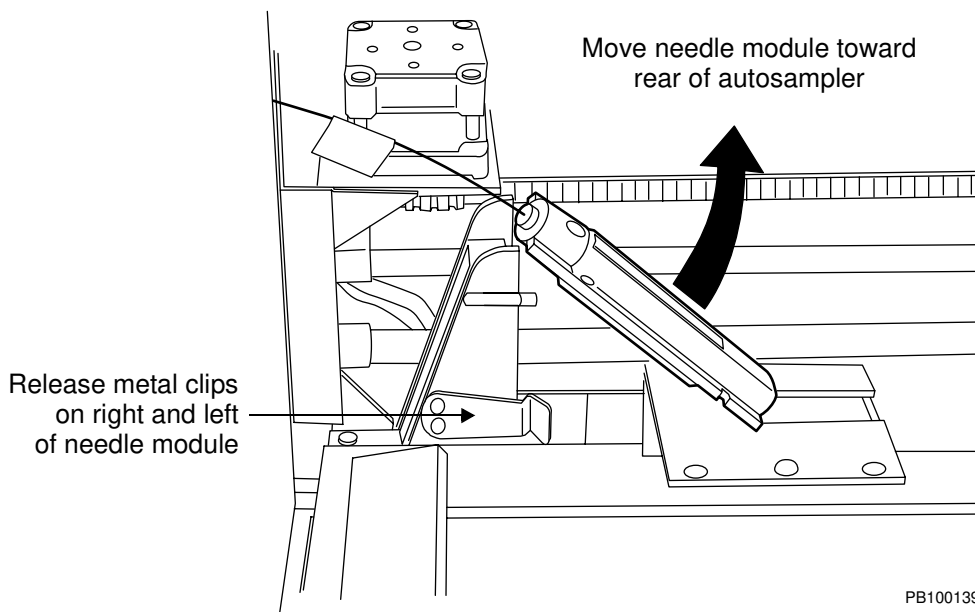


Figure 9-19 Removing the Autosampler Needle Module (Right Side View of Autosampler Area)

4. Look at the needle tip. If the bore is plugged, unscrew the needle line from the autosampler injector valve (port 2) using a 1/4-inch open-end wrench. Clean the needle module as described in “Cleaning the needle module” on page 9-40.

CAUTION

The needle line is very fragile. Do not bend or kink the line. The sample needle module is glued during manufacture. Do not try to disassemble.

Cleaning the needle module

To clean the autosampler needle module, flush it using the BioCAD 700E solvent pump:

WARNING

Wear safety glasses when performing this procedure.

AVERTISSEMENT

Veillez porter des lunettes de sécurité lors de cette opération.

1. Connect the needle line to the pump filter on the BioCAD 700E.

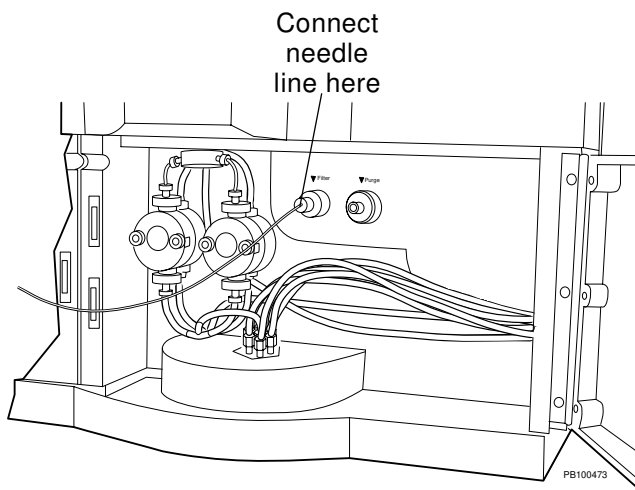


Figure 9-20 BioCAD 700E Pump Filter

2. Place buffer/solvent line A in water. Place the needle in a waste container.
3. On the BioCAD 700E Control Panel, select buffer/solvent channel A and start the pump at 0.1 ml/min.

NOTE: Observe the pump filter connection. If the connection is leaking, tighten with a wrench. Do not overtighten.

4. Do one of the following:

If fluid flows out of the needle	<ol style="list-style-type: none"> 1. Increase flow rate to 2 ml/min or 5 ml/min until you see a steady stream of liquid. 2. Stop pump flow. 3. Reinstall the needle module.
If fluid does not flow out of the needle	<ol style="list-style-type: none"> 1. Stop pump flow. 2. Disconnect the needle line from the pump filter. 3. Sonicate the needle module for 5 minutes. 4. Flush the needle again and check for flow. 5. If there is no flow, install a new needle module.

Installing the needle module

To install the needle module:

1. Snap the needle module into place on the sample tower. Position the needle module so that its label, "This side out", faces the sample tray area.
2. Thread the needle line through the hole in the front of the autosampler.
3. Screw the needle line into the autosampler injector valve (port 2). Tighten with a 1/4-inch open-end wrench.

If you are installing a new needle module, use the fitting and ferrule provided with the needle.

4. If you are installing a new needle module, adjust the needle height. See Section 9.9.2, Adjusting the Needle Height.

9.9.2 Adjusting the Needle Height

Adjust the needle height when you install a new needle.

To adjust the needle height:

1. Prime the autosampler lines with autosampler flush solution. For more information, see Section 3.6.5, Priming the Autosampler.

2. Close the BioCAD software if it is open.

3. Click the **Start** button, then click **Run**.

The Run dialog box is displayed.

4. Type **C:\BIOCAD\SYSDIAG.EXE** in the Open text box and click **OK**.

The BioCAD 700E System Level Diagnostics window is displayed.

5. Select **Autosampler Display** from the Windows menu.

The Autosampler window is displayed (Figure 9-21).

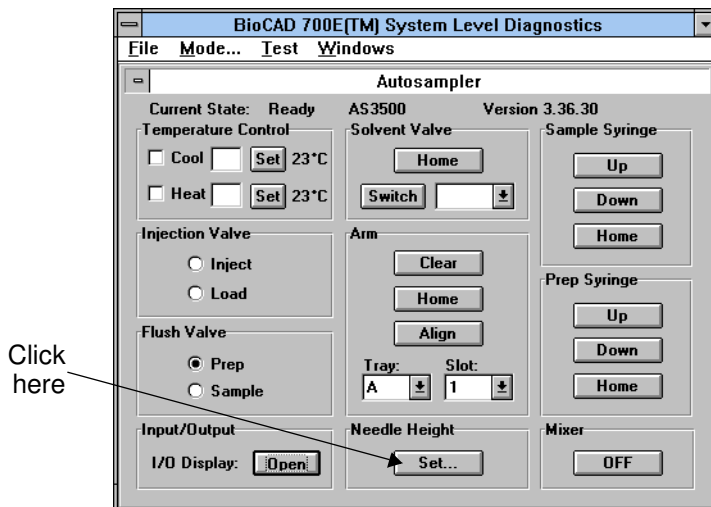


Figure 9-21 Autosampler Window

- When the Current State field displays Ready, click the **Set** button in the Needle Height section of the window.

A dialog box is displayed (Figure 9-22) listing the equipment you need to adjust the needle height.

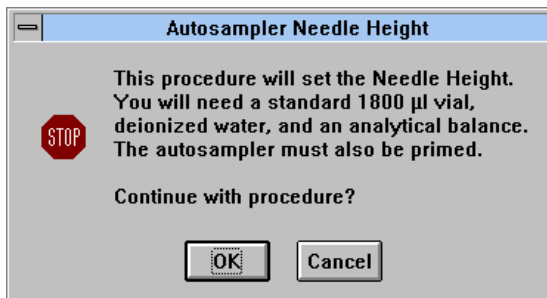


Figure 9-22 Autosampler Needle Height Equipment

- Click **OK** to continue the procedure.

The following Autosampler Needle Height dialog box is displayed (Figure 9-23).

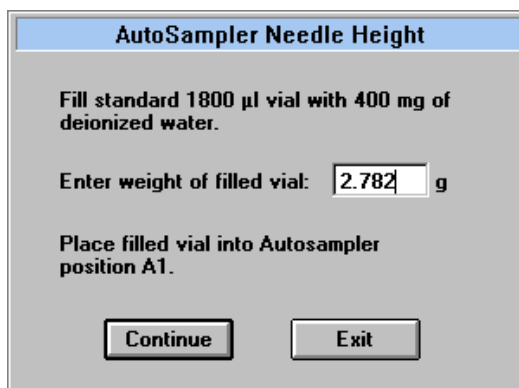


Figure 9-23 Autosampler Needle Height—Vial Weight

8. Fill a standard 1800 μl vial with 400 mg of deionized water, weigh it, and place the vial in autosampler position A1. Type the **weight** of the vial and contents in the weight text box. Click **Continue** and wait for the autosampler to make an injection from the vial in position A1.

The following Autosampler Needle Height dialog box is displayed (Figure 9-24).

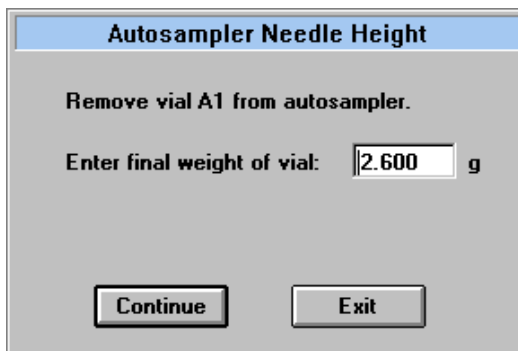


Figure 9-24 Autosampler Needle Height—Vial Weight

9. Remove the vial from position A1 and weigh the vial and contents. Type the **final weight** in the weight text box and click **Continue**.

The autosampler compares the pre-injection weight to the post-injection weight. The following Autosampler Needle Height dialog box is displayed with the adjusted needle height, which can be between 0.0 and 2.0 mm.



Figure 9-25 Autosampler Needle Height

10. Repeat step 6 through step 9.

After repeating the procedure, if the needle height adjustment is the same, a dialog box is displayed stating that the needle height is properly adjusted.

After repeating the procedure, if the needle height adjustment is not the same, repeat the procedure until you get the same result. If you cannot get the same result, check the following:

- Vial weighed correctly
- Weights entered correctly
- Needle installed properly
- Autosampler primed properly
- Leaks in the system

9.9.3 Cleaning the Sample Tray Area

Clean the sample tray area once a week.

To clean the sample tray area:

1. Remove autosampler trays.
2. Clean the autosampler tray area with a damp paper towel and dry out.
3. Clean the trays and replace.

9.9.4 Cleaning the Autosampler Rails and Roller

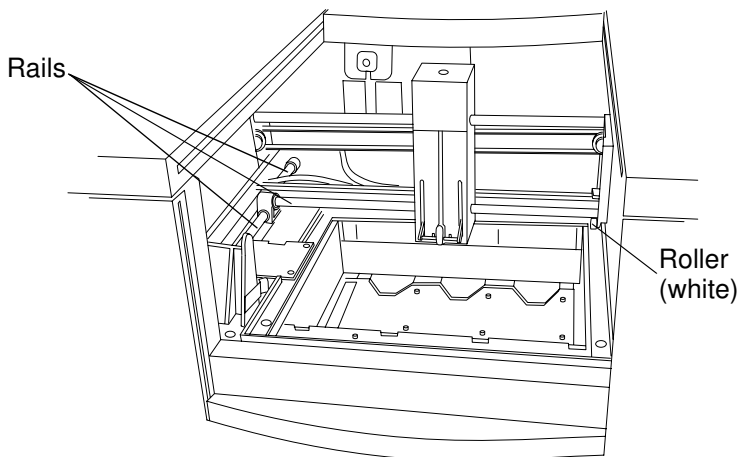
Every three months, clean the autosampler rails and rollers to ensure smooth operation of the autosampler arm.

CAUTION

Do not oil the rails or roller. Oiling these parts will cause dust to collect and jam the mechanism.

To clean the autosampler rails and rollers:

1. Remove autosampler trays.
2. Using a lint-free tissue, wipe the rails and rollers (Figure 9-26).



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Figure 9-26 Cleaning Autosampler Rails and Rollers

9.10 Hard Disk Maintenance

This section includes procedures for maintaining and configuring the hard disk on your workstation. It describes:

- Backing up and archiving data
- Testing hard disk integrity
- Defragmenting the hard disk

CAUTION

Do not compress the hard disk. Hard disk compression slows system performance. Also, if you have a hard disk failure, it is very difficult to retrieve data if the hard disk is compressed.

Operating system requirements

To perform the procedures in this section, your workstation must be running Windows 95.

BioCAD 700E Workstations are currently shipped with Windows 95 installed.

Viewing files

Use Windows Explorer to view the files stored in specific drives and directories.

Use the commands in the View menu to:

- View file details, such as the file size and date and time files were collected.
- Sort files. You can sort and display files by date, name, type, or size.

9.10.1 Backing Up and Archiving Data

Why back up data?

Back up data weekly, or as needed. Back up and archive data if the free disk space on your hard drive is less than 20%.

To run properly, the BioCAD software requires:

- Disk space for the data recorder
- Disk space for data files and method files
- Disk space for printing

If the disk becomes full, writing data files, printing, or saving methods may fail and can result in loss of data. The system also operates more slowly as free disk space decreases.

To maintain adequate disk space, back up and archive data on a routine basis:

- **Back up**—Temporarily store data in case of a hard disk problem.
- **Archive**—Permanently store important data for retrieval at a later time, and remove the original data from the hard drive.

Backing up Use the Backup utility to back up data.

The first time you run the Backup utility, it automatically configures itself for your system. You will need two blank 1.44 MB floppy disks so the Backup utility can test the floppy drive.

NOTE: *It is necessary to copy only data files, group files, method files, and multi-method files. You can retrieve configuration files from data files.*

To back up data:

1. Start Windows.
2. Click **Start**, point to **Programs**, point to **Accessories**, point to **System Tools**, and then click **Backup**.

The *Welcome to Microsoft Backup* window is displayed.

3. Follow the onscreen instructions.

Archiving On a regular basis (frequency depends on your workload and amount of data), copy data files from completed projects to a floppy disk or other backup media. Use Windows Explorer to copy data. For instructions on using Windows Explorer, see the *Microsoft Windows User's Guide*.

After copying the data to backup media, delete it from your hard disk. Make sure to properly label the media.

9.10.2 Testing Hard Disk Integrity

Once a week, run ScanDisk to verify the hard disk drive integrity and repair it if necessary.

To test disk integrity:

1. Start Windows.
2. Click **Start**, point to **Programs**, point to **Accessories**, point to **System Tools**, and then click **ScanDisk**.

The ScanDisk dialog box is displayed.

3. Select **Thorough** and click **Start**.

NOTE: *Selecting Thorough can take over one hour to complete. Run ScanDisk when you will not be using the workstation.*

4. Follow the onscreen instructions.

9.10.3 Defragmenting the Hard Disk

Why defragment the hard disk?

Once a month (or as needed), defragment your hard disk to optimize disk performance.

When your workstation is first installed, each file uses sequential (contiguous) blocks on the hard disk. The free space on the disk is also sequential. This provides the fastest access because the whole file can be read without searching more than one area of the disk.

As files are created and deleted from the disk, and disk blocks are reused, the disk becomes fragmented. New files no longer use sequential blocks on the disk, and it takes longer to access these files. In addition, the system must search the disk for free space when creating new files.

Defragmenting the disk reorganizes the files so they are sequential on the disk. Defragmenting can substantially improve system speed, especially if the disk is badly fragmented.

Defragmenting

To defragment the hard disk:

1. Free up as much disk space as possible by backing up and deleting unneeded files. For more information, see Section 9.10.1, Backing Up and Archiving Data.
2. Delete all files from the C:\BIOCAD\TEMP directory.

NOTE: Files in the C:\BIOCAD\TEMP directory are Strip Chart Recorder files. You lose the contents of the Strip Chart Recorder when you delete these files.

3. Click **Start**, point to **Programs**, point to **Accessories**, point to **System Tools**, then click **Disk Defragmenter**.

The Select Drive dialog box is displayed.

4. Follow the onscreen instructions.

9.11 Long Term Shutdown

If you are shutting down the BioCAD 700E Workstation for more than one week:

1. Click the **Bypass** option button in the Column section of the Control Panel, to take the column out of line.

NOTE: *If you select Low Dispersion, Three Columns, or Direct Control column plumbing configurations, you cannot bypass the column. Replace the columns with unions before running the SYSCLEAN method.*

2. Purge the system with a 1 M salt solution to prepare the pH and conductivity monitors for shutdown. For more information, see Section 5.2.4, Purging the System.
3. Run the **SYSCLEAN** method as described on page 2-38.

NOTE: *As an alternative to running the SYSCLEAN method, you can purge the system with water from the Control Panel. Take the pH monitor offline by clicking the **Bypass** option button in the pH/Cond section of the Control Panel. Then purge the system with filtered deionized water. For more information, see Section 5.2.4, Purging the System.*

4. Purge the system with a solution of water and isopropanol (80:20). For more information, see Section 5.2.4, Purging the System.
5. Remove the pH probe and store as described in Section 9.5, pH and Conductivity Monitor Maintenance.
6. Exit the BioCAD software by selecting **Exit BioCAD** from the File menu in the Control Panel.
7. Exit Microsoft Windows by clicking **Start**, then **Shut Down**.

8. Power down the components of the workstation in any order.
9. Set the Helium On/Off switch to the **Off** position.
10. Power down the BioCAD 700E Workstation.
11. Turn off all gas and helium sources.

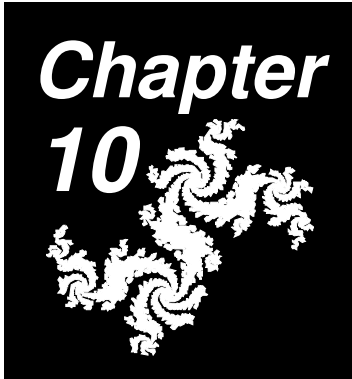
CAUTION

Do not close the helium tank without turning off the helium switch. Helium diffuses out of the line, forming a vacuum, which pulls liquid into the sparging system.

NOTE: *If you shut down the system for an extended time period, you may want to remove the pH probe. Store the probe in the storage solution (saturated KCl) and protective container provided with the probe.*

9

10 Troubleshooting and Error Codes



This chapter includes the following sections:

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10.1.2	Control Panel Troubleshooting	10-12
10.1.3	Status Window Troubleshooting	10-15
10.1.4	Strip Chart Recorder Troubleshooting	10-15
10.1.5	Fraction Collector Troubleshooting	10-18
10.1.6	Integrated Autosampler (AS 3500) Troubleshooting	10-20
10.1.7	Sample Table Troubleshooting	10-28
10.1.8	Method Editor Troubleshooting	10-29
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10.2	Error Codes.....	10-37

10.1 Troubleshooting

This section contains troubleshooting information for:

- System
- Control Panel
- Status window
- Strip Chart Recorder
- Fraction Collector
- Integrated autosampler
- Sample Table
- Method Editor
- Templates
- Computer

Technical support

If you cannot solve problems using the guidelines in the following sections, call Applied Biosystems Technical Support. To reach Applied Biosystems Technical Support, refer to the back cover of this book.

10.1.1 System Troubleshooting

Table 10-1 System Troubleshooting

Symptom	Possible Cause	Action
No fluid flowing from the BioCAD 700E waste line	Buffer/solvent line not in reservoir	Place line in reservoir and secure. Prime lines. See Section 3.4.2, Priming the Buffer/Solvent Lines.
	Reservoirs empty	Fill reservoir. Prime lines. See Section 3.4.2, Priming the Buffer/Solvent Lines.
	Lines not primed	Prime buffer/solvent lines. See Section 3.4.2, Priming the Buffer/Solvent Lines.
	Leak in flow path	Check all fittings for leaks.
	Wrong valve plumbed to waste	Check both ports on fraction/waste valve. Bottom port should be plumbed to waste. Replumb if necessary.
Erratic flow and short-term pressure fluctuations	Buffers/solvents not sparged	Sparge buffers/solvents. See Section 3.2.1, Preparing Buffers/Solvents.
	Air bubbles in line	Prime buffer/solvent lines. See Section 3.4.2, Priming the Buffer/Solvent Lines.
	Check valves leaking	Clean check valves. See Section 9.6.2, Cleaning the Check Valves.



Table 10-1 System Troubleshooting (Continued)

Symptom	Possible Cause	Action
High pressure shutdown	Tubing plugged or crimped	Flush tubing, uncrimp. Replace tubing if necessary. See Section 3.1.3, Plumbing Tubing and Columns.
	High Pressure limit is set too low in the Configuration dialog box	Increase high pressure limit. See Section 4.2.1, Configuring the Pump.
	Column option set in the Configuration dialog box does not match physical plumbing. For example, the workstation is plumbed with two columns, and you have one column configured.	Check column plumbing and column option selected in the Configuration dialog box. Adjust. See Section 4.2.4, Configuring Columns and Plumbing.
	Column pressure set too low in configuration dialog box	Adjust column pressure limit. See Section 4.5.3, Customizing the Column Library.
	Column or column frit plugged	Clean column.
		Wash column, replace column frit.
		Replace column.
Pump filter frit on BioCAD 700E Workstation plugged	Clean or replace frit. See Section 9.6.3, Cleaning the Filter Frit.	

Table 10-1 System Troubleshooting (Continued)

Symptom	Possible Cause	Action
High pressure shutdown (continued)	Ports on sample injection valve or column switching valves are blocked	Identify which port is blocked and backflush valve by reversing appropriate plumbing.
		Clean or replace stators. See page 9-37.
	UV/Vis detector flow cell blocked	Backflush detector flow cell by switching inlet and outlet connections and starting pump.
	Wrong tubing or flow cell for flow rate range	Change tubing. See Section 3.1.1, Selecting Tubing Diameter and Length, for tubing diameter/flow rate ranges.
Change flow cell. See Section 9.4.3, Changing the UV/Vis Detector Flow Cell.		
Gradient not linear	Buffers/solvents not sparged	Sparge buffers/solvents. See Section 3.2.1, Preparing Buffers/Solvents.
	Buffer/solvent lines not primed	Prime buffer/solvent lines. See Section 3.4.2, Priming the Buffer/Solvent Lines.
Gradient delayed	Tubing diameter too large for application	Change tubing. See Section 3.1.1, Selecting Tubing Diameter and Length, for tubing diameter/flow rate ranges.

Table 10-1 System Troubleshooting (Continued)

Symptom	Possible Cause	Action
Unexpected gradient performance	Gradient not programmed properly	Check method and Blend Graph. Run gradient from Control Panel to duplicate error.
	Blend settings on Control Panel Settings not activated until you click Set .	Click Set .
	See “Gradient not linear” causes and actions	_____
No peaks	Buffer/solvent lines not purged properly, you did not click Set after changing blend settings	When purging from the Control Panel, click Set after changing settings.
	UV/Vis lamp off	Turn UV/Vis lamp on.
	No flow	Start pump.
	Reservoirs empty	Fill reservoirs. Prime lines. See Section 3.4.2, Priming the Buffer/Solvent Lines.
	Wrong buffer/solvent configured for your application	Adjust configuration. See Section 4.2.2, Configuring Buffers/Solvents.
	Wrong buffer/solvent used for your application, no peaks eluting from column	Change buffer/solvents.
	Wrong wavelength set in method or Control Panel	Adjust wavelength.

Table 10-1 System Troubleshooting (Continued)

Symptom	Possible Cause	Action
No peaks (continued)	Problems with chemistry or application	Address problems with chemistry or application.
	Sample proteins bound to filter during sample preparation	Use low-protein-binding filter.
	Non-specific absorbance to column	Determine by bypassing column and making injection from Control Panel. If peak is present when column bypassed, reevaluate column or mobile phase chemistry.
	No sample injected	See “No sample injected” causes and actions below.
No sample injected	Wrong sample channel selected	Select proper sample channel (1, 2, 3, or 4) in the Control Panel or method. See Section 5.3.1, Making Autoloader Injections, or Section 6.3.2, Making Autoloader Injections in a Method.
	Sample lines not primed	Prime autoloader sample lines. See Section 3.4.3, Priming and Flushing the Autoloader Sample Lines, or “Sample Loader Events” on page 6-42.

Table 10-1 System Troubleshooting (Continued)

Symptom	Possible Cause	Action
Chromatogram run in single wavelength mode between 366 nm to 450 nm differs from chromatogram run in dual-wavelength mode at same wavelength.	In single wavelength mode, wavelength ranges for lamps are: <ul style="list-style-type: none"> • Deuterium—190 to 365 • Tungsten—366 to 800 In dual-wavelength mode, wavelength ranges for lamps are: <ul style="list-style-type: none"> • Deuterium—190 to 450 • Tungsten—366 to 700 	No action. Lower sample rate in dual mode caused by the grating movement between wavelengths.
Beeping and clicking noise from internal UV/Vis detector	Motorized grating is switching between wavelengths in dual-wavelength mode	No action, normal occurrence.
Pump shuts down	“Pump Off” or “Set Flow Rate = 0” events added to method	Check method for “Pump Off” or “Set Flow Rate = 0” events.
	See “High pressure shutdown” causes and actions	_____
No signal collected on UV/Vis #2 detector channel	UV/Vis #2 channel not enabled	Enable UV/Vis #2 channel in Configuration dialog box. See Section 4.2.5, Configuring Detectors.
	No external detector selected in BioCAD hardware setup	Update hardware setup. See Section 2.5.2, Updating BioCAD Hardware Setup Options.

Table 10-1 System Troubleshooting (Continued)

Symptom	Possible Cause	Action
Excessive noise in chromatogram	Buffers/solvents not sparged	Spurge buffers/solvents. See Section 3.2.1, Preparing Buffers/Solvents.
	Electronic interference	Make sure other equipment connected to outlet is well-grounded. Use surge protector.
	UV/Vis flow cell plumbed incorrectly	Remove the flow cell cover and verify the inlet tubing is plumbed to the bottom of the flow cell and outlet tubing to the top of the flow cell. Reversed connections may trap air bubbles in the flow cell. See Section 9.4.3, Changing the UV/Vis Detector Flow Cell
	Dirty or cracked UV/Vis flow cell	Clean or replace flow cell. See Section 9.4.2, Cleaning the UV/Vis Detector Flow Cell, or Section 9.4.3, Changing the UV/Vis Detector Flow Cell.
	Low UV/Vis detector lamp energy	Replace UV/Vis lamp. See Section 9.4.1, Changing the UV/Vis Detector Lamps.
	Buffer in use contains UV-absorbing compound	Use buffer/solvent without UV-absorbing compound.
	Tubing, column, injection or column switching valves dirty	Replace tubing. Clean column. Clean valve stators See page 9-37.



Table 10-1 System Troubleshooting (Continued)

Symptom	Possible Cause	Action
pH response slow or not accurate	Dirty pH probe	Clean pH probe. See Section 9.5.1, Cleaning the pH Probe and Cell.
	pH probe not seated properly	Reinstall the pH probe. See "Replacing pH probe" on page 9-18.
	pH probe not properly calibrated	Calibrate the pH probe. See Section 9.5.2, Calibrating the pH Probe.
	pH monitor bypassed	Place pH monitor inline by doing one of the following: <ul style="list-style-type: none"> Adjust General Settings in method to place pH monitor inline. Click Inline option button in pH/Cond section of Control Panel.
	pH cell dirty	Clean pH cell. See Section 9.5.1, Cleaning the pH Probe and Cell.
	Buffers do not have buffering capacity at the working pH	Monitor pH with pump flowing and column bypassed.
	PH.CAL file corrupt	Delete PH.CAL file (in BIOCAD directory) and reboot computer.

Table 10-1 System Troubleshooting (Continued)

Symptom	Possible Cause	Action
Conductivity monitor response not accurate	Conductivity monitor not properly calibrated	Calibrate conductivity monitor. See Section 9.5.4, Calibrating the Conductivity Monitor.
	Conductivity monitor bypassed	Place conductivity monitor inline by doing one of the following: <ul style="list-style-type: none"> • Adjust General Settings in method to place conductivity monitor inline. • Click Inline option button in pH/Cond section of Control Panel.
	Blockage in conductivity flow cell	Backflush flow cell by switching inlet and outlet connections and starting pump.
No data collected during Inject or Autosampler Inject segment	There is a Data Collection End event in the method before the Inject or Autosampler Inject segment	Delete the Data Collection End event.
		Move the Data Collection End event after the Inject or Autosampler Inject segment.
UV/Vis detector resets to single wavelength at end of method	When you run a method with dual wavelength enabled, the setting changes to single wavelength at the end of a method to reduce wear of the detector grating	No action, normal occurrence.

10.1.2 Control Panel Troubleshooting

Table 10-2 Control Panel Troubleshooting

Symptom	Possible Cause	Action
In pH blend mode, no pH text box in Blend section of Control Panel (% text box instead)	Buffers/solvents configured are uncalibrated custom pH blend buffers	Configure either of the following buffers/solvents: <ul style="list-style-type: none"> • Calibrated custom pH blend buffers • pH blend buffers See Section 3.2.2, Preparing pH Blend Mode Buffers, and Section 4.2.2, Configuring Buffers/Solvents.
pH dimmed on Blend menu	pH mode is available only if you specify buffer/solvents as the following types in the Buffer/Solvent Library: <ul style="list-style-type: none"> • pH buffers—must be of type Buffer • Water—must be of type H₂O • Salt solution—must be of type Eluent 	Adjust type of buffers/solvents in Buffer/Solvent Library and add them to the Configuration dialog box. See Section 4.2.2, Configuring Buffers/Solvents, and Section 4.5.1, Customizing the Buffer/Solvent Library.
Eluent dimmed on Blend menu	Eluent mode is available only if you specify a buffer/solvent as type Eluent in the Buffer/Solvent Library	

Table 10-2 Control Panel Troubleshooting (Continued)

Symptom	Possible Cause	Action
Changes you make to blend settings in Blend section of Control Panel are not activated	Settings not activated until you click Set	Click Set after changing blend settings on Control Panel.
Flow rate units CV/min and cm/hr dimmed in Pump section of Control Panel	Column not configured	Configure a column in the Configuration dialog box. See Section 4.2.4, Configuring Columns and Plumbing.
When pump is running, flow change you make not initiated	Change not initiated until you click on another area on the Control Panel	Click on another area of Control Panel.
Method section of Control Panel blank	No method running	No action, normal occurrence.
Fraction/waste valve on BioCAD 700E not responding when you click option buttons in Collector section of Control Panel	Fraction collector in use. When fraction collector is configured, option buttons control the diverter valve on the fraction collector, not the BioCAD 700E fraction/waste valve.	Disable fraction collector in configuration by selecting Built In Fraction/Waste Valve in Collector section of Configuration dialog box.
No Fraction Collector Setup command on Config menu	Fraction Collector not configured	Adjust configuration. See Section 4.6, Configuring and Preparing the BioCAD Software for a Fraction Collector.

Table 10-2 Control Panel Troubleshooting (Continued)

Symptom	Possible Cause	Action
Cannot select UV/Vis Dual Wavelength from the Detector menu	An external fluorescence detector is enabled in hardware setup	Disable the external fluorescence detector in the hardware setup. See Section 2.5.2, Updating BioCAD Hardware Setup Options.
Cannot select UV/Vis Single Wavelength from the Detector menu	An external UV/Vis detector is enabled in hardware setup	Disable the external UV/Vis detector in the hardware setup. See Section 2.5.2, Updating BioCAD Hardware Setup Options.

10.1.3 Status Window Troubleshooting

Table 10-3 Status Window Troubleshooting

Symptom	Possible Cause	Action
Wrong detectors displayed in Status window	Status window not set to display desired signals	Select the signals to display from the View menu. Selected signals are checked. Only the first three selected signals are displayed.

10.1.4 Strip Chart Recorder Troubleshooting

Table 10-4 Strip Chart Recorder Troubleshooting

Symptom	Possible Cause	Action
Cannot scroll Strip Chart Recorder	Strip Chart Recorder is in Real-Time mode	Change Strip Chart Recorder to History mode by doing one of the following: <ul style="list-style-type: none"> • Deselect Real-Time from Display menu in Strip Chart Recorder. • Click on the Strip Chart Recorder.

Table 10-4 Strip Chart Recorder Troubleshooting (Continued)

Symptom	Possible Cause	Action
pH/conductivity signal on Strip Chart Recorder does not change	Buffers do not have buffering capacity at the working pH	Use appropriate buffers.
	Detectors bypassed in General Settings in method	Place monitors inline in General Settings section of method. See Section 6.2.3, Specifying General Settings.
	Detectors bypassed on Control Panel	Click Inline option button in pH/Cond section of Control Panel.
pH/conductivity signal not shown on Strip Chart Recorder	pH and conductivity channels are not selected in Strip Chart Recorder Settings	Change Strip Chart Recorder Settings by selecting Options from the Display menu. See Section 8.3, Using the Strip Chart Recorder.
	pH and conductivity channels are not configured	Enable the pH and conductivity channels in the Configuration dialog box. See Section 4.2.5, Configuring Detectors.

Table 10-4 Strip Chart Recorder Troubleshooting (Continued)

Symptom	Possible Cause	Action
Data not displayed on Strip Chart Recorder as it is acquired	Strip Chart Recorder is not in Real-Time mode	Place Strip Chart Recorder in Real-Time mode by selecting Real-Time from the Display menu.
	Data offscale	Adjust scale by selecting Options from the Display menu and changing scale. See “Customizing the Strip Chart Recorder” on page 8-9.
	No channels selected in Strip Chart Settings	Enable channels by selecting Options from the Display menu. See “Customizing the Strip Chart Recorder” on page 8-9.
	Detector data channels are not enabled in the Recorder section of the Configuration dialog box	Enable data channels in the Recorder section of the Configuration dialog box. See Section 4.2.5, Configuring Detectors.

10.1.5 Fraction Collector Troubleshooting

Table 10-5 Fraction Collector Troubleshooting

Symptom	Possible Cause	Action
No Fraction Collector Setup command on Config menu	Fraction Collector not configured	Adjust configuration. See Section 4.6, Configuring and Preparing the BioCAD Software for a Fraction Collector.
Unable to change settings on Fraction Collector front panel	Cannot use Fraction Collector front panel when it is configured in BioCAD software	To program the fraction collector front panel, you must do one of the following: <ul style="list-style-type: none"> • Disable fraction collection in the configuration by selecting Built In Fraction/Waste Valve in Collector section of Configuration dialog box • Disconnect fraction collector cable from the BioCAD 700E Workstation • Exit the BioCAD software
Fraction Collector settings in Control Panel not used during method run	During a run, fraction collector settings in method override settings in Control Panel	No action, normal occurrence.
Non-peak fractions not collected at proper times	Incorrect delay volume set for Fraction Valve to Fraction Collector	Adjust delay volumes in Configuration dialog box. See “Setting delay volumes” on page 4-13.

Table 10-5 Fraction Collector Troubleshooting (Continued)

Symptom	Possible Cause	Action
Fractions not deposited cleanly into tubes	Rack not positioned properly in fraction collector	Test tube rack has tabs that line up with the base of the fraction collector. Make sure to push the rack all the way back against the base.
	Tubes not positioned properly in rack	Make sure all test tubes are centered in the slots and are pointing straight up.
	Outlet on fraction collector diverter valve is dirty or plugged	Clean diverter valve.
	Fraction collector cables or tubing caught on fraction collector arm	Untangle cables or tubing.
Fraction collector is not filling tubes in the correct order	Number of tubes in fraction collector software not set to 120 (if using Simple mode)	Change number of tubes in fraction collector software. See Section 3.5.3, Preparing Fraction Collector Software.
	Wrong number or wrong type of tubes, or wrong mode (Simple or Bottle) programmed in fraction collector software	Reprogram fraction collector. See Section 3.5.3, Preparing Fraction Collector Software.
Fraction collector tubes overfilling	Volume (ml/tube) set in Configuration dialog box exceeds tube volume.	Adjust configuration. See Section 4.6, Configuring and Preparing the BioCAD Software for a Fraction Collector.

Table 10-5 Fraction Collector Troubleshooting (Continued)

Symptom	Possible Cause	Action
When you click the Advance button on the Control Panel with a fraction collector configured, fraction collector does not respond immediately	When the pump is flowing, the fraction collector does not advance until the delay volume specified in configuration is exceeded	No action, normal occurrence.

10.1.6 Integrated Autosampler (AS 3500) Troubleshooting

Table 10-6 Integrated Autosampler (AS 3500) Troubleshooting

Symptom	Possible Cause	Action
Autosampler arm jam	After the autosampler picked up a vial, you placed a new vial in the temporarily empty location	<ol style="list-style-type: none"> 1. Remove vial from the current position and from the autosampler arm. 2. Place vial removed from arm in proper tray position. 3. Select Edit Configuration from the Control Panel and click OK after the configuration is displayed. 4. Restart method.

Table 10-6 Integrated Autosampler (AS 3500) Troubleshooting (Continued)

Symptom	Possible Cause	Action
Autosampler arm jam (continued)	Vial dropped	<ol style="list-style-type: none"> 1. Pick up vial and place in proper tray position. 2. Select Edit Configuration from the Control Panel and click OK after the configuration is displayed. 3. Restart method.
	Vial not seated properly in tray	<ol style="list-style-type: none"> 1. Pick up vial and place in proper tray position. 2. Select Edit Configuration from the Control Panel and click OK after the configuration is displayed. 3. Restart method.
	Sample tray misaligned	Check that sample tray is seated properly on pins. It may be necessary to remove the tray and then place it back in position. See “Installing sample trays” on page 3-55.
	Vial stuck in autosampler tower	See “Vial stuck in autosampler tower” on page 10-26.
No vial in autosampler arm	Vial not in correct position of tray	Place vial in the correct position. Restart method.
	Vial dropped	Replace vial and check the sample tray alignment. Restart method.

Table 10-6 Integrated Autosampler (AS 3500) Troubleshooting (Continued)

Symptom	Possible Cause	Action
Autosampler drops vial when returning to sample tray	Autosampler arm is misaligned	Call Applied Biosystems Technical Support.
Sample Table does not appear in Window menu	Integrated autosampler is not configured	Select an AS 3500 integrated autosampler in hardware setup. See Section 2.5.2, Updating BioCAD Hardware Setup Options.
Autosampler warm when autosampler cooler (tray) is turned on	Normal operation. Air above sample trays is often warm.	Touch bottom of tray with your hand to check temperature. If it is cool, cooling is operating properly. If it is not cool, select Sample Cooler from the Sample menu in the Sample Table window. Verify that the cooler is turned on and the temperature is set correctly.
	Temperature control is turned off or setpoint temperature is higher than ambient temperature	Select Sample Cooler from the Sample menu in the Sample Table window. Verify that the cooler is turned on and the temperature is set correctly.
	You did not allow sufficient time for autosampler cooler (tray) to reach temperature	Allow 30 minutes for autosampler cooler (tray) to reach temperature.

Table 10-6 Integrated Autosampler (AS 3500) Troubleshooting (Continued)

Symptom	Possible Cause	Action
<p>Selected temperature does not match temperature displayed in Sample Table window</p>	<p>Temperature control is turned off</p>	<p>Select Sample Heater or Sample Cooler from the Sample menu in the Sample Table window. Verify that the heater or cooler is turned on and the temperature is set correctly. See “Controlling sample heating and cooling” on page 5-50.</p>
	<p>You did not allow sufficient time for autosampler heater (heat chamber) or cooler (tray) to reach temperature</p>	<p>Allow 30 minutes for autosampler heater (heat chamber) or cooler (tray) to reach temperature.</p>
<p>Method runs, but autosampler does not inject sample</p>	<p>Sample Table has incorrect sample vial volume</p>	<p>Check sample vial volume in configuration by double-clicking on a vial position in the Sample Table to display the Vial Editor. Make sure sample vial volume is greater than injection volume set in method Autosampler Inject (AI) segment. For more information, see Section 4.7.3, Entering Vials in Sample Table.</p>
<p>Autosampler does not pick up vial when running a method</p>	<p>Autosampler heater (heat chamber) or cooler (tray) has not reached set temperature</p>	<p>Allow 30 minutes for autosampler heater (heat chamber) or cooler (tray) to reach temperature.</p>
	<p>Autosampler door is open</p>	<p>Close autosampler door.</p>

Table 10-6 Integrated Autosampler (AS 3500) Troubleshooting (Continued)

Symptom	Possible Cause	Action
Autosampler chooses wrong vial when you run a template other than the Analysis template	If the sample volume in the vial you select in the template is insufficient to make the injection, the system automatically injects from another vial with the same sample name and sufficient volume (if one exists)	No action. Normal occurrence.
Air bubbles in autosampler syringes	Autosampler solvent or flush bottles are empty	Fill bottles and prime lines. See Section 3.6.5, Priming the Autosampler.
	Autosampler lines not primed	Prime autosampler lines. See Section 3.6.5, Priming the Autosampler.
	Fittings above autosampler syringes loose	Hand-tighten fittings.
	Autosampler syringes loose	Hand-tighten syringes.
	Autosampler syringes leaking	Call Applied Biosystems Technical Support.
Autosampler syringes leaking, and you can turn the syringe barrel without turning the metal band at the top of the barrel	Seal broken between barrel and metal band	Call Applied Biosystems Technical Support.

Table 10-6 Integrated Autosampler (AS 3500) Troubleshooting (Continued)

Symptom	Possible Cause	Action
Integrated AS 3500 not available in Autosampler section of Configuration dialog box	Integrated AS 3500 autosampler is not specified in hardware setup	Update hardware setup. See Section 2.5.2, Updating BioCAD Hardware Setup Options.
Autosampler does not run startup routine when BioCAD 700E Workstation is powered up	Hardware fault	Power down and power up autosampler. If symptom still present, call Applied Biosystems Technical Support.
Autosampler does not respond when you control it from the BioCAD software	Communications failure	Reboot BioCAD software.
		Power down and power up BioCAD 700E Workstation.
		Call Applied Biosystems Technical Support.
Loud buzzing noise from autosampler syringe area	Defective switch	Call Applied Biosystems Technical Support.
Loud noise prior to injection	Autosampler is mixing sample	No action. Normal occurrence.
Autosampler motor does not stop after autosampler syringe returns to home position	Defective sensor	Call Applied Biosystems Technical Support.

Table 10-6 Integrated Autosampler (AS 3500) Troubleshooting (Continued)

Symptom	Possible Cause	Action
Autosampler hook does not lift vial	Autosampler arm motor failed	Call Applied Biosystems Technical Support.
Vial stuck in autosampler tower	Autosampler needle bent	Replace needle. See the <i>Autosampler Reference Manual</i> .
	Non-recommended vial or septa	Remove vial and replace with recommended vial and septa. For more information, see Section 3.6.6, Preparing Sample Vials.
Vial stuck in mixer	Communications error	<ol style="list-style-type: none"> 1. Power down workstation. 2. Move autosampler arm out of the way. 3. Remove vial. 4. Power up and restart method.
Septa on sample vials torn	Autosampler needle bent	Replace needle. See the <i>Autosampler Reference Manual</i> .
Autosampler stops during an injection	Autosampler door is open	Close autosampler door.
Autosampler is not making an injection when instructed to do so by a method or from the Sample Table	Communication error between computer and autosampler	Reset configuration by displaying the Configuration dialog box, then clicking OK .

Table 10-6 Integrated Autosampler (AS 3500) Troubleshooting (Continued)

Symptom	Possible Cause	Action
Autosampler not injecting accurate volume	Air bubbles in autosampler lines	Prime autosampler lines. See Section 3.6.5, Priming the Autosampler.
	Air bubbles in autosampler syringes	
	Teflon plunger in autosampler syringe (sample or prep syringe, depending on injection volume) not sealing	Call Applied Biosystems Technical Support.
Autosampler makes incomplete injection	You installed a new sample needle on the autosampler, but did not adjust the needle height	Adjust the needle height. See Section 9.9.2, Adjusting the Needle Height.
Autosampler aspirates air instead of injecting sample		

10.1.7 Sample Table Troubleshooting

Table 10-7 Sample Table Troubleshooting

Symptom	Possible Cause	Action
Sample Table does not appear in Window menu	Integrated autosampler is not configured	Select an AS 3500 integrated autosampler in hardware setup. See Section 2.5.2, Updating BioCAD Hardware Setup Options.
Autoname check box is dimmed in the Vial Editor dialog box	You did not use the Add Vials button to add sample vials to the Sample Table	Click Add Vials after selecting vials in the Sample Table. See “Autonaming batch-entered vials” on page 4-50.

10.1.8 Method Editor Troubleshooting

Table 10-8 Method Editor Troubleshooting

Symptom	Possible Cause	Action
In Method Editor, values for column volume too large, or duration too long	Column dimensions set incorrectly in Configuration dialog box	Adjust column dimensions in Configuration dialog box. See Section 4.2.4, Configuring Columns and Plumbing, and Section 4.5.3, Customizing the Column Library.
Method events not displayed in blocks	Method Editor is in Block view, and events displayed in Sequence view only	Select Sequence View from the Display menu.
Changes to system configuration not recognized by open method in Method Editor	Changes made to system configuration are not recognized by method configuration unless you update the method configuration	To recognize changes to the system configuration after method is saved, select Update Configuration from the Options menu in the Method Editor.
System not using method configuration when running method	During a run, the workstation <i>always</i> uses the system configuration set in the Configuration dialog box, not the method configuration saved with the method. For a description of each configuration, see Section 4.1, Configuration Overview.	Load the method configuration as the system configuration. See Section 4.4, Loading an Existing Configuration.

Table 10-8 Method Editor Troubleshooting (Continued)

Symptom	Possible Cause	Action
During a run, method Step segment skipped	In Step segment, you set thresholds, but did not set a delay. At beginning of segment, peak has not yet eluted therefore threshold criteria met (signal is above or below the threshold) and so segment is skipped.	Set Delay for threshold in Step segment. See "Setting thresholds and delay durations" on page 6-22.
Block buttons at top of window dimmed	Buttons not active when blocks or segments selected	Click on line below the last block in method to activate block buttons.
Segment buttons at top of window dimmed	Buttons active only when appropriate block selected	Click on block in method to activate appropriate segment buttons.
Second Data Collection Start event in method ignored	Method can have only one contiguous segment of recorded data	Remove second Data Collection Start event from method.
In Blend Graph Setup, pH and Eluent dimmed in Mode section	pH mode is available only if you specify two pH buffers as type Buffer, water as type H ₂ O, and a salt solution as type Eluent in the Buffer/Solvent Library. Eluent mode is available only if you specify a buffer/solvent as type Eluent in the Buffer/Solvent Library.	Adjust configuration. See Section 4.2.2, Configuring Buffers/Solvents, and Section 4.5.1, Customizing the Buffer/Solvent Library.

Table 10-8 Method Editor Troubleshooting (Continued)

Symptom	Possible Cause	Action
During a run, software prompts you for a data file name before every injection	You did not specify Data Storage options	Before a run, select Data Storage from the Options menu. Specify file name, extension, and directory. See Section 6.2.10, Specifying Data Storage Options.
	In Data Storage Options, you selected Name File When Run	Select Auto file extension in Data Storage Options. See Section 6.2.10, Specifying Data Storage Options.
Method duration longer or shorter than expected	Column dimensions set incorrectly in Configuration dialog box	Adjust column dimensions in Configuration dialog box. See Section 4.2.4, Configuring Columns and Plumbing, and Section 4.5.3, Customizing the Column Library.
	Column bypassed in General Settings of method	Place column inline in General Settings. See Section 6.2.3, Specifying General Settings.
Segment duration 0 CV	Column not set inline after Purge segment	In Purge segment, set Flow Direction at End of Purge to something other than Bypass. See "Purge Segments" on page 6-25.
All segment durations 0	No flow rate and no column inline in General Settings	Adjust General Settings. See Section 6.2.3, Specifying General Settings.

Table 10-8 Method Editor Troubleshooting (Continued)

Symptom	Possible Cause	Action
In pH mode, Step segment requests percent value instead of pH value	Buffers/solvents configured are uncalibrated custom pH blend buffers	Configure either of the following buffers/solvents: <ul style="list-style-type: none"> • Calibrated custom pH blend buffers • pH blend buffers See Section 3.2.2, Preparing pH Blend Mode Buffers, and Section 4.2.2, Configuring Buffers/Solvents.
Method run stops before end of method	Pump Off event included before the end of the last segment	Delete Pump Off event and insert at end of segment.
Multi-Method button dimmed	Multi-Method button is not active unless a multi-method is open	Select New Multi Method or Open Multi Method from the Multi Method menu.
Cannot enable dual wavelength in General Settings or “Set UV Detector Wavelength” event	An external fluorescence detector is enabled in hardware setup	Disable the external fluorescence detector in the hardware setup. See Section 2.5.2, Updating BioCAD Hardware Setup Options.
Cannot disable dual wavelength in General Settings or “Set UV Detector Wavelength” event	An external UV/Vis detector is enabled in hardware setup	Disable the external UV/Vis detector in the hardware setup. See Section 2.5.2, Updating BioCAD Hardware Setup Options.

Table 10-8 Method Editor Troubleshooting (Continued)

Symptom	Possible Cause	Action
Purge (P) segment button dimmed	You configured Low Dispersion, Three Columns, or Direct Control for the Column Option. Because column bypass is not available with these column options, the Purge segment is not available.	Change column option in the Configuration dialog box. See Section 4.2.4, Configuring Columns and Plumbing.
Prep block button (PR) is not displayed on Method Editor toolbar	Integrated AS 3500 autosampler is not configured	Configure an integrated AS 3500 autosampler. See Section 2.5.2, Updating BioCAD Hardware Setup Options.
	Autosampler does not support sample preparation	You cannot use the Prep block feature.
Prep block segment buttons (D, C, HM) are not displayed on Method Editor toolbar	Prep block is not selected in method	Select a Prep block in the method.

Table 10-8 Method Editor Troubleshooting (Continued)

Symptom	Possible Cause	Action
Prep block segment buttons (D, C, HM) are dimmed	Sample vials or empty vials are not loaded in the Sample Table	Load sample vials and empty vials in the Sample Table. See Section 4.7.3, Entering Vials in Sample Table.
	No autosampler solvents are configured in the Sample Preparation Configuration dialog box	Configure autosampler solvents. See “Configuring sample prep” on page 4-42.
	Different vial shape (Standard or Micro) is selected for empty vials in the Prep block and empty vials in the Sample Table	Select the same vial shape (Standard or Micro) for empty vials in the Prep block and empty vials in the Sample Table.
	Method configuration is not updated with Sample Table information	Update configuration from the Method Editor by selecting Update Configuration from the Options menu.
Autosampler Inject (AI) segment button is dimmed	Sample vials are not loaded in the Sample Table when an integrated autosampler is configured	Load sample vials in the Sample Table. See Section 4.7.3, Entering Vials in Sample Table.
Add Event button is dimmed	A segment (Step, Gradient, Inject, Autosampler Inject, or Purge) is not selected	Select a segment (Step, Gradient, Inject, Autosampler Inject, or Purge).

10.1.9 Templates Troubleshooting

Table 10-9 Templates Troubleshooting

Symptom	Possible Cause	Action
In pH mode, pH Map Template dialog box requests percent value instead of pH value	Buffers/solvents configured are uncalibrated custom pH blend buffers	Configure either of the following buffers/solvents: <ul style="list-style-type: none"> • Calibrated custom pH blend buffers • pH blend buffers See Section 3.2.2, Preparing pH Blend Mode Buffers, and Section 4.2.2, Configuring Buffers/Solvents.
Values do not appear under a parameter in the Combinatorial Optimization Template dialog box	Method or system requirements are not met for the parameter	Make sure method and system requirements are met for the desired parameter. See Section 7.1.9.1, System and Method Requirements.
Parameter information that defines each template run is not fully visible in the Method Queue	Label column in Method Queue is not wide enough	Widen Label column in Method Queue. See Section 8.1, Using the Method Queue.

10.1.10 Computer Troubleshooting

Table 10-10 Computer Troubleshooting

Symptom	Possible Cause	Action
Nothing appears on monitor	Not powered on	Power on.
	Contrast not adjusted	Adjust contrast.
Computer performance very slow	Too many windows open	Close windows you are not using.
	Hard disk is fragmented	Defragment the disk using a computer utility program. See Section 9.10.3, Defragmenting the Hard Disk.
	Too many data points collected in data file	See Section 4.2.5, Configuring Detectors, for guidelines on setting data rate.

10.2 Error Codes

Error codes may be displayed in warning boxes during normal operation. Codes are also displayed in the Event Log window, described in Section 8.5, Using the Event Log.

This section describes the following types of error codes:

- File
- Blend
- Hardware
- Resource/Memory
- Configuration
- Autoloader
- Autosampler (AS 3500)
- Sample Table
- Syringe Pump
- Detector
- Method
- Supervisor Option
- Program Loading
- Communications
- COM Port

10.2.1 File Error Codes

Table 10-11 File Error Codes

Error	Possible Cause	Action
"Error in Writing to <file>"	Disk full	Delete unneeded files from disk.
	File marked as "Read-only"	Change file access.
"Data Loss to file <file>"	Disk full, cannot store all data	Delete unneeded files from disk.
"Cannot create Directory <dir>—Unknown Error!"	You are running on a network and do not have rights to create directories	Choose another drive. See your network manager.
"Too many files with same base name: <name>"	You are running methods using auto file extension feature, and will create more than 100 data files	Run fewer than 100 data files. Change the base file name.
"Cannot open method."	Not enough memory	Close windows. Reboot computer.
	Not a method file	Select BioCAD method file (.MET).
	File damaged	Retrieve file from backup diskette.
"Cannot Read Method <name>"	Not enough memory	Close windows. Reboot computer.
	File does not exist, or file damaged	Retrieve file from backup diskette.

Table 10-11 File Error Codes (Continued)

Error	Possible Cause	Action
"Cannot Add method <name> to group"	Group file or multi-method file contains maximum of 100 files	Create new group file or multi-method file.
"Cannot access method file <name>"	File does not exist	Select valid file name.
	File damaged	Retrieve file from backup diskette.
"Cannot create group file <name>"	Disk full	Delete unneeded files.
	You are running on a network and do not have rights to create directories	Choose another drive. See your network manager.
"Cannot create data file <name>"	Disk full	Delete unneeded files.
	You are running on a network and do not have rights to create directories	Choose another drive. See your network manager.
"<dataFile> is currently in the queue and cannot be overwritten"	Starting a run that duplicates a data file name already in the Method Queue	Delete the method in the Method Queue.
		Change the data file name.

Table 10-11 File Error Codes (Continued)

Error	Possible Cause	Action
"Drive A does not exist"	You opened a data file from a floppy disk in the Group Analysis window. Group Analysis keeps the file open, even after you close the Group Analysis window.	To correct the error, do one of the following: <ul style="list-style-type: none"> • Open another file from the C drive • Exit the BioCAD software To prevent the error from occurring again, copy files to the C drive before opening.
	Hardware problem	Call Applied Biosystems Technical Support.

10.2.2 Blend Error Codes

Table 10-12 Blend Error Codes

Error	Possible Cause	Action
“- Invalid Blend: Changed from... to...”	Buffers in Configuration do not match buffers in method	Adjust buffers in method to match system configuration, or load configuration from method as system configuration, or change system configuration.
“- Illegal Blend: Changed from... to...”	Method programmed in blend mode that system configuration does not support (example, method programmed in pH mode, but configuration does not contain two buffers/ water/ salt)	Load configuration from method as system configuration, or change system configuration.

10.2.3 Hardware Error Codes

Table 10-13 Hardware Error Codes

Error	Possible Cause	Action
“Illegal Hardware Configuration for Detectors. Run the BioCAD Setup Program Again.”	Hardware setup does not match software setup	Update hardware setup. See Section 2.5.2, Updating BioCAD Hardware Setup Options.
“BioCAD Hardware is not functional”	Internal problem	Call Applied Biosystems Technical Support.

10.2.4 Resource/Memory Error Codes

Table 10-14 Resource/Memory Error Codes

Errors	Possible Cause	Action
"Insufficient resources for <n> hours of Recorder Buffering. Using <m> hours."	Not enough memory or hard disk space to accommodate Buffer Duration	Close unneeded applications.
		Delete unneeded files from disk.
		Decrease data rate.
		Decrease buffer duration.
"Insufficient memory to initialize data recording for <name>. Exit Windows and restart."	Ran out of disk space or memory during a method run	Stop method, close unneeded applications, delete unneeded files, restart method.
	Ran out of memory during initialization of software	Check Windows virtual memory settings.
"Not enough system resources to initialize data recording for <n> hours. Maximum of <m> hours is selected."	Ran out of disk space or memory during a method run	Stop method, close unneeded applications, delete unneeded files, restart method.
	Ran out of memory during initialization of software	Check Windows virtual memory settings.

Table 10-14 Resource/Memory Error Codes (Continued)

Errors	Possible Cause	Action
“Insufficient system resources to open BioCAD. Try closing applications or documents.”	Too many windows open	Close windows.
“Insufficient system resources to run BioCAD. Try closing applications or documents.”		
“Only <n> bytes of virtual memory are available out of a maximum of <n> bytes. Please close applications, free up disk space or check virtual memory settings.”	Not enough disk space free to run BioCAD software (need 15 to 20 MB free disk space)	Delete unneeded files.
“Not enough memory to copy Method.”	Too many windows open	Close windows. Reboot computer.

10.2.5 Configuration Error Codes

Table 10-15 Configuration Error Codes

Error	Possible Cause	Action
<p>“Illegal Hardware Configuration for Detectors. Run the BioCAD Setup Program Again.”</p>	<p>Hardware setup does not match software setup</p>	<p>Update hardware setup. See Section 2.5.2, Updating BioCAD Hardware Setup Options.</p>
<p>“Available Detectors are different from Configuration. Change configuration to match?”</p>	<p>You have installed an external detector and have changed the BioCAD hardware setup. Message displayed the first time you start software after installing device, or when you load an old configuration.</p>	<p>Click OK.</p>
<p>“Configured Recorder Channels are not available. Change configuration?”</p>	<p>You removed an external detector and have changed the BioCAD hardware setup. Message displayed the first time you start software after removing device, or when you load an old configuration.</p>	<p>Click OK.</p>

Table 10-15 Configuration Error Codes (Continued)

Error	Possible Cause	Action
“Configured Recorder Rates are not available. Change configuration?”	Data rate selected incorrect for installed detector (for example, data rate of 20 pps set for Model 204). May occur if you share files between workstations containing different detector models, or you change hardware.	If sharing files, change data rate in configuration. If you installed a new detector, update BioCAD hardware setup. See Section 2.5.2, Updating BioCAD Hardware Setup Options.
“Solvent Pump Configuration does not match instrument. Change configuration?”	You have installed a new solvent pump and have changed the BioCAD setup. Message displayed the first time you start software after installing device.	Click OK .
“System Configuration does not match instrument. Change configuration?”	You have changed BioCAD hardware setup	Click OK .
“Loader Configuration does not match instrument. Change configuration?”	You have changed BioCAD hardware setup	Click OK .
“Plumbing Configuration does not match instrument. Change configuration?”	You have changed BioCAD hardware setup	Click OK .

Table 10-15 Configuration Error Codes (Continued)

Error	Possible Cause	Action
"Blend Configuration does not match instrument. Change configuration?"	Method configuration does not match system configuration	Load configuration from method as system configuration, or change system configuration.
"Collector Configuration does not match instrument. Change configuration?"	Method configuration does not match system configuration	Load configuration from method as system configuration, or change system configuration.
"Sample Loader Configuration does not match instrument. Change configuration?"	You have changed BioCAD hardware setup	Click OK .
"Changing Configuration will shut down the system. All methods will be canceled."	You displayed the Configuration dialog box while a method is running, or pump is flowing.	Click Cancel to allow the method or pump to continue at present settings OR Click OK to abort run and change settings.
"Configuration Mis-match"	Method configuration does not match system configuration	Run method using system configuration OR Load configuration from method as system configuration, or change system configuration.

10.2.6 Autoloader Error Codes

Table 10-16 Autoloader Error Codes

Error	Possible Cause	Action
"Injector valve stuck in counter clockwise position"	Compressed dry gas (air or nitrogen) source for valve operation not turned on, or regulated below 80 psi	Turn on or adjust gas (air or nitrogen) pressure.
	Valve not switching	Call Applied Biosystems Technical Support.

10.2.7 Autosampler (AS 3500) Error Codes

Table 10-17 Autosampler (AS 3500) Error Codes

Error	Possible Cause	Action
"Connection # 108" error on autosampler front panel	You powered up a direct wire autosampler before powering up BioCAD 700E Workstation	Power up the workstation before the direct wire autosampler.
	You have an autosampler connected to the BioCAD 700E Workstation, and the BioCAD 700E Workstation is configured for an autoloader	Adjust configuration for autosampler. See Section 4.8, Configuring and Preparing the BioCAD Software for a Direct Wire Autosampler.

Table 10-17 Autosampler (AS 3500) Error Codes (Continued)

Error	Possible Cause	Action
“AutoSampler Invalid Load Amount”	Injection volume entered in BioCAD software zero or negative value	Enter positive value for injection volume.
“AutoSampler is Busy”	Autosampler not ready to make injection	Wait for device to complete cycle before making injection.
“Input Fault: <fault_code>, <fault Text>”	Autosampler error	Depends on error.
“RealTime Fault: <fault_code>, <fault Text>”	Fatal autosampler error	Power down autosampler. Call Applied Biosystems Technical Support.
“Power-Up Fault(s): <fault_code>, <fault Text>”	Autosampler hardware error	Power down autosampler. Call Applied Biosystems Technical Support.
“Initialization Fault: <errorCode>, <errorText>”	Autosampler power up error, hardware problem	Depends on error. Power down autosampler and correct problem before continuing.
“RealTime Fault: <errorCode>, <errorText>”	Fatal error	Power down, power up.

10.2.8 Sample Table Error Codes

Table 10-18 Sample Table Error Codes

Error	Possible Cause	Action
"No name has been specified. Enter the name then exit."	You did not enter a Short Name in the Sample Editor dialog box	Enter a name in the Short Name text box of the Sample Editor dialog box. See Section 4.7.2, Customizing the Sample Library.
"Cannot edit Sample Table while method is running!"	Method is running	Wait until method finishes, or abort method before editing Sample Table.
"No vial at current location!"	You clicked Load Loop when a blank vial position (no sample loaded) is selected in the Sample Table	Select a vial position in the Sample Table that has a sample loaded (Sample, Standard, Control, or Reagent vial type).
"Invalid vial at current location!"	You clicked Load Loop when an Empty vial type is selected in the Sample Table	Select a vial position in the Sample Table that has a sample loaded (Sample, Standard, Control, or Reagent vial type). OR Add sample to vial and load vial in Sample Table before clicking Load Loop .

Table 10-18 Sample Table Error Codes (Continued)

Error	Possible Cause	Action
“Required volume of .. μ l exceeds volume in Vial!”	You clicked Load Loop when the volume entered in the Volume text box exceeds the volume available in the vial	Enter a smaller volume in the Volume text box of the Sample Table.
“Cannot specify zero volume”	You clicked Load Loop when the volume entered in the Volume text box is 0.0	Enter a value larger than 0.0 in the Volume text box before clicking Load Loop .
“Sample has already been loaded into loop”	You are trying to load the sample loop a second time before injecting the contents of the loop	Inject previously loaded sample.
“Injection volume exceeds syringe capacity, amount set to .. μ l”	The volume you entered in the Volume text box is greater than: <ul style="list-style-type: none"> • 200 μl (PushLoop) • 2 ml (other modes) 	Click OK to allow the software to adjust the injection volume.
“Prep Syringe Over expel” (doesn't occur during proper operation)	A previous incomplete action left the autosampler prep syringe plunger down, and it cannot pull the required volume	Reboot to reset autosampler.

Table 10-18 Sample Table Error Codes (Continued)

Error	Possible Cause	Action
"Loading .. μ l into .. μ l loop"	The volume you entered in the Volume text box is greater than the maximum loop volume for Pull or Push modes	Click Cancel and adjust the volume. <i>Note: If you click OK to continue, the system injects the maximum loop volume. It does not inject the volume entered in the Sample Table Volume text box.</i>
"Cannot perform an accurate full loop injection, the .. μ l loop will be under filled."	Installed sample loop is too large for the injection volume	Click OK , change the installed sample loop, and reconfigure the sample loop size by selecting Change Loop Size from the Sample command in the Sample Table.

10.2.9 Syringe Pump Error Codes

Table 10-19 Syringe Pump Error Codes

Error	Possible Cause	Action
“Syringe Pump Busy”	Syringe pump in use	Wait for syringe pump to complete cycle before using.
“Amount is Greater than Syringe Capacity”	Injection volume entered in BioCAD software exceeds capacity of installed syringe	Change injection volume. Change to a larger syringe. See Section 5.2.1, Changing the Syringe and Sample Loop.
“Syringe Pump: Failed to find home position”	Syringe not moving properly	Make sure syringe is properly installed, plunger not bent, nothing obstructing motion.
“Syringe Pump: Microprocessor failure”	Internal problem	Call Applied Biosystems Technical Support.
“Syringe Pump: EPROM check sum error”		
“Syringe Pump: Software error”		
“Syringe Pump Initialization Error”		

Table 10-19 Syringe Pump Error Codes (Continued)

Error	Possible Cause	Action
"Syringe Pump: Serial link hardware failure"	Communication lost between BioCAD software and autoloader syringe	Reset configuration by displaying the Configuration dialog box, then clicking OK .
"Syringe Pump: Serial link framing error"		
"Syringe Pump Communications Error"	Communication problem with pump	Power down BioCAD 700E Workstation, then power up.
"Syringe Pump Off Line"	Usually displayed after Offline message. Pump not responding	Power down BioCAD 700E Workstation, then power up.
"Syringe Pump: Pump not Responding" or "Syringe pump failed to respond"	Communication lost between BioCAD software and autoloader syringe	Reset configuration by displaying the Configuration dialog box, then clicking OK .
		Exit BioCAD software and restart. If problem persists, call Applied Biosystems.
	Wrong syringe pump specified in hardware setup	Update hardware setup. <ul style="list-style-type: none"> • On SB1200 syringes, inlet and outlet tubing is perpendicular to the syringe • On XL300 syringes, inlet and outlet tubing is at an angle to the syringe

Table 10-19 Syringe Pump Error Codes (Continued)

Error	Possible Cause	Action
“Syringe Pump: Operation Timeout”	Communication problem with pump	Call Applied Biosystems.
“Syringe Pump Communications Error”	Communication problem with pump	Power down BioCAD 700E Workstation, then power up.
“Syringe Pump Off Line”	Usually displayed after Offline message. Pump not responding	Power down BioCAD 700E Workstation, then power up.
“Syringe Pump Not Initialized”	You tried to use the autoloader before it completed initialization cycle	Wait for autoloader to complete cycle before using.
“Syringe Pump Plunger Overload”	Syringe cannot find home position. Syringe plunger bent or dirty	Clean or replace plunger.
	Syringe not properly screwed into position	Tightly screw syringe into position.
	Sample loop clogged	Replace sample loop.
“Syringe Pump Valve Overload”	Valve dirty	Clean valve by forcing water through lines attached to syringe.

10.2.10 Detector Error Codes

Table 10-20 *Detector Error Codes*

Error	Possible Cause	Action
"UV Detector Offline"	Problem with communication between BioCAD 700E hardware and BioCAD software	Reset configuration by displaying the Configuration dialog box and clicking OK .
		Exit BioCAD software and restart. If problem persists, call Applied Biosystems Technical Support.
"UVIS-201 Detector Offline"	Communication problem	Reset Configuration by displaying Configuration dialog box, then clicking OK .
"UVIS-201 Detector: <errorText>"	Error from detector	Depends on error.
"Real-time Fault: <code>, Low light on D2 lamp."	UV/Vis flow cell dirty	Clean UV/Vis flow cell. See Section 9.4.2, Cleaning the UV/Vis Detector Flow Cell.
	Deuterium lamp aging	Replace lamp. See Section 9.4.1, Changing the UV/Vis Detector Lamps.
	Optics misaligned	Call Applied Biosystems Technical Support.

Table 10-20 Detector Error Codes (Continued)

Error	Possible Cause	Action
“UV/VIS Detector: Deuterium Lamp Light Level Low”	See “Real-time Fault: <code>, Low light on D2 lamp” causes and actions	_____
“Real-time Fault: <code>, Low light on W lamp.”	UV/Vis flow cell dirty	Clean UV/Vis flow cell. See Section 9.4.2, Cleaning the UV/Vis Detector Flow Cell.
	Tungsten lamp aging	Replace lamp. See Section 9.4.1, Changing the UV/Vis Detector Lamps.
	Optics misaligned	Call Applied Biosystems Technical Support.
“UV/VIS Detector: Tungsten Lamp Light Level Low”	See “Real-time Fault: <code>, Low light on W lamp” causes and actions	_____
“D2 Lamp Not Present or Failed”	Lamp burned out, or not installed	Try turning on by pressing Lamp button on front panel. Replace lamp. See Section 9.4.1, Changing the UV/Vis Detector Lamps.

Table 10-20 Detector Error Codes (Continued)

Error	Possible Cause	Action
"W Lamp Not Present or Failed"	Lamp burned out, or not installed	Try turning on by pressing Lamp button on front panel. Replace lamp. See Section 9.4.1, Changing the UV/Vis Detector Lamps.
"UV/VIS Detector: Could not turn ON lamp"	Bad lamp	Replace lamp. See Section 9.4.1, Changing the UV/Vis Detector Lamps.
"UV/VIS Detector: Stepper Motor Error"	Internal problem	Call Applied Biosystems Technical Support.
"Detector bypass valve stuck in counter clockwise position"	Compressed dry gas (air or nitrogen) source for valve operation not turned on, or regulated below 80 psi	Turn on or adjust gas (air or nitrogen) pressure.
	Valve not switching	Call Applied Biosystems Technical Support.
"Conductivity Reference <value> is not within expected range."	Problem with analog board	Call Applied Biosystems Technical Support.

Table 10-20 Detector Error Codes (Continued)

Error	Possible Cause	Action
“FL-304/305 COM <> Timeout Error” when you start the BioCAD software	Fluorescence detector not powered on	Power on Fluorescence detector.
	Fluorescence detector not connected to COM port on BioCAD 700E Workstation	Connect Fluorescence detector to COM port.
	Fluorescence detector connected to different COM port than you specified in hardware setup	Connect to correct COM port, or change hardware setup. See Section 2.5.2, Updating BioCAD Hardware Setup Options.
“FL-304/305 Detector <error text>”	Error from detector	Depends on error.
“FL-304/305 Detector Offline”	Communication problem between BioCAD hardware and BioCAD software	Reset configuration by displaying the Configuration dialog box, then clicking OK .
“Fluorescence Detector Offline”	Communication problem between BioCAD hardware and BioCAD software	Reset configuration by displaying the Configuration dialog box, then clicking OK .
		Exit and restart BioCAD software. If problem persists, call Applied Biosystems Technical Support.

10.2.11 Method Error Codes

Table 10-21 Method Error Codes

Error	Possible Cause	Action
“Illegal Event <event> in Method”	Event in method does not match configuration	Delete event, or load configuration from method as system configuration.
“The data recorder Buffer Duration is less than the method duration. Loss of data may result... Run method anyway?”	Buffer duration shorter than method run	Increase Buffer Duration, or run the method anyway, but you may not capture all data in the Strip Chart Recorder.
“Event [<event>] is illegal for this system configuration.”	System configuration does not allow a method event	Delete event, or load configuration from method as the system configuration.
“Event [<event>] is invalid for this system configuration.”	Method event settings do not match configuration	Change method event settings.
“Method: <name> aborted due to file error!”	File corrupted	Retrieve file from backup diskette.

Table 10-21 Method Error Codes (Continued)

Error	Possible Cause	Action
“Method’s configuration does not match current System Configuration... Update Method?”	Method configuration does not match system configuration	Load configuration from method as system configuration, or change system configuration.
	Sample names and volumes you entered in the Sample Table do not match the sample names and volumes requested in the method	Change the sample name and volume information in the Sample Table or the method.
“File... does not contain a valid BioCAD Method.”	You are exporting or printing a file that does not contain a valid BioCAD method, for example, a data file saved from the Strip Chart Recorder	No action. Normal occurrence.
“<fileName> Does not contain a valid method.”		
“Injection volume exceeds limits, amount set to ... μ l”	Injection volume in Inject segment is greater than the maximum volume that the autosampler syringe can draw	Click OK to allow the system to adjust the injection volume.
“Sample Loader: Loading ... μ l into ... μ l loop.”	Injection volume is greater than the loop volume for Pull or Push modes. Injected volume will be smaller than the volume you entered.	Click Cancel and adjust the injection volume. Note: If you click OK to continue, the system injects a volume less than the volume you entered.

Table 10-21 Method Error Codes (Continued)

Error	Possible Cause	Action
"No <sample> available. Volume set to 0"	Sample listed in method is not listed in the currently loaded Sample Table	Select different sample in Inject segment, or add sample to Sample Table.
	Sample listed in method has same Short Name as another sample listed in the Sample Table, but has different attributes. These two samples are treated as different samples, even though Short Name is the same.	Select same sample in method and Sample Table. For more information on attributes of a sample, see Section 4.7.2, Customizing the Sample Library.
"<sample> not found"	Sample listed in method is not listed in the currently loaded Sample Table	Select different sample in Inject segment, or add sample to Sample Table.
"Only .. µl of sample <sample> available"	Not enough specified sample available in sample vial	Adjust injection volume in method, or add sample to vial and adjust volume in Vial Editor dialog box. See Section 4.7.3, Entering Vials in Sample Table.
"Not enough sample available. Vial contains ..µl."		
"Insufficient volume of <sample> to perform dilution."		
"No diluent configured on channel ..."	You are creating or editing a Dilute segment or Combine segment with no diluent configured	Edit configuration. In the Sample Preparation Configuration dialog box, select a reagent. See "Configuring sample prep" on page 4-42.

Table 10-21 Method Error Codes (Continued)

Error	Possible Cause	Action
“Final Concentration must be greater than zero and less than the initial concentration.”	In a Dilute or Combine segment, you entered a final concentration greater than the initial concentration of a sample	Enter a final concentration less than the initial concentration of the sample.
“Number of Serial Dilution Steps must be between 1 and 20”	In a Dilute segment, you entered a number greater than 20 in the Serial Dilution Steps text box	Enter a number between 1 and 20 in the Serial Dilution Steps text box. See “Dilute Segments” on page 6-57.
“Not enough empty vials. Number of dilution steps changed from .. to ...”	Not enough empty vials of the required shape	Add enough empty vials of the required vial shape (Standard or Micro) to the autosampler tray and to the Sample Table for each Dilute segment. The number of empty vials needed is # Serial Dilution Steps.
“This dilution requires .. empty vial(s). <#> empty vials are available for this segment.”		
“Combination requires 1 empty vial. No empty vials are available for this segment.”	Not enough empty vials of the required shape for Combine or Dilute segment	Add enough empty vials of the required vial shape (Standard or Micro) to the autosampler tray and to the Sample Table for each Combine or Dilute segment. The number of empty vials needed for each Dilute segment is # Serial Dilution Steps. The number of empty vials needed for each Combine segment is 1.

Table 10-21 Method Error Codes (Continued)

Error	Possible Cause	Action
“Total percentage of samples is greater than 100%. Modify sample concentrations.”	In a Combine segment, you have entered Final Concentrations for samples that exceed a total of 100%	Change percentages to total 100% in the Combine Segment Setup dialog box. See “Combine Segments” on page 6-61.
“Insufficient volume for Loading Template”	Volume in vial not sufficient to run template	Adjust injection volume in method, or add sample to vial and adjust volume in Vial Editor dialog box. See Section 4.7.3, Entering Vials in Sample Table.
“Not enough sample <sample> for Run #... Template canceled.”		

10.2.12 Supervisor Option Error Codes

Table 10-22 Supervisor Option Error Codes

Error	Possible Cause	Error
“Selected Option not Available. Protection key not found.”	Hardware key not installed for Supervisor software option	Install hardware key. See Section 2.5.3, Installing the Hardware Key.
“Selected Option not Available. Protection key not found or incorrect key type.”		
“Selected Option not Available. This option is disabled by the Protection Key.”	Option not available with Supervisor standalone software	No action. Normal occurrence.

10.2.13 Program Loading Error Codes

Table 10-23 Program Loading Error Codes

Error	Possible Cause	Action
NOTE: The following messages can be generated when you attempt to open another application (for example, Microsoft Excel) from the BioCAD Window menu.		
"Cannot Load <appName>: Insufficient Memory"	Too many windows open	Close unneeded windows.
"Cannot Load <appName>: File Not Found"	File missing	Copy file back onto system from floppy diskette.
"Cannot Load <appName>: Path Not Found"	Path corrupted	Fix path and reload application.
"Cannot Load <appName>: Invalid.EXE file"	Executable file missing	Reload application.
"Cannot Load <appName>: Application created for earlier version of Windows"	Software not compatible with current version of Microsoft Windows	No action. Cannot run.
"Cannot Load <appName>: Error <errcode>"	Depends on error	Depends on error.

10.2.14 Communication Error Codes

Table 10-24 Communications Error Codes

Error	Possible Cause	Action
“Data Overrun”	BioCAD 700E Workstation not able to receive all data sent by device, for example, when two detectors are sending data at same time	Power down unneeded devices.
“Receive Buffer Overflow”	Data collection rate too high for method run time	Reduce data collection rate. See “Configuring data channels” on page 4-16.
	Too many applications open when running a method	Close some applications.
“Parity Error”	Problem with communication between BioCAD 700E hardware and BioCAD software	Check hardware and software configuration. Make sure configuration matches physical setup.
“Framing Error”		
“Transmit Buffer Overflow”	External device not receiving messages fast enough from BioCAD 700E Workstation. Device not powered up. Device not functioning.	Power up device. Check function.
“CommLink error: <deviceName> TimeOut”	BioCAD 700E component not responding within an internal timeout period	Exit BioCAD software. Check that device is powered up. Start software.

Table 10-24 Communications Error Codes (Continued)

Error	Possible Cause	Action
“CommLink error: <deviceName> <errorname>”	Problem with device	Depends on problem and device.
“Packet Error: <errorCode>”		
“Can't Handle ASCII Message: <text>”		
“Input Fault: <errorCode>, <errorText>”		
“CommLink error: Unable to write packet (probable queue full)”	Problem with communication between BioCAD 700E hardware and BioCAD software	Reset configuration by displaying the Configuration dialog box and clicking OK .
		Exit software, start software.

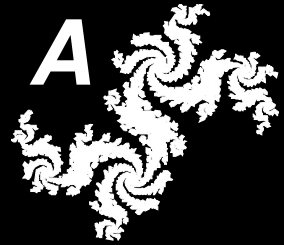
10.2.15 COM Port Error Codes

Table 10-25 COM Port Error Codes

Error	Possible Cause	Action
“Comm Port Conflict: Registering port <n>. That port already exists.”	Same COM port selected for more than one device.	Update hardware setup. Specify different COM ports. See Section 2.5.2, Updating BioCAD Hardware Setup Options.
“Comm Port Conflict: <device1> Requested Comm Port <n>. That port is already owned by <device2>”		

Appendix

A



A Specifications

This appendix includes specifications for:

- General
- Detection
- Miscellaneous

NOTE: The specifications for this instrument are subject to change without notice.

Table A-1 General

Condition	Specification
Weight	275 pounds (125 kg)
Space requirements	Space required for workstation, computer monitor, keyboard, mouse, and buffers: <ul style="list-style-type: none"> • Height—36 inches (91 cm) • Depth—36 inches (91 cm) • Width—72 inches (183 cm)
Pumping system	Dual piston, positive displacement with 6 inlets, pressure safety, and built-in seal flush
Flow rate	0.2–100 ml/min Accuracy is +/- 1% of setpoint or 0.05 ml/min (whichever is greater)
Maximum system pressure	3,000 psi (204 bar, 20.4 MPa)
Gradient capability	Up to six solvents, with low-pressure quaternary blending in Percentage, Eluent, or pH blend modes with 0.1% increments. Compositional accuracy: <ul style="list-style-type: none"> • 0.2–90 ml/min: ±1% • 90–100 ml/min: ±1.5%

Table A-1 General (Continued)

Condition	Specification
Sample handling	<p>Six injection modes:</p> <ul style="list-style-type: none"> • Autoloader—Up to four sample channels, with a sample loading range of 1 μl to 25 ml, \pm1% accuracy at full stroke • Direct Autoloader—Reduces prime volume (minimum 5 μl), single sample channel, with a sample loading range of 1 μl to 25 ml, \pm1% accuracy at full stroke • Manual—Eliminates prime volume, needle port adapter included • Pump—Unlimited volume loading • Integrated Autosampler (optional)—Inject from multiple samples, with complete control over autosampler functions • Direct Wire Autosampler (optional)—Inject from multiple samples, control autosampler from autosampler front panel
Column sizes	<ul style="list-style-type: none"> • POROS® Perfusion Chromatography® columns—2.1 to 25 mm diameter • Conventional chromatography columns—no limit on column size
Column configurations	<ul style="list-style-type: none"> • Single column, with bypass and reverse • Tandem columns, with bypass • Three columns • Low dispersion • Direct control <p>NOTE: Use up to eight columns with optional SCOUT Column Selector.</p>
Fraction collection	<ul style="list-style-type: none"> • Internal fraction/waste valve for manual and automated control of single fraction collection • Integrated control of optional external fraction collectors, which accommodate tubes or larger bottles. Tube-to-tube switching time is 0.1 seconds.

Table A-1 General (Continued)

Condition	Specification
Materials	Biocompatible and solvent-resistant—PEEK, Teflon®, Kel F®, Kynar®, sapphire, stainless steel, aluminum oxide, ruby
Auxiliary inputs and outputs	<ul style="list-style-type: none">• Inputs—2 TTL• Outputs—2 TTL
Auxiliary driver outputs	<ul style="list-style-type: none">• Spareout LC—12 V (0.25 A)• Spareout HC—24 V (1 A)

Table A-2 Detection

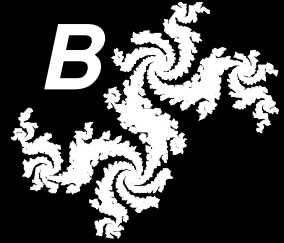
Condition	Specification
Pressure	Transducer on pump discharge—0 to 3,000 psi
Auxiliary input	12-bit A/D for additional detector
Monitor bypass	Automated valve switches pH/conductivity probes in and out of line
pH probe	<ul style="list-style-type: none"> • Range—2 to 12 pH units • Flow cell volume—50 μl
Conductivity probe	<ul style="list-style-type: none"> • Range—0 to 200 mS • Flow cell volume—7 μl
UV/Visible	<p>Model 201 (single wavelength)—190 nm to 800 nm</p> <p>Model 205 (dual wavelength)</p> <ul style="list-style-type: none"> • 190 nm to 800 nm (single wavelength range) • 190 to 450 nm OR 366 to 700 nm (dual wavelength ranges) <p>Flow cells available:</p> <ul style="list-style-type: none"> • Analytical—6 mm path length, 9 μl volume, 45 ml/min flow rate maximum • Semi-prep—3 mm path length, 4.5 μl volume, 100 ml/min flow rate maximum

Table A-3 Miscellaneous

Standard	Specification
Hardware (standard)	<ul style="list-style-type: none"> • Internal Pentium/133 MHz processor (minimum) • 3.2 gigabyte hard disk drive (minimum) • 64 megabyte RAM (minimum) • 3.5-inch floppy disk drive • 14-inch Super VGA color monitor • Keyboard • Mouse • Network card • SCSI card
Hardware (options)	<ul style="list-style-type: none"> • 17-inch color monitor • 21-inch color monitor • Laser and color printers • SCOUT Column Selector • External detector (UV/Vis or fluorescence) • Fraction collector • Autosampler • Kits of selected POROS columns
Software (standard)	<ul style="list-style-type: none"> • BioCAD Software • Windows 95 Version OSR 2.5 (minimum)
Software (options)	<ul style="list-style-type: none"> • Supervisor™ Software (stand-alone software version) • Microsoft Office 97

Appendix

B



B Spare Parts

This appendix contains part numbers for the following spare parts:

- Solvent pump
- Fittings and tubing
- Detector and monitor
- Fraction collector
- Miscellaneous
- Spare Parts Kits

The parts listed in this appendix are recommended for customer installation.

Procedures other than those listed in Chapter 2, Installing the BioCAD 700E Workstation, and Chapter 9, Maintenance, may require a trained service representative.

CAUTION

Damage resulting from unauthorized work on your workstation may invalidate your warranty.

Table B-1 100 ml/min Solvent Pump Spare Parts

Item	Where used	Part Number
Check valve kit, TSS-100 <ul style="list-style-type: none"> • Inlet check valve housing (1) • Outlet check valve housing (1) • Check valves (2) 	Primary solvent (front) pump head	Contact your technical service representative
Check valves, primary head, TSS-100 (1)	Primary solvent (front) pump head	PH602848
Check valves, seal wash, TSS-100 (1)	Pump seal wash system	PH602840
Fitting, stainless steel, compression ring, and ferrule (10/pkg)	Columns, prime/purge valve, filter/mixer, union	GEN602135
Fitting, wing nut, 10-32, with ferrule, PEEK (5/pkg)	Columns, prime/purge valve, filter/mixer, union	GEN602301
Inlet tubing/solvent barb kit, TSS-100 <ul style="list-style-type: none"> • Barb fittings, 3/32-inch (4) • Barb fittings, 1/8-inch (10) • Spacers, solvent valve (10) • Inlet tubing 	Accumulator solvent (front) pump head	Contact your technical service representative
Piston, primary pump head, TSS-100	Primary pump assembly	PH602836
Piston, accumulator pump head, TSS-100	Accumulator pump assembly	PH602837

Table B-1 100 ml/min Solvent Pump Spare Parts (Continued)

Item	Where used	Part Number
Pump head seal kit, TSS-100 <ul style="list-style-type: none"> • Solvent pump seal, primary (1) • Solvent pump seal, accumulator (1) • Wash pump seal (2) • O-rings (2) • Seal insertion and removal tools • Scouring pad • Installation instructions 	Primary and accumulator solvent (front) and seal wash (back) pump heads	Contact your technical service representative
Seal wash line kit, TSS-100 <ul style="list-style-type: none"> • Barb fittings, 1/8-inch (10) • Tubing, Tygon®, 102 inches • Installation instructions 	Pump seal wash system	Contact your technical service representative
Solvent proportioning valve kit	Pump compartment	GEN 601114



Table B-2 60 ml/min Solvent Pump Spare Parts

Item	Where used	Part Number
Check valve assembly kit (housing, inlet valve, outlet valve)	Pump head	P5-1006-00-5030
Mixer filter frit replacement kit <ul style="list-style-type: none"> • Filter frit • Fitting, extra long, with ferrule, for 1/16-in tubing, 1.03-in length, 10-32 threads, PEEK (1 each) • Fitting, barbed, 1/8-in, 10-32 threads, PP • Tubing, Tygon®, 3-in, 1/8-in ID, 1/4-in OD • Fitting, Luer-Lok, for 1/8-in barb • Seal, filter/mixer 	Filter/mixer	GEN 601907
Fitting, extra-long, with ferrule, for 1/16-in tubing, 1.03-in length, 10-32 threads, PEEK (10 each)	Filter/mixer	GEN 601933
Fitting, compression ring, with stainless steel nut, stainless steel ring, and PEEK ferrule, for 1/16-in tubing (10 each)	Filter/mixer, prime/purge valve	GEN 602135
Piston	Pump head	P5-1006-06-5160
Seal, filter/mixer	Filter/mixer	GEN 602376
Seal kit, pump head (complete to refurbish 2 pump heads)	Pump head	P5-1006-06-5020
Seal wash line kit	Seal wash lines	P5-1006-06-5040
Solvent proportioning valve kit	Pump compartment	GEN 601114
Check valve assembly kit (housing, inlet valve, outlet valve)	Pump head	P5-1006-00-5030

Table B-3 Fittings and Tubing

Item	Where used	Part Number
Fitting, 10-32 threads, with ferrule, PEEK (5 each)	Flow cell	GEN 602301
Fitting, Rheodyne Rheflex (6 each)	Injection and column switching valves	P5-1017-06
Seal wash line kit	Seal wash lines	P5-1006-06-5040
Sample loop, 5.0 µl 1/16-in	Injection valve	P5-1016-00-0005
Sample loop, 10 µl 1/16-in	Injection valve	P5-1016-00-0010
Sample loop, 20 µl 1/16-in	Injection valve	P5-1016-00-0020
Sample loop, 50 µl 1/16-in	Injection valve	P5-1016-00-0050
Sample loop, 100 µl 1/16-in	Injection valve	P5-1016-00-0100
Sample loop, 200 µl 1/16-in	Injection valve	P5-1016-00-0200
Sample loop, 500 µl 1/16-in	Injection valve	P5-1016-00-0500
Sample loop, 1 ml 1/16-in	Injection valve	P5-1016-00-1000
Sample loop, 2 ml 1/16-in	Injection valve	P5-1016-00-2000
Sample loop, 5 ml 1/16-in	Injection valve	P5-1016-00-5000
Tubing, PEEK 0.010-in blue, 5 ft	Connections to injection and switching valves	P5-1023-06-0010
Tubing, PEEK 0.020-in orange, 5 ft	Connections to injection and switching valves	P5-1023-06-0020

B

Table B-3 Fittings and Tubing (Continued)

Item	Where used	Part Number
Tubing, PEEK 0.030-in green, 5 ft	Connections to injection and switching valves	P5-1023-06-0030
Tubing, Teflon 1/16-in, 25 ft	Waste line from autoloader and waste valve	P5-1020-06-0031
Tubing, Teflon 1/8-in, 25 ft	Air supply line	P5-1020-12-0062
Tubing cutter	Tubing	P5-1030-00-0000

Table B-4 Detector and Monitor Spare Parts

Item	Where used	Part Number
Lamp, tungsten	UV/Vis detector	P5-1085-00-5010
Lamp, deuterium assembly	UV/Vis detector	P5-1085-00-5020
Flow cell, Semi-prep	UV/Vis detector	P5-1085-51-0000
Flow cell, Analytical	UV/Vis detector	P5-1085-52-0000
pH probe*	pH monitor	P5-1086-02-0000
pH flow cell*	pH monitor	P5-2085-02-0000
Conductivity cell	Conductivity monitor	P5-1087-11-0000

* Call Technical Support to determine the pH probe and flow cell to order for your workstation.

Table B-5 Fraction Collector Spare Parts

Item	Where used	Part Number
Cable, Advantec fraction collector	Advantec fraction collector	P5-1908-00-0000
Cable, Gilson fraction collector	Gilson fraction collector	P5-2082-00-0000
Cart, mobile	Advantec fraction collector	P5-1260-51
Nozzle, high flow	Advantec fraction collector	P5-1260-00-5140
Nozzle, low flow	Advantec fraction collector	P5-1260-00-5130
Rack, Eppendorf	Advantec fraction collector	P5-1260-11
Rack, funnel	Advantec Fraction Collector	P5-1260-31
Rack, prep funnel	Advantec fraction collector	P5-1260-32
Rack, vial, 4 ml	Advantec fraction collector	P5-1260-21
Tubing, prep	Advantec fraction collector	P5-1260-33
Valve, Advantec fraction collector	Advantec fraction collector	P5-1260-01-0000
Valve, Gilson fraction collector	Gilson fraction collector	P5-1924-21-0000



Table B-6 Miscellaneous Spare Parts

Item	Where used	Part Number
Fan Guard, 4.69-in	Fan on rear panel of workstation	P5-1242-00-0000
Keyboard, Windows 95 104-key	Computer	GEN 602329
Syringe, reagent, 500 µl	Autoloader syringe	P5-1012-00-0005
Syringe, reagent, 2.5 ml	Autoloader syringe	P5-1012-00-0025
Syringe, reagent, 5 ml	Autoloader syringe	P5-1012-00-0050
Syringe, reagent, 25 ml	Autoloader syringe	P5-1012-00-0250
Rotor Seal, Rheodyne, Tefzel	6-port injection valve or column switching valves	P5-1013-00-5110
Stator, Rheodyne, PEEK	6-port injection valve	P5-1013-00-5120
Stator, Rheodyne, PEEK	6-port column switching valves	P5-1013-00-5200
Stator assembly, white, ceramic	6-port injection valve or column switching valves	P5-1013-00-5160

Table B-7 Spare Parts Kits

Item	Where used	Part Number
<p>BioCAD Accessory Kit</p> <ul style="list-style-type: none"> • Sample loop, 100 µl 1/16-in • Sample loop, 5 ml 1/16-in • Fitting, 10-32 thread, with ferrule, PEEK • Tubing, PEEK 0.010-in blue, 5 ft (2) • Tubing, PEEK 0.020-in orange, 5 ft (2) • Tubing, PEEK 0.030-in green, 5 ft (2) • Adapter, brass, male, 1/8-in Swagelok® to 1/4-in NPT (2) • Syringe, 60 ml plastic • Adaptor, needle port • BioCAD Fitting Kit • BioCAD Tubing Label Kit • Accessory Kit instructions 	<p>Miscellaneous areas of BioCAD 700E Workstation</p>	<p>P5-2305-01-0000</p>



Table B-7 Spare Parts Kits (Continued)

Item	Where used	Part Number
BioCAD Fitting Kit <ul style="list-style-type: none"> • Ferrule, 1/16-in, plastic, blue (10) • Nut, 1/16-in, low pressure, plastic, black (6) • Nut, 1/16-in, low pressure, long, plastic, blue (2) • Ferrule, 1/8-in, plastic, yellow (10) • Nut, 1/8-in, low pressure, plastic, black (10) • Union, 0.020-in, high pressure, PEEK (2) • Union, 1/4-in, low pressure, Tefzel (2) • Tubing cutter 	Miscellaneous connections on the BioCAD 700E Workstation	P5-1937-01-0000
BioCAD Sparge Kit <ul style="list-style-type: none"> • Bottle cap, plastic (6) • Bottle cap insert, plastic, white (6) • Vent plug, plastic, white (6) • Sparge element (6) • Tubing, 1/8-in, 4 ft, Teflon (6) • Tubing, 1/4-in, 2-in, Urethane (2) • Sparge kit instructions 	Buffer/Solvent lines and sparge panel	P5-2296-02-0000
BioCAD Solvent Line Kit <ul style="list-style-type: none"> • Spacer, 0.209 x 0.125 x 0.160, Kel-F (10) • Fitting, barbed, small, polypropylene, (10) • Tubing, 1/8-in, 4 ft, Teflon (6) 	Buffer/Solvent connections	P5-2303-02-0000
Column Clamp Kit	Injection and column switching valves	GEN 601113

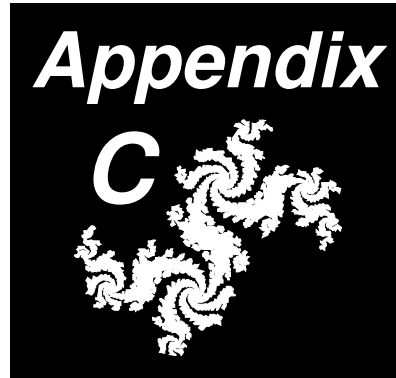
Table B-7 Spare Parts Kits (Continued)

Item	Where used	Part Number
Fuse Kit	Main power and internal printed circuit board fuses	P5-1953-02-0000
Gas Fitting Kit <ul style="list-style-type: none">• Brass fittings for regulator, 1/8 to 1/4 NPT (2)• Brass ferrules (10)	Gas regulator	GEN 601116
Seal Wash Line Kit	Seal wash lines	P5-1006-06-5040
Syringe Seal Kit (2.5 ml)	Syringe pump	GEN 601111
Syringe Seal Kit (0.5 ml)	Syringe pump	GEN 601112



B

C Warranty/Service Information



Applied Biosystems supplies or recommends certain configurations of computer hardware, software, and peripherals for use with its instrumentation. Applied Biosystems reserves the right to decline support for or impose charges for supporting nonstandard computer configurations or components that have not been supplied or recommended by Applied Biosystems. Applied Biosystems also reserves the right to require that computer hardware and software be restored to the standard configuration prior to providing service or technical support. For systems such as BioCAD[®], VISION[™], SPRINT[™], or INTEGRAL[™], which have built-in computers, installing unauthorized hardware or software may void the Warranty or Service Plan.

C.1 Limited Product Warranty

Limited warranty

Applied Biosystems warrants that all standard components of the BioCAD Workstation will be free of defects in materials and workmanship for a period of ninety (90) days. Applied Biosystems will repair or replace, at its discretion, all defective components during this warranty period. After this warranty period, repairs and replacement components may be purchased from Applied Biosystems at its published

rates. Applied Biosystems also provides service agreements for post-warranty coverage. Applied Biosystems reserves the right to use new, repaired, or refurbished instruments or components for warranty and post-warranty service agreement replacements. Repair or replacement of products or components under warranty does not extend the original warranty period.

Applied Biosystems warrants that all optional accessories supplied with its BioCAD Workstation, such as peripherals, printers, and special monitors, will be free of defects in materials and workmanship for a period of ninety (90) days. Applied Biosystems will repair or replace, at its discretion, defective accessories during this warranty period. After this warranty period, Applied Biosystems will pass on to the buyer, to the extent that it is permitted to do so, the warranty of the original manufacturer for such accessories.

With the exception of consumable and maintenance items, replaceable products or components used on the instrument are themselves warranted to be free of defects in materials and workmanship for ninety (90) days.

Applied Biosystems warrants that chemicals and other consumable products will be free of defects in materials and workmanship when received by the buyer, but not thereafter, unless otherwise specified in documentation accompanying the product.

Applied Biosystems warrants that for a period of ninety (90) days from the date of installation, the software designated for use with the product will perform substantially in accordance with the function and features described in its accompanying documentation when properly installed on the product. Applied Biosystems does not warrant that the operation of the instrument or software will be uninterrupted or error free. Applied Biosystems will provide any software corrections or "bug-fixes" if and when they become available, for a period of ninety (90) days after installation.

**Warranty
period effective
date**

Any applicable warranty period under these sections will begin on the date of installation for hardware and software installed by Applied Biosystems personnel, unless that date has been delayed at the buyer's request. In that case, and for all hardware and software installed by the buyer, and for all other products, the applicable warranty period begins the date the component is received by the buyer.

**Warranty
exceptions**

The above warranties shall not apply to defects resulting from misuse, neglect, or accident, including without limitation: operation with incompatible solvents or samples in the system; operation outside of the environmental or use specification instructions for the product or accessories; performance of improper or inadequate maintenance by the user; installation of software or interfacing not supplied by Applied Biosystems; and modification or repair of the product or the software not authorized by Applied Biosystems.

The foregoing provisions set forth Applied Biosystems' sole and exclusive representations, warranties, and obligations with respect to its products, and Applied Biosystems makes no other warranty of any kind whatsoever, expressed or implied, including without limitation, warranties of merchantability and fitness for a particular purpose, whether arising from a statute or otherwise in law or from a course of dealing or usage of trade, all of which are expressly disclaimed. Such limited warranty is given only to buyer or any third party in the event of use of products furnished hereunder by any third party.

**Warranty
limitations**

The remedies provided herein are the buyer's sole and exclusive remedies. Without limiting the generality of the foregoing, in no event shall Applied Biosystems be liable, whether in contract, in tort, warranty, or under any statute (including without limitation, any trade practice, unfair competition, or other statute of similar import) or on any other basis, for direct, indirect, punitive, incidental, multiple, consequential, or special damages sustained by the buyer or any other person, whether or not foreseeable and whether or not Applied Biosystems is advised of the possibility of such damage, including without limitation, damage arising from or



related to loss of use, loss of data, failure or interruption in the operation of any equipment or software, delay in repair or replacement, or for loss of revenue or profits, loss of good will, loss of business or other financial loss or personal injury or property damage.

No agent, employee, or representative of Applied Biosystems has any authority to bind Applied Biosystems to any affirmation, representation, or warranty concerning the product that is not contained in this Limited Warranty Statement. Any such affirmation, representation, or warranty made by any agent, employee, or representative of Applied Biosystems will not be binding on Applied Biosystems.

This warranty is limited to the buyer of the product from Applied Biosystems and is not transferable.

C.1 Damages, Claims, Returns

Damages Unpack shipments promptly after receipt to check for any concealed damage.

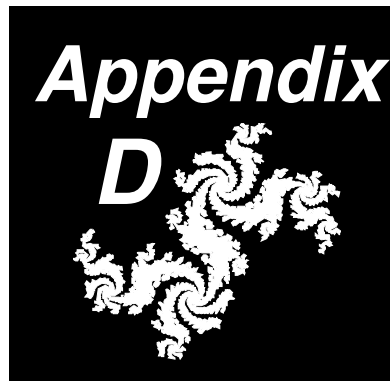
If you discover damage, stop unpacking. Contact the shipping carrier and request inspection by a local agent. Secure a written report of the findings to support any claim. Do not return damaged goods to Applied Biosystems without first securing an inspection report and contacting Applied Biosystems Technical Support for a Return Authorization (RA) number.

Claims After a damage inspection report is secured, Applied Biosystems will supply the replacements and process claims that are initiated by either party.

Returns Do not return any material without prior notification and authorization.

If for any reason it becomes necessary to return material to Applied Biosystems, contact Applied Biosystems Technical Support or your nearest Applied Biosystems subsidiary or distributor for a return authorization (RA) number and forwarding address. Place the RA number in a prominent location on the outside of the shipping container, and return the material to the appropriate address.

D BioCAD 700E Sample Methods



Sample method description

The BioCAD 700E includes sample methods which are stored on your hard drive in the C:\BIOCAD\METHOD directory.

You can use these sample methods:

- For demonstrations and training.
- As templates for creating new methods. Methods are designed so you can substitute different column sizes and types or different flow rates without affecting the chromatography.

Sample method configuration

Your system configuration may differ from the method configuration set in the sample methods. When you open a sample method, a configuration mismatch error may be displayed.

Click **Update Method with System Configuration** to update the method with the current system configuration.

Click **Do Not Update Method with System Configuration** to leave the method configuration unchanged. If you want to run the sample method using the method configuration, you must load the method configuration as the system configuration. For more information, see Section 4.4, Loading an Existing Configuration.

Printing You can print the sample methods from the Method Editor by selecting **Print Method** from the File menu. All methods are stored on your hard drive in the C:\BIOCAD\METHOD directory.

List of sample methods This appendix describes the following sample methods:

Sample Method Name	Purpose
AFFSTD.MET	Separation of standard proteins on POROS A and POROS G columns
HICSTD.MET	Separation of standard proteins on POROS HP, PE, and ET columns
RPSTD.MET	Separation of standard proteins on POROS R1 and R2 columns
ANIONSTD.MET	Separation of standard proteins on POROS HQ, QE, and PI columns
CATIONSD.MET	Separation of standard proteins on POROS HS, SP, S, and CM columns
ANIONPH.MET	Anion exchange pH map with standard proteins
CATPH.MET	Cation exchange pH map with standard proteins
CUSTOMPH.MET	Custom pH map with standard proteins (for use with uncalibrated pH buffers)
SYSCLEAN.MET	Automated system cleaning and shutdown

D.1 AFFSTD.MET—Separation on POROS A and POROS G

Configuration Configuration settings are listed below:

Instrument Configuration	
High/Low pressure	3,000 psi, 0 psi
Buffers/Solvents	<ul style="list-style-type: none"> • Channel A—Phosphate buffered saline (PBS) • Channel B—Acid - 12 mM HCl + 0.15 M NaCl
Plumbing	Single Column (can use other column configurations)
Column	4.6 mmD/100mmL POROS A/M
Tubing ID	0.010" (blue)
Flow Cell	Analytical
Sample Loader	Autoloader, 0.5 ml syringe, 100 µl sample loop
Sample	Sample Channel 1—BSA and human IgG, protein concentration 10 mg/ml each
Prime/Flush volumes	500 µl, 100 µl
Recorder	Detector Channel UV/Vis #1



Method settings Method settings are listed below, including:

- General Settings
- Block/Segment/Added Event Settings

General Settings	
Flow rate	5 ml/min. You can specify an 1,800 cm/hr flow rate so that when you configure a new column, the method maintains the same linear velocity for the new column.
Initial Column State	Column 1 inline
UV/Vis Wavelength	280 nm
Initial Detector State	pH/conductivity inline

Block	Segments and Added Events
Equil	<ul style="list-style-type: none"> • Purge segment—Equilibrates system with PBS (Channel A) before placing column 1 inline to equilibrate. • Step segment—Equilibrates column for 10 column volumes (CV). If you select a column with different dimensions in the configuration, the method updates itself and maintains a 10 CV equilibration based on the new column dimensions.
Load	<ul style="list-style-type: none"> • Inject segment—Injects sample onto column. Flush Loader is selected to flush sample lines with sample channel 1 before loading loop with 20 µl of sample. • Zero UV Detector Event—Zeroes UV/Vis detector after injection. • Step segment—Flows standard on column for 0.5 min. Ensures sample passes through column and non-retained components are washed off before Elute block begins.
Elute	<ul style="list-style-type: none"> • Purge segment—Flushes system with acid (Channel B). This minimizes column elution time (because you are not waiting for the acid front to reach the column head). It also performs a true step change to acid, rather than a gradual change from PBS to acid. • Step segment—Holds acidic conditions for 5 CV, to allow peak to elute and pass through the detector before method ends.



D.2 HICSTD.MET—Separation on POROS HP, PE, and ET

Configuration Configuration settings are listed below:

Instrument Configuration	
High/Low pressure	3,000 psi, 0 psi
Buffers/Solvents	<ul style="list-style-type: none"> Channel A—20 mM phosphate pH 7 + 3M ammonium sulfate Channel B—Phos - consists of 20 mM Phosphate, pH 7
Plumbing	Single Column (can use other column configurations)
Column	4.6 mmD/100mmL POROS PE/M
Tubing	0.010" (blue)
Flow Cell	Analytical
Sample Loader	Autoloader, 0.5 ml syringe, 100 µl sample loop
Sample	Sample Channel 1—ribonuclease and lysozyme, protein concentration 5 mg/ml
Prime/Flush volumes	500 µl, 100 µl
Recorder	Detector Channel UV/Vis #1

Method settings Method settings are listed below, including:

- General Settings
- Block/Segment/Added Event Settings

General Settings	
Flow rate	5 ml/min. You can specify an 1,800 cm/hr flow rate so that when you configure a new column, the method maintains the same linear velocity for the new column.
Initial Column State	Column 1 inline
UV/Vis Wavelength	280 nm
Initial Detector State	pH/conductivity inline

Block	Segments and Added Events
Equil	<ul style="list-style-type: none"> • Purge segment—Equilibrates system with ammonium sulfate buffer (Channel A) before placing column 1 inline to equilibrate. • Step segment—Equilibrates column for 10 column volumes (CV). If you select a column with different dimensions in the configuration, the method updates itself and maintains a 10 CV equilibration based on the new column dimensions.
Load	<ul style="list-style-type: none"> • Inject segment—Injects sample onto column. Flush Loader is selected to flush sample lines with sample channel 1 before loading loop with 20 µl of sample. • Zero UV Detector Event—Zeroes UV/Vis detector after injection.

D.3 RPSTD.MET—Separation on POROS R1 and POROS R2

Configuration Configuration settings are listed below:

Instrument Configuration	
High/Low pressure	3,000 psi, 0 psi
Buffers/Solvents	<ul style="list-style-type: none"> • Channel A—0.1% v/v TFA in water • Channel B—Acetonitrile
Plumbing	Single Column (can use other configurations)
Column	4.6 mmD/100mmL POROS R/M
Tubing	0.010" (blue)
Flow Cell	Analytical
Sample Loader	Autoloader, 0.5 ml syringe, 100 µl sample loop
Sample	Sample Channel 1—cytochrome C and soybean trypsin inhibitor (STI), protein concentration 10 mg/ml
Prime/Flush volume	500 µl, 100 µl
Recorder	Detector Channel UV/Vis #1

Method settings Method settings are listed below, including:

- General Settings
- Block/Segment/Added Event Settings

General Settings	
Flow rate	5 ml/min. You can specify an 1,800 cm/hr flow rate so that when you configure a new column, the method maintains the same linear velocity for the new column.
Initial Column State	Column 1 inline
UV/Vis Wavelength	220 nm
Initial Detector State	pH/conductivity bypassed NOTE: Take pH/conductivity monitors out of line when performing reversed-phase separations.

Block	Segments and Added Event
Equil	<ul style="list-style-type: none"> • Purge segment—Equilibrates system with 85% 0.1% TFA (Channel A) and 15% acetonitrile (Channel B) before placing column 1 inline to equilibrate. • Step segment—Equilibrates column for 10 column volumes (CV). If you select a column with different dimensions in the configuration, the method updates itself and maintains a 10 CV equilibration based on the new column dimensions.
Load	<ul style="list-style-type: none"> • Inject segment—Injects sample onto column. Flush Loader is selected to flush sample lines with sample channel 1 before loading loop with 20 µl of sample. • Zero UV Detector Event—Zeroes UV/Vis detector after injection.

D.4 ANIONSTD.MET—Separation on POROS HQ, QE, and PI

Configuration Configuration settings are listed below:

Instrument Configuration	
High/Low pressure	3,000 psi, 0 psi
Buffers/Solvents	<ul style="list-style-type: none"> Channel A—20 mM Tris, pH 8 Channel B—20 mM Tris, pH 8 + 0.5 M NaCl
Plumbing	Single Column (can use other column configurations)
Column	4.6 mmD/100mmL POROS HQ/M
Tubing	0.010" (blue)
Flow Cell	Analytical
Sample Loader	Autoloader, 0.5 ml syringe, 100 µl sample loop
Sample	Sample Channel 1—BSA and ovalbumin, protein concentration 10 mg/ml
Prime/Flush volume	500 µl, 100 µl
Recorder	Detector Channel UV/Vis #1

Method settings Method settings are listed below, including:

- General Settings
- Block/Segment/Added Event Settings

General Settings	
Flow rate	5 ml/min. You can specify an 1,800 cm/hr flow rate so that when you configure a new column, the method maintains the same linear velocity for the new column.
Initial Column State	Column 1 inline
UV/Vis Wavelength	280 nm
Initial Detector State	pH/conductivity inline

Block	Segments and Added Event
Equil	<ul style="list-style-type: none"> • Purge segment—Equilibrates system with Tris buffer (Channel A) before placing column 1 inline to equilibrate. • Step segment—Equilibrates column for 10 column volumes (CV). If you select a column with different dimensions in the configuration, the method updates itself and maintains a 10 CV equilibration based on the new column dimensions.
Load	<ul style="list-style-type: none"> • Inject segment—Injects sample onto column. Flush Loader is selected to flush sample lines with sample channel 1 before loading loop with 20 µl of sample. • Zero UV Detector Event—Zeroes UV/Vis detector after injection.
Elute	<ul style="list-style-type: none"> • Gradient segment—A 15 CV gradient changes from 100% Tris (Channel A) to 100% Tris and salt (Channel B). Gradient segment can be programmed in time (min) or volume (ml). There is a 2.5 ml delay before the gradient front reaches the column, due to delay volumes inherent in the proportioning/blending system. Therefore, it is not necessary to program a wash segment before gradient start, unless you need more than 2.5 ml of wash. • Step segment—Holds end of the gradient for 10 ml to ensure the gradient front is through column and detector before method ends.

D.5 CATIONSD.MET—Separation on POROS HS, SP, S, and CM

Configuration Configuration settings are listed below:

Instrument Configuration	
High/Low pressure	3,000 psi, 0 psi
Buffers/Solvents	<ul style="list-style-type: none"> Channel A—20 mM MES, pH 6.2 Channel B—20 mM MES, pH 6.2 + 0.5 M NaCl (substitute 100 mM acetate pH 4.5 and 100 mM acetate pH 4.5 + 1.0 M NaCl for POROS CM)
Plumbing	Single Column (can use other column configurations)
Column	4.6 mmD/100mmL POROS HS/M
Tubing	0.010" (blue)
Flow Cell	Analytical
Sample Loader	Autoloader, 0.5 ml syringe, 100 µl sample loop
Sample	Sample Channel 1—ribonuclease and lysozyme (substitute ovalbumin and bovine serum albumin for POROS CM), protein concentration 5 mg/ml
Prime/Flush volume	500 µl, 100 µl
Recorder	Detector Channel UV/Vis #1

Method settings Method settings are listed below, including:

- General Settings
- Block/Segment/Added Event Settings

General Settings	
Flow rate	5 ml/min. You can specify an 1,800 cm/hr flow rate so that when you configure a new column, the method maintains the same linear velocity for the new column.
UV/Vis Wavelength	280 nm
Initial Column State	Column 1 inline
Initial Detector State	pH/conductivity inline

Block	Segments and Added Events
Equil	<ul style="list-style-type: none"> • Purge segment—Equilibrates system with MES buffer (Channel A) before placing column 1 inline to equilibrate. • Step segment—Equilibrates column for 10 column volumes (CV). If you select a column with different dimensions in configuration, the method updates itself and maintains a 10 CV equilibration based on the new column dimensions.
Load	<ul style="list-style-type: none"> • Inject segment—Injects sample onto column. Flush Loader is selected to flush sample lines with sample channel 1 before loading loop with 20 µl of sample. • Zero UV Detector Event—Zeroes UV/Vis detector after injection.
Elute	<ul style="list-style-type: none"> • Gradient segment—A 15 CV gradient changes from 100% MES (Channel A) to 100% MES and salt (Channel B). Gradient segment can be programmed in time (min) or volume (ml). There is a 2.5 ml delay before the gradient front reaches the column, due to delay volumes inherent in proportioning/blending system. Therefore, it is not necessary to program a wash segment before gradient start, unless you need more than 2.5 ml of wash. • Step segment—Holds end of the gradient for 10 ml to ensure the gradient front is through column and detector before method ends.

D.6 ANIONPH.MET— Anion Exchange pH Map

Configuration Configuration settings are listed below:

Instrument Configuration	
High/Low pressure	3,000 psi, 0 psi
Buffers/Solvents	<ul style="list-style-type: none"> Channel A—100 mM anion buffer, pH 6 (pH blend anion buffer); configure as type Buffer in Buffer/Solvent Library Channel B—100 mM anion buffer, pH 9 (pH blend anion buffer); configure as type Buffer in Buffer/Solvent Library Channel C—Water; configure as type H2O in Buffer/Solvent Library Channel D—3 M NaCl; configure as type Eluent in Buffer/Solvent Library <p>NOTE: You can also use your own custom pH blend buffers, if you calibrate them.</p>
Plumbing	Single Column (can use other column configurations)
Column	4.6 mmD/100mmL POROS HQ/M
Tubing	0.010" (blue)
Flow Cell	Analytical
Sample Loader	Autoloader, 0.5 ml syringe, 100 µl sample loop
Sample	Sample Channel 1—BSA and ovalbumin, protein concentration 10 mg/ml
Prime/Flush volume	500 µl, 100 µl
Recorder	Detector Channel UV/Vis #1

Method settings All segments are programmed in the pH blend mode.

In pH blend mode, you specify:

- Blend concentration (mM)

NOTE: *The blend concentration is typically a two fold or greater dilution of the concentrated buffers in channels A and B.*

- Blend pH
- Eluent concentration (mM)

NOTE: *The eluent concentration is typically a two fold or greater dilution of the concentrated eluent in channel D (in this case NaCl).*

You can select Percentage blend mode in each segment to see actual percentages that the system is blending in response to the pH mode specification.

The ANIONPH.MET method was designed for use with the pH Map template. Note that the easiest way to change the pH setting may be by running the pH Map template, even if the template consists of only one run.

Method settings are listed below, including:

- General Settings
- Block/Segment/Added Event Settings

D

General Settings	
Flow rate	5 ml/min. You can specify an 1,800 cm/hr flow rate so that when you configure a new column, the method maintains the same linear velocity for the new column.
Initial Column State	Column 1 inline
UV/Vis Wavelength	280 nm
Initial Detector State	pH/conductivity inline

Block	Segments and Added Events
Equil	<ul style="list-style-type: none"> • Purge segment—Equilibrates system with the pH blend settings (50 mM buffer, pH 7) before placing column 1 inline to equilibrate. • Step segment—Equilibrates column for 10 column volumes (CV). If you select a column with different dimensions in configuration, the method updates itself and maintains a 10 CV equilibration based on the new column dimensions.
Load	<ul style="list-style-type: none"> • Inject segment—Injects sample onto column. Flush Loader is selected to flush sample lines with sample channel 1 before loading loop with 20 µl of sample. • Zero UV Detector Event—Zeroes UV/Vis detector after injection.
Elute	<ul style="list-style-type: none"> • Gradient segment—A 15 CV gradient changes from 0 mM NaCl to 1000 mM NaCl. Gradient segment can be programmed in time (min) or volume (ml). When specifying the gradient segment in pH blend mode, specify buffer concentration (50 mM), pH gradient (in this case, pH is held constant at 7), and eluent gradient (in this case, 0 to 1000 mM NaCl). There is a 2.5 ml delay before the gradient front reaches the column, due to delay volumes inherent in the proportioning/blending system. Therefore, it is not necessary to program a wash segment before gradient start, unless you need more than 2.5 ml of wash. • Step segment—Holds end of the gradient for 10 ml to ensure the gradient front is through column and detector before method ends.

D

D.7 CATPH.MET— Cation Exchange pH Map

Configuration Configuration settings are listed below:

Instrument Configuration	
High/Low pressure	3,000 psi, 0 psi
Buffers/Solvents	<ul style="list-style-type: none"> Channel A—100 mM cation buffer, pH 4.5 (pH blend cation buffer); configure as type Buffer in Buffer/Solvent Library Channel B—100 mM cation buffer, pH 7.5 (pH blend cation buffer); configure as type Buffer in Buffer/Solvent Library Channel C—Water; configure as type H2O in Buffer/Solvent Library Channel D—3 M NaCl; configure as type Eluent in Buffer/Solvent Library <p>NOTE: You can also use your own custom pH blend buffers, if you calibrate them.</p>
Plumbing	Single Column (can use other column configurations)
Column	4.6 mmD/100mmL POROS HS/M
Tubing	0.010"
Flow Cell	Analytical
Sample Loader	Autoloader, 0.5 ml syringe, 100 µl sample loop
Sample	Sample Channel 1—ribonuclease and lysozyme, protein concentration 5 mg/ml
Prime/Flush volume	500 µl, 100 µl
Recorder	Detector Channel UV/Vis #1

Method settings All segments are programmed in the pH blend mode.

In pH blend mode, you specify:

- Blend concentration (mM)

NOTE: *The blend concentration is typically a two fold or greater dilution of the concentrated buffers in channels A and B.*

- Blend pH
- Eluent concentration (mM)

NOTE: *The eluent concentration is typically a two fold or greater dilution of the concentrated eluent in channel D (in this case NaCl).*

You can select Percentage blend mode in each segment to see actual percentages that the system is blending in response to the pH mode specification.

The CATPH.MET method was designed for use with the pH Map template. Note that the easiest way to change the pH setting may be by running the pH Map template, even if the template consists of only one run.

Method settings are listed below, including:

- General Settings
- Block/Segment/Added Event Settings



General Settings	
Flow rate	5 ml/min. You can specify an 1,800 cm/hr flow rate so that when you configure a new column, the method maintains the same linear velocity for the new column.
Initial Column State	Column 1 inline
UV/Vis Wavelength	280 nm
Initial Detector State	pH/conductivity inline

Block	Includes
Equil	<ul style="list-style-type: none"> • Purge segment—Equilibrates system with the pH blend settings (50 mM buffer, pH 5) before placing column 1 inline to equilibrate. • Step segment—Equilibrates column for 10 column volumes (CV). If you select a column with different dimensions in configuration, the method updates itself and maintains a 10 CV equilibration based on the new column dimensions.
Load	<ul style="list-style-type: none"> • Inject segment—Injects sample onto column. Flush Loader is selected to flush sample lines with sample channel 1 before loading loop with 20 µl of sample. • Zero UV Detector Event—Zeroes UV/Vis detector after injection.
Elute	<ul style="list-style-type: none"> • Gradient segment—A 15 CV gradient changes from 0 mM NaCl to 1,000 mM NaCl. Gradient segment can be programmed in time (min) or volume (ml). When specifying the gradient segment in pH blend mode, specify buffer concentration (50 mM), pH gradient (in this case, pH is held constant at 5), and eluent gradient (in this case, 0 to 1,000 mM NaCl). There us a 2.5 ml delay before the gradient front reaches the column, due to delay volumes inherent in the proportioning/blending system. Therefore, it is not necessary to program a wash segment before gradient start, unless you need more than 2.5 ml of wash. • Step segment—Holds end of the gradient for 10 ml to ensure the gradient front is through column and detector before method ends.

D.8 CUSTOMPH.MET— Custom pH Map

Configuration Configuration settings are listed below:

Instrument Configuration	
High/Low pressure	3,000 psi, 0 psi
Buffers/Solvents	<ul style="list-style-type: none"> • Channel A—100 mM phosphate, pH 6; configure as type Buffer in Buffer/Solvent Library • Channel B—100 mM phosphate, pH 9; configure as type Buffer in Buffer/Solvent Library • Channel C—Water; configure as type H2O in Buffer/Solvent Library • Channel D—3 M NaCl; configure as type Eluent in Buffer/Solvent Library
Plumbing	Single Column (can use other column configurations)
Column	4.6 mmD/100mmL POROS HQ/M
Tubing/	0.010" (blue)
Flow Cell	Analytical
Sample Loader	Autoloader, 0.5 ml syringe, 100 µl sample loop
Sample	Sample Channel 1—BSA and ovalbumin, protein concentration 10 mg/ml
Prime/Flush volume	500 µl, 100 µl
Recorder	Detector Channel UV/Vis #1



Method settings

All segments are programmed in pH mode.

Because the CUSTOMPH.MET method configuration does not use pH Blend Buffers or calibrated custom pH blend buffers, the pH blending uses a percentage, instead of a pH specification.

In pH mode with uncalibrated pH blend buffers, you specify:

- Blend percentage of the two pH buffers. The resulting pH is equal to the percent of the pH values. For example, if buffers are pH 4 and pH 6, and you enter 50%, the resulting pH is 5.
- Blend concentration (mM)

NOTE: *The blend concentration is typically a two fold or greater dilution of the concentrated buffers in channels A and B.*

- Eluent concentration (mM)

NOTE: *The eluent concentration is typically a two fold or greater dilution of the concentrated eluent in channel D (in this case NaCl).*

You can select Percentage blend mode in each segment to see actual percentages that the system is blending in response to the pH mode specification.

The CUSTOMPH.MET method was designed for use with the pH Map template. Note that the easiest way to change the pH setting may be by running the pH Map template, even if the template consists of only one run.

Method settings are listed below, including:

- General Settings
- Block/Segment/Added Event Settings

General Settings	
Flow rate	5 ml/min. You can specify an 1,800 cm/hr flow rate so that when you configure a new column, the method maintains the same linear velocity for the new column.
Initial Column State	Column 1 inline
UV/Vis Wavelength	280 nm
Initial Detector State	pH/conductivity inline

Block	Includes
Equil	<ul style="list-style-type: none"> • Purge segment—Equilibrates system with the pH blend settings (50 mM buffer, 20% low pH buffer/80% high pH buffer) before placing column 1 inline to equilibrate. • Step segment—Equilibrates column for 10 column volumes (CV). If you select a column with different dimensions in the configuration, the method updates itself and maintains a 10 CV equilibration based on the new column dimensions.
Load	<ul style="list-style-type: none"> • Inject segment—Injects sample onto column. Flush Loader is selected to flush sample lines with sample channel 1 before loading loop with 20 µl of sample. • Zero UV Detector Event—Zeroes UV/Vis detector after injection.
Elute	<ul style="list-style-type: none"> • Gradient segment—A 15 CV minute gradient changes from 0 mM NaCl to 1,000 mM. Gradient segment can be programmed in time (min) or volume (ml). When specifying the gradient segment in the pH mode, specify buffer concentration (50 mM), pH gradient (in this case, pH blend of buffers is held constant at 20/80) and eluent gradient (0 to 1,000 mM NaCl). There is a 2.5 ml delay before the gradient front reaches the column, due to delay volumes inherent in the proportioning/blending system. Therefore, it is not necessary to program a wash segment before gradient start, unless you need more than 2.5 ml of wash. • Step segment—Holds end of the gradient for 10 ml to ensure the gradient front is through column and detector before method ends.



D.9 SYSCLEAN.MET—Shutdown

Configuration The SYSCLEAN.MET method is for automated system cleaning and shutdown. However, the method does not include steps to clean inline columns. All flow is in the bypass column mode. Set High Pressure limit to 3,000 psi.

CAUTION

The system must be in Single Column or Tandem Column plumbing configuration to run SYSCLEAN.MET. You cannot bypass columns in other plumbing configurations.

Method settings To run this method, place all solvent and sample lines in a beaker of water (or other appropriate flush solution).

Method settings are listed below, including:

- General Settings
- Block/Segment/Added Event Settings

General Settings	
Flow rate	30 ml/min. Depending on tubing diameter and detector flow cell, you may not be able to run at 30 ml/min without exceeding 3,000 psi and shutting down the pump. If this occurs, reduce the flow rate in General Settings and in all Purge segments.
Initial Column State	Columns bypassed
UV/Vis Wavelength	254 nm
Initial Detector State	pH/conductivity bypassed

Block	Includes
Equil	<ul style="list-style-type: none"> • Purge segments A through F—Purges all buffer/solvent lines (A–F). • Prime Sample Loader events—Primes sample lines (1–4). • Set Detectors Inline event—Places conductivity/pH monitors inline. • Turn UV Detector Lamp Off event—Turns off UV/Vis detector. • Pump Off event—Turns off pump.

E External Instrument Connections

Appendix

E



The BioCAD 700E Workstation has three connectors on the left side panel:

- Fraction Collector
- Autosampler
- Auxiliary

Connectors are mounted vertically with Pin 1 at the top. Pin assignments are listed in the following tables.

Table E-1 Fraction Collector Connector Pin Assignments

Pin	Assignment
1	Integrator Output (+)—1 Volt/AU
2	Integrator Output (-)—1 Volt/AU
3	Logic Ground Output
4	Fraction Collector Start—TTL
5	Fraction Collector End—TTL
6	Advance Tube—TTL
7	Event Mark Input—TTL
8	Event Mark Ground
9	Valve Power Output +12 V, 0.25 Amp
10	Valve Switch Output

Table E-2 Autosampler Connector Pin Assignments

Pin	Assignment
1	Logic Input Ground
2	Input Injection Trigger—TTL
3	Input Stop—TTL
4	Busy Output—TTL
5	Start Output—TTL
6	Stop Output—TTL
7	Logic Output Ground
8	No Connection
9	No Connection
10	No Connection

Table E-3 Auxiliary Connector Pin Assignments

Pin	Assignment
1	Analog Input (+) 0 To 1 Volt Full Scale
2	Analog Input (-) 0 To 1 Volt Full Scale
3	Output Valve Power # 1 +24 Volt, 1 Amp
4	Output Valve Switch # 1
5	Output Valve Power # 2 +12 Volt, 0.25 Amp
6	Output Valve Switch # 2
7	Logic Input Ground
8	Input # 1—TTL
9	Input # 2—TTL
10	Output # 1—TTL
11	Output # 2—TTL
12	Logic Output Ground
13–16	Do Not Connect

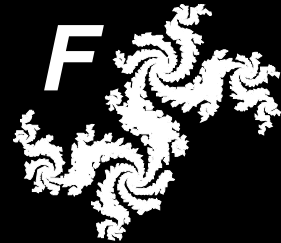
NOTE: When connecting a device to the Auxiliary connector, use a shielded cable with a ferrite bead to maintain CE compliance. Recommended source and part number for the ferrite bead is:
 Fair-Rite Products Corporation, No. 2643164151.



F Autosampler Injection Types

Appendix

F



This appendix describes how the AS 3500 autosampler makes each injection type.

NOTE: For more information on plumbing the autosampler injector valve, see Section 3.6.2, Plumbing Autosampler Injector Valve.

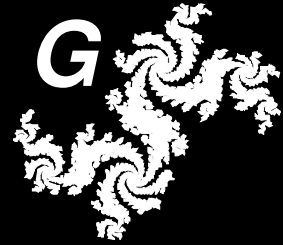
NOTE: For information on selecting and configuring injection type for an integrated autosampler, see Section 4.8, Configuring and Preparing the BioCAD Software for a Direct Wire Autosampler.

Table F-1 AS 3500 Autosampler Injections

Injection Type	Autosampler Function
Pull Loop ($< 200 \mu\text{l}$) (default)	<ul style="list-style-type: none"> • An air bubble is drawn into the needle to isolate the sample slug from the flush solvent in the line. • With injector valve in Load position, sample is drawn into needle. • The sample vial is lowered below needle tip, and sufficient air is drawn into the needle to pull the sample slug into the loop. • The injector valve rotates to Inject position and sample is carried from the loop onto the column.
PushLoop [®]	<ul style="list-style-type: none"> • An air bubble is drawn into the needle to isolate the sample slug from the flush solvent in the line. • With the injector valve in Inject position, sample is pulled up to and past the injector valve. • The injector valve rotates to Load position and the desired sample volume is pushed into the loop. • The injector valve rotates to Inject position and sample is carried from the loop onto the column.
Full Loop ($\leq 100 \mu\text{l}$)	<ul style="list-style-type: none"> • An air bubble is drawn into the needle to isolate the sample slug from the flush solvent in the line. • With the injector valve in Load position, the syringe retracts to pull sample from the vial into the sample loop. The volume of sample drawn is approximately two times the loop volume to ensure the loop is completely filled. • The injector valve rotates to Inject position and sample is carried from the loop onto the column.
Pull Loop ($> 200 \mu\text{l}$) OR Full Loop ($> 100 \mu\text{l}$)	<ul style="list-style-type: none"> • With the injector valve in Inject position, the autosampler sample syringe pulls sample into needle, past injector valve. • The injector valve rotates to Load position. • The autosampler prep syringe pulls sample into the loop. • The injector valve rotates to Inject position and sample is carried from the loop onto the column.

Appendix

G



G Maintenance Log

The following pages include a log sheet listing maintenance procedures. Copy these pages and keep them by your BioCAD 700E Workstation.

Instructions for performing maintenance procedures are listed in:

- Section 2.6, Starting Up and Shutting Down
- Section 3.2.3, Preparing Pump Seal Wash Solution
- Chapter 9, Maintenance

Maintenance Log

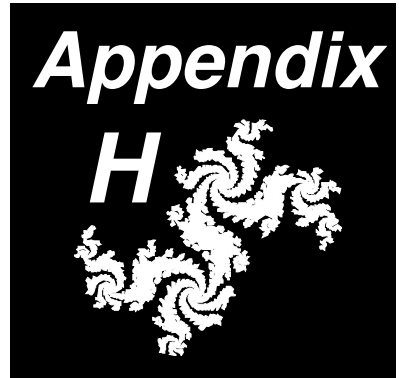
for BioCAD 700E System Serial Number _____

Record the date and your initials when you perform maintenance procedures.

Daily	Jan	Feb	Mar	Apr	May	June	July	Aug	Sept	Oct	Nov	Dec
Flush system with SYSCLEAN.MET												
Calibrate pH probe and conductivity monitor (If operating under GLP)												
Weekly												
Change pump seal wash solution												
Calibrate pH probe												
Calibrate conductivity monitor												
Back up data												
Run SCANDISK												

Monthly	Jan	Feb	Mar	Apr	May	June	July	Aug	Sept	Oct	Nov	Dec
Clean fan filter												
Clean pH probe												
Clean pump filter/mixer frit												
Run DEFRAG												
6 Months	Jan	Feb	Mar	Apr	May	June	July	Aug	Sept	Oct	Nov	Dec
Replace pump seals, clean pistons, clean pump heads												
Clean check valves												
Clean UV/Vis detector flow cell												
Clean fluorescence flow cell (See Fluorescence Detectors Manual)												
Inspect autoloader syringe seal												
Clean injection and column switching valve stators												

H Autoloader Flow Path



This appendix includes diagrams of the flow path of the autoloader during the following operations:

- Priming and Flushing
- Filling the sample loop with buffer
- Loading the sample loop with sample
- Injecting sample (emptying the sample loop)

NOTE: For information on making autoloader injections, see Section 5.3, Making Injections From the Control Panel.

H.1 Priming and Flushing

Figure H-1 illustrates the autoloader flow path when priming and flushing the sample lines.

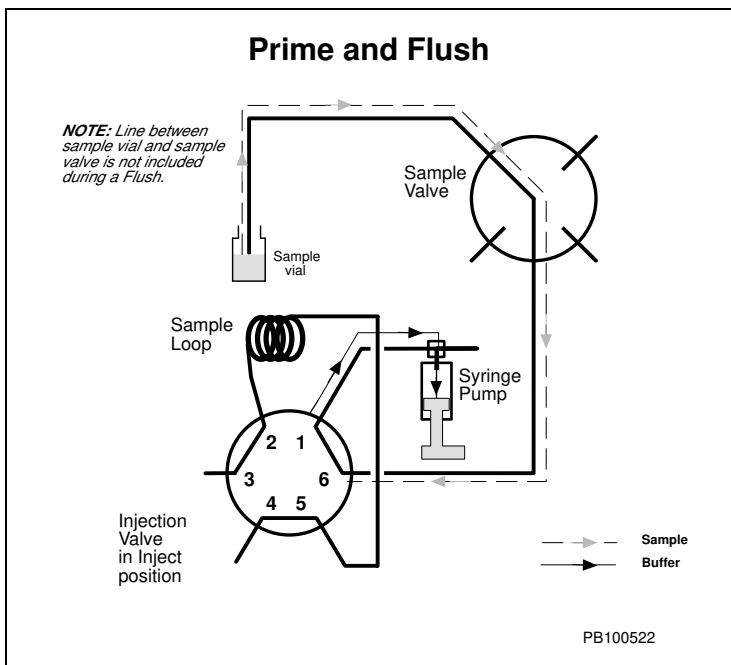


Figure H-1 Autoloader Flow Path When Priming and Flushing Sample Lines

To prime or flush the sample lines, select the appropriate command from the Sample menu in the Control Panel. For more information, see Section 3.4.3, Priming and Flushing the Autoloader Sample Lines.

During priming, the syringe withdraws and pulls sample through the line between the sample vial and the Injection valve.

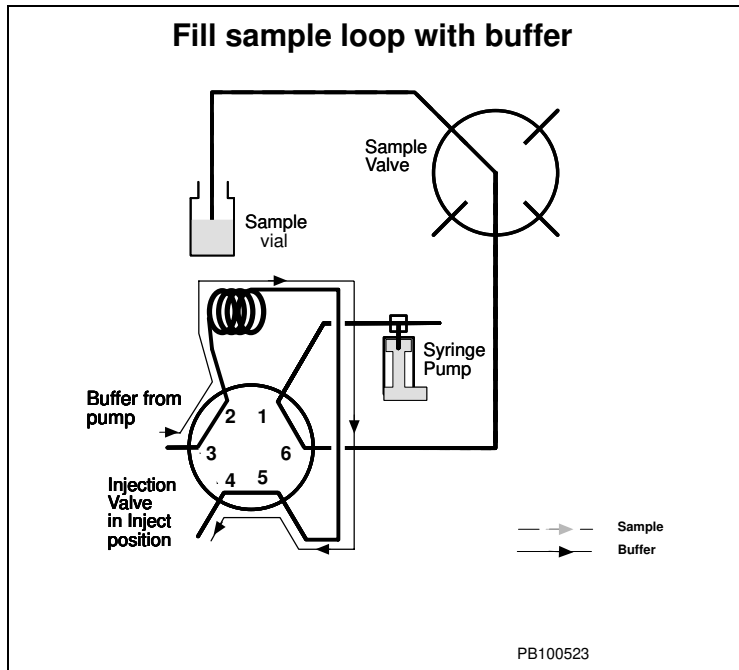
During flushing, the syringe withdraws a smaller volume of sample than priming. Flushing pulls a volume of sample sufficient to fill the line between the Sample valve and the Injection valve.

NOTE: *If you prime only once using default volumes, sample does not enter the syringe. If you prime more than one time, or increase the prime volume, sample enters the syringe.*



H.2 Filling the Sample Loop with Buffer

Figure H-2 illustrates the autoloader flow path when filling the sample loop with buffer.

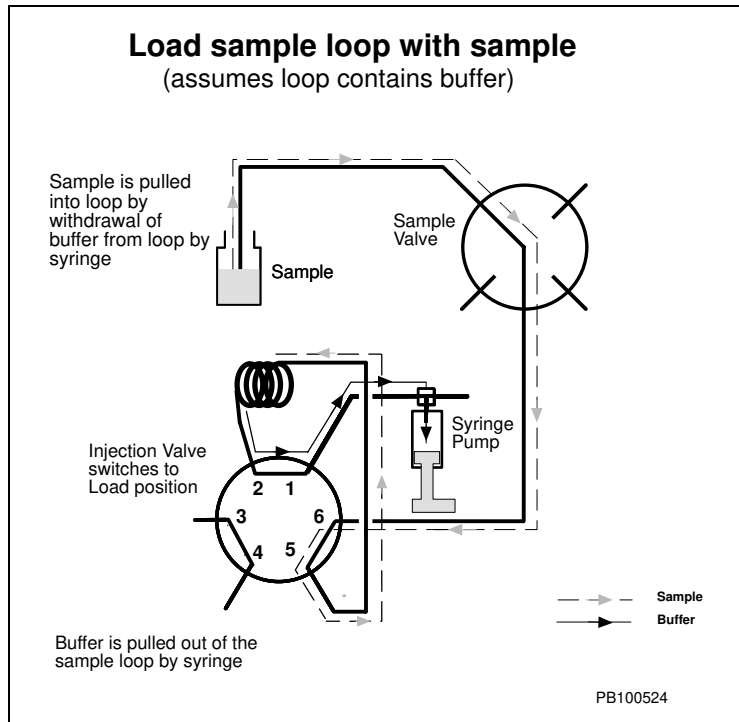


**Figure H-2 Autoloader Flow Path
When Filling the Sample Loop With Buffer**

To fill the sample loop with buffer, place the Injection valve in the Inject position while the pump is running.

H.3 Loading the Sample Loop with Sample

Figure H-3 illustrates the autoloader flow path when loading the sample loop with sample.



**Figure H-3 Autoloader Flow Path
When Loading the Sample Loop With Sample**

NOTE: Figure H-3 is not drawn to scale. The actual sample loop path between port 5 and port 2 is shorter than it appears in the illustration.

To load the sample loop with sample, click the **Load Loop** button in the Control Panel. For more information, see Section 5.3.1, Making Autoloader Injections.

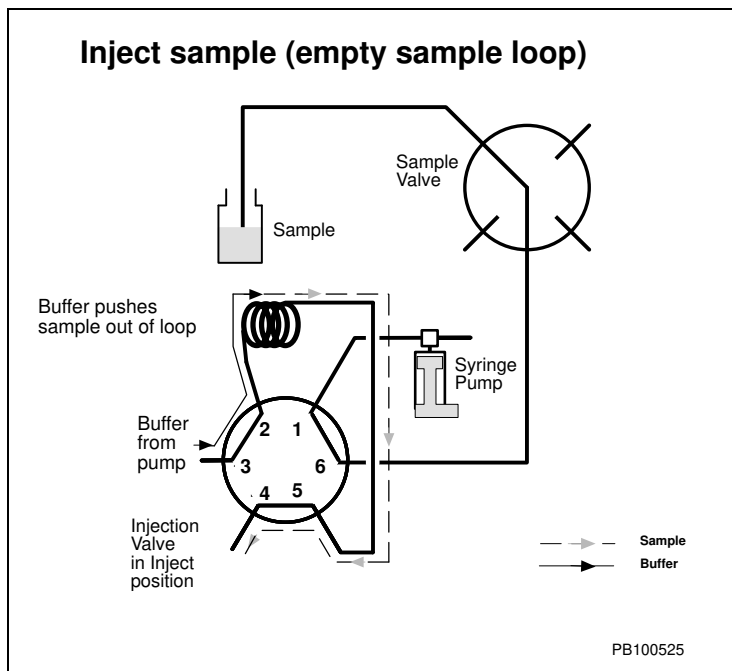
The Injection valve switches to the Load position. As the syringe withdraws, it pulls buffer out of the sample loop and into the syringe. The displaced buffer *pulls* sample into the sample loop.

NOTE: *Sample does not enter the syringe, unless the injection volume exceeds the sample loop volume.*



H.4 Injecting Sample (Emptying the Sample Loop)

Figure H-4 illustrates the autoloader flow path when injecting sample (emptying the sample loop).



**Figure H-4 Autoloader Flow Path When Injecting Sample
(Emptying the Sample Loop)**



To inject sample (empty the sample loop):

- Prime or flush the sample line as described in Section H.1, Priming and Flushing
- Fill the sample loop as described in Section H.2, Filling the Sample Loop with Buffer
- Load the sample loop as described in Section H.3, Loading the Sample Loop with Sample
- With the pump running, click the **Inject** option button in the Sample section of the Control Panel.

For more information, see Section 5.3.1, Making Autoloader Injections.

The Injection valve switches to the Inject position and places the sample loop in the fluid path. Buffer from the pump pushes the sample out of the sample loop and through the system. The switching valve on the autoloader syringe diverts buffer in the syringe to waste as the syringe plunger returns to the home position.



Glossary

Analysis—Integration, calibration, and reporting of data. In BioCAD, analysis is performed in the Group Analysis window and Data Analysis module.

Area percent—Percent of area value contributed by a given integrated peak, as compared to all integrated peaks in a chromatogram.

Asymmetry—Shape of a chromatographic peak. Peak asymmetry is a value you can export from the BioCAD software, and is described by B/A where $B = RT$ at rear of peak at 10% of peak height and $A = RT$ at front of peak at 10% of peak height.

AU—Absorbance unit, a measure of UV/Vis light absorbance.

.BIO or .B## file—Data file generated by BioCAD software. ## are sequential numbers generated with the auto file extension feature.

Blocks—Six types of operations which you can easily assemble to create a method. Blocks include: Prep, Equilibrate, Load, Wash, Elute, and Clean.

Bypass valve—A six-port selection valve that lets you bypass the conductivity and pH monitors so that output from the absorbance detector flows directly to the fraction collection valve.

Calibration curve—Plots peak area versus amounts of known pure standards. By comparing the peak area of unknowns to the curve, you can calculate amounts.

Calibration level—Concentration of each calibrated peak in calibration standards.

Capacity factor - k' —Chromatographic value that indicates the number of column volumes that a peak is retained.

.CFG file—BioCAD system configuration file, created in the Configuration dialog box.

CV—Column volumes.

Defragment—To eliminate non-contiguous disk space. Can improve computer performance.

Desktop—Screen background for windows. Windows, icons, and dialog boxes are displayed on the desktop.

Direct autoloader injection—Automatically injects from one sample vial with minimum sample loss. Effective range of injection is the same as the autoloader, but dead volume and priming volume are minimized.

Directory—Structure that organizes your files on disk. A directory can contain files or subdirectories.

DOS prompt—Letter designation for the active drive. In the DOS window, the prompt appears at the left side of the computer screen and is followed by a colon.

.DRC file—BioCAD strip chart recorder file.

Eluent mode—BioCAD blend programming mode that allows you to specify an eluent concentration, and select a buffer and an eluent to accomplish the blend.

External detector—External detector controlled by the BioCAD software (UV Channel 2).

External Standard—Unknowns are compared to standards without correction. With external standard calibration, it is important that injection size is accurate and reproducible.

Flush—Automated action for the BioCAD autoloader. Flushes line between sample selection valve and injection valve.

.GMM file—BioCAD multi-method file.

.GRO file—BioCAD group file. A group file is a directory of files created using a multi-method or a template.

.G## file—Method file generated by BioCAD Multi-Method Editor. ## are sequential numbers that represent subsequent methods.

Group—In BioCAD software, a file that contains a directory of file names. A group is created automatically when you run a template or multi-method.

Group integration—Integration in the BioCAD Data Analysis software, where all files are integrated with one set of analysis parameters.

Hardware key—Protection key required to run Supervisor stand-alone software option.

Height percent—Percent of height value contributed by a given integrated peak, as compared to all integrated peaks in a chromatogram.

Icon—Graphical representation of a program or window. When a window is minimized, it is reduced to an icon.

Individual integration—Integration in the BioCAD Data Analysis software, where each file is integrated with its own set of analysis parameters.

Integration—Calculations that determine area or height of peak.

Internal dual wavelength—A model 205 detector is installed in the workstation. The detector includes deuterium and tungsten lamps. Only one lamp is used at one time.

Internal Standard—Samples (standards and unknowns) are spiked with a known amount of a known compound. When samples are run, areas are “corrected” using the internal standard. The internal standard technique corrects for errors in sample preparation and injection.

Linear velocity—Expression of flow rate in cm/hr, calculated as volumetric flow rate (ml/hr) divided by column cross-sectional area (cm²).

.MET file—Method file generated by BioCAD Method Editor.

Method—Set of instructions BioCAD software needs to collect data. Includes blocks, segments, and events that control settings and operation of instruments.

Method configuration—Configuration information stored with the method. You can think of this as the inactive configuration. Method configuration not used when you run a method unless you load the configuration before running.

MS-DOS®—Microsoft Disk Operating System.

Microsoft® Windows®—Graphical user interface (GUI) in which the BioCAD software runs.

Multi-method—Feature in the Method Editor that allows you to create your own templates by:

- Making sequential modifications to the current method and saving them in a multi-method file
- Linking a group of different methods together in a multi-method file

Path—Specifies location of a file in the directory. Example: the path for data files in the BioCAD software is C:\BIOCAD\DATA.

Peak width—Width of peak in minutes. In BioCAD, defined by the formula:

$$\text{Peak Width} = 1.6 \left(\frac{\text{Peak Area}}{\text{Peak Height}} \right)$$

PEEK—Acronym for polyetheretherketone. Chemically inert, flexible polymer that can withstand high pressures and temperatures.

Percentage mode—BioCAD blend programming mode. Conventional % (v/v) technique used on most conventional HPLC instruments. Allows you to select a percentage of solvent and blend up to four of the six configured solvents.

pH mode—BioCAD blend programming mode that uses two concentrated buffers at different pH values, water and a concentrated eluent such as salt. This mode is required for running pH maps.

When you use special PerSeptive pH buffers, the BioCAD software automatically calculates the required percentages needed to obtain the programmed pH buffer and eluent concentrations.

When you use any other buffers, you regulate the proportions of the two buffers in the total blend.

Prime—Automated action for the BioCAD autoloader. Primes line between sample vessels, through sample selection valve, to injection valve.

POROS[®] media—Patented media that contains two types of pores: *throughpores* (allow convective flow through the particle) and *diffusive* pores (provide high surface area).

Perfusion Chromatography[®]—Flow-through particle chromatography that uses POROS[®] columns and media. Patented technology that takes advantage of convective and diffusive flow. Overcomes the speed limitations of standard LC and HPLC.

Plate count—Measure of column efficiency.

Purge—Automated BioCAD feature that allows you to run the system at high flow rates for efficient solvent changeover. Purge:

- Switches the columns out of line
- Changes the blend to the new value
- Increases the flow rate
- Runs for a set duration

Quantitation—Calculation of amounts for unknowns by comparing to a calibration curve.

RAM—Random access memory.

Response—Quantitative value of a detector output, often chromatographically measured in peak height or area.

Response Factor—Measurement of detector response to a specific compound. Ideally follows a linear curve.

Segments—Actions within each method block. Segments include: Step, Gradient, Inject, Autosampler Inject, Purge, Dilute, Combine, and Heat/Mix.

Supervisor[™] software—A stand-alone version of the BioCAD software, designed to run on a computer without a BioCAD workstation. The Supervisor software option contains all software modules except the Control Panel, and lets you create and edit methods and configurations, and analyze data.

System configuration—

Information currently set in the Configuration dialog box (accessed from the Control Panel). System configuration is used when you run the BioCAD workstation. You can think of this as the active configuration.

Systematic methods development—

Using the BioCAD workstation and POROS columns and media, methods development in which key variables are identified, then systematically evaluated and optimized.

Template—A predefined program that is set up to perform a sequence of runs in which one or more variables change from run to run. BioCAD templates include:

- **pH Map**—Changes pH
- **Loading Study**—Changes injection volume
- **Linear Gradient**—Changes gradient slope
- **Flow Rate Study**—Changes flow rate
- **Auto Cycling**—Runs the method multiple times
- **Column Switching**—Changes columns
- **Analysis**—Changes the sample that is injected and the number of injections
- **Step Optimization**—Changes blending conditions for column equilibration and step elution
- **Combinatorial Optimization**—Changes up to six parameters

Window—Area on the desktop in which a software application (for example, the BioCAD software) runs.

G L O S S A R Y

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