ÄKTA*design*

ÄKTA*prime*

User Manual

18-1135-24

Amersham Biosciences

Important user information

Meaning: Consult the instruction manual to avoid personal injury or damage to the product or other equipment.

WARNING!
The Warning sign is used to call attention to the necessity to follow an instruction in detail to avoid personal injury. Be sure not to proceed until the instructions are clearly understood and all stated conditions are met.

CAUTION!
The Caution sign is used to call attention to instructions or conditions that shall be followed to avoid damage to the product or other equipment. Be sure not to proceed until the instructions are clearly understood and all stated conditions are met.

Note
The Note sign is used to indicate information important for trouble-free or optimal use of the product.

Should you have any comments on this instruction, we will be pleased to receive them at:

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Contents

About this manual

This manual describes the operation of the ÄKTA™*prime* system.

System description, system installation, maintenance and trouble-shooting are also found in this manual. It also contains information on how to operate the system in different purification applications.

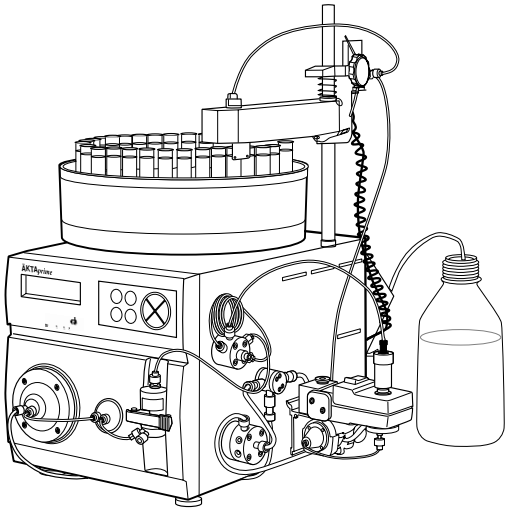
1 Introduction

1.1 General

ÄKTA™*prime* is a compact, automated liquid chromatography system. It is designed for standard separation applications.

ÄKTA*prime* features:

- Easy unpacking and installation.
- Application templates ready to use.
- Method templates as a basis for creating customized methods.
- Cue cards for simple and quick operation.
- Flow rates up to 50 ml/min and pressures up to 1 MPa.
- High accuracy and reproducibility.



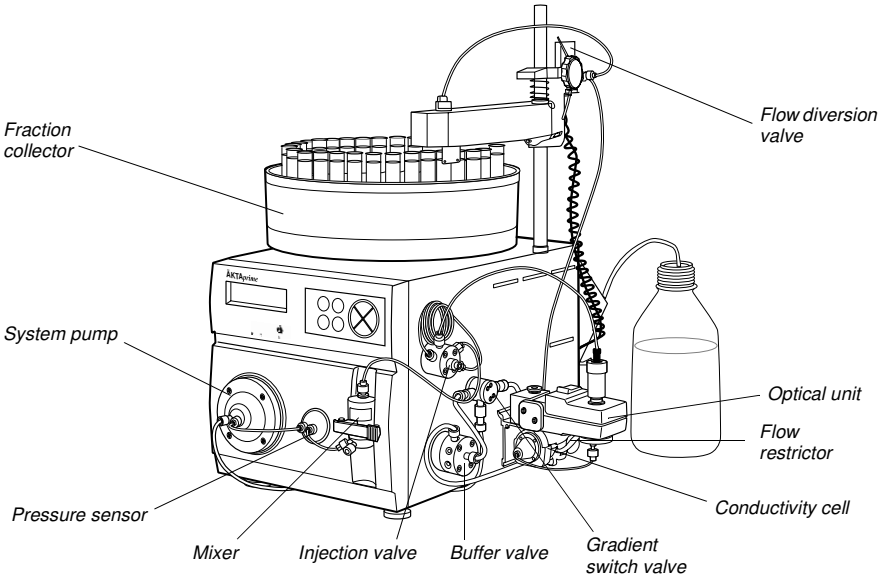
ÄKTA*prime* is a "one-box" system, including components for measuring UV and conductivity, generating gradients and collecting fractions. The user interface at the front panel consists of an LCD display and membrane push buttons. A probe for pH measurement is available as an accessory.

The package also includes a recorder, REC 112, which is used for printing the curves obtained during a run.

ÄKTA*prime* is described in detail in section *A* of *Reference information*. Optional components are also described there.

1.2 Description

The location of each of the system components is shown in the following illustration:



ÄKTA*prime* comprises the following components:

Buffer valve and gradient switch valve

The buffer valve is used for selecting the buffer solution to use, and for application of large sample volumes with the system pump. The gradient switch valve is used to create gradients.

System pump

The system pump is used for pumping fluids, such as sample or buffer solutions, through the system. The fluid is introduced into the flow path either through the buffer valve, the gradient switch valve, or through the injection valve.

Pressure sensor

The pressure sensor allows measurement of the liquid pressure on-line. It is also used as a pressure level guard.

Mixer

The mixer is used for mixing binary gradients. It mixes the solutions in two steps for optimum result. As an option, the mixer chambers are interchangeable with chambers of different volumes.

Injection valve

The injection valve is used for loading the sample loop and for injecting the sample onto the column.

Monitor

The purpose of the monitor is to measure UV absorption, conductivity and pH (optional) of the liquid exiting the column. The flow cells used for these measurements are attached to the system on the right-hand side.

Fraction collector with flow diversion valve

The fraction collector is used for collecting sample fractions in tubes for further analysis. The flow diversion valve switches the flow between waste and the collection tubes.

1.3 Safety

- The system is designed for indoor use only.
- Do not use in dusty atmosphere or close to spraying water.
- Operate in accordance with local safety instructions.
- Do not block the air inlet or outlet of the system.

WARNING! The system must be connected to a grounded mains socket.

WARNING! The system must not be opened by the user. It contains high voltage circuits that can give a lethal electric shock.

WARNING! Always disconnect the power supply before attempting to replace any item during maintenance.

WARNING! When the lamp power is on, the lamp socket carries a dangerous voltage. Do not connect/disconnect with the system switched on.

WARNING! The system uses high intensity ultra-violet light. Do not remove the UV lamp while the system is running.
Before replacing a UV lamp, ensure that the lamp cable is disconnected from the rear of the system to prevent injury to eyes. If the mercury lamp is broken, make sure that all mercury is removed and disposed according to national and local environmental regulations.

WARNING! When using hazardous chemicals, all suitable protective measures, such as protective glasses, must be taken.

WARNING! There must always be a sample loop connected to ports 2 and 6 of the injection valve. This is to prevent liquid spraying out of the ports when switching the valve. This is especially dangerous if hazardous chemicals are used.

WARNING! If there is a risk that large volumes of spilt liquid have penetrated the casing of the system and come into contact with the electrical components, immediately switch off the system and contact an authorised service technician.

WARNING! NaOH is injurious to health. Avoid spillage.

WARNING! When using hazardous chemicals, ensure that the entire system has been flushed thoroughly with distilled water before service and maintenance.

WARNING! Only spare parts that are approved or supplied by Amersham Biosciences may be used for maintaining or servicing the system.

WARNING! Use ONLY tubings supplied by Amersham Biosciences to ensure that the pressure specifications of the tubings are fulfilled.

WARNING! If the system is turned, the external capillaries and other tubing may become entangled in nearby objects and be pulled from their connections causing leakage.

2 Unpacking and installation

2.1 General

This chapter describes unpacking and installation of the ÄKTA*prime* system. After the installation procedure has been performed, ÄKTA*prime* is ready for purification work.

ÄKTA*prime* is assembled and fully tested before shipping.

For safe transportation, however, some components have been secured and need to be released from strappings.

Accessories like fittings, tubing, column holders, etc., are enclosed in separate packages.

The system may be operated at normal ambient temperatures in the range +4 to +40 °C.

Installation procedure overview

2.2 Pre-requisites

WARNING! ÄKTA*prime* must be connected to a grounded mains socket.

- To install ÄKTA*prime,* a working area of about 120 x 80 cm (width x depth) is required.
- The system should be located in a place of low temperature variations, away from heat sources, draughts and direct sunlight.
- The system should be installed on a stable laboratory bench. To ensure correct ventilation, a free space of 0.1 m is required behind and in front of the system. Do not place soft material under the system. It may block the ventilation inlet.
- ÄKTA*prime* requires 100–120/220-240 V~, 50-60 Hz electrical supply with safety grounding.
- Flasks for buffers and waste are needed.

2.3 Unpacking ÄKTA*prime*

CAUTION! Read the following information carefully to ensure that the system is installed correctly.

Begin by creating a dry and clean working area of 120 x 80 cm that allows easy access. Then follow the step-by-step instructions below.

Note: *Save all the original packing material. If the system has to be repacked, for transportation or otherwise, it is important that the system can be safely packed using the original packing material.*

1 After removing the cardboard hood, the red strap that secures the system to the pallet, and other packing material, check the contents against the enclosed packing list. Also check enclosed packages, including the recorder. Store all boxes and plastic bags in a convenient nearby place.

CAUTION! Take care not to damage any capillaries or components while lifting the instrument or when opening the plastic cover.

CAUTION! Do not lift the system by the pillar.

2 Grip the instrument between the cushions and gently lift it onto the work area. Take care not to damage any capillaries or components while doing this.

3 Open the plastic cover from the top and fold down to uncover the system.

4 Remove the plastic cover by gently tilting the system back and forth while pulling out the plastic cover.

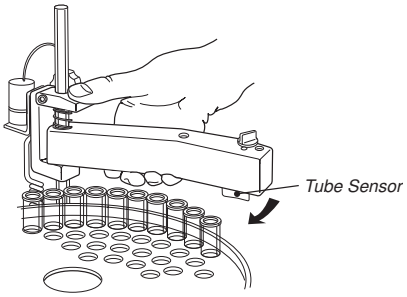
5 Remove the protection pad placed under the fraction collector bowl.

6 Put some collection tubes in the bowl, starting at the first position.

7 Raise the column holder to the top position.

8 Loosen the knob holding the delivery arm and raise the arm.

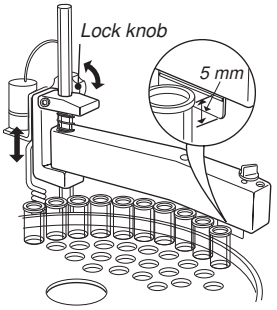
9 Adjust the delivery arm so that the tube sensor touches the collection tubes of the outer track.

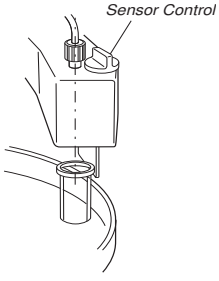


10 Adjust the arm bracket so that the bottom of the tube sensor is about 5 mm below the top of the tubes. The tubes should always be below the horizontal mark on the tube sensor

11 Lock the arm bracket at this height with the lock knob.

12 Rotate the rack counter-clockwise by hand, until the rear half of the tube sensor rests against tube 1. When the fraction collector is started, the bowl moves to the correct position to collect the first fraction in tube 1.





13 Check that the sensor is in the correct position for the tube size. The eluent tubing should be over the centre of the collection tube. Use the red sensor control to position the tube holder.

14 Remove the inlet tubings and the brown waste capillaries from the plastic bag. Route the inlet tubings to buffer vessels and the waste capillaries to a waste flask.

2.4 Connecting the mains cable

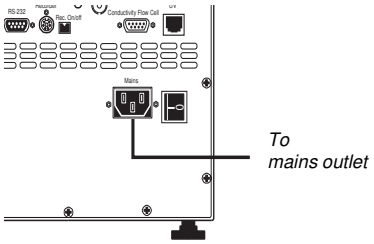
1 Turn ÄKTA*prime* to access the rear.

2 Remove all tape holding the cables.

3 Connect the system mains cable from the mains inlet to a properly grounded mains socket.

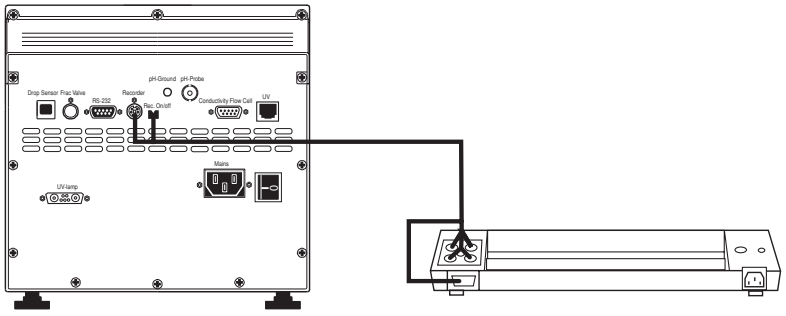
4 Ensure that the other cables are connected properly to the rear panel.

5 Turn ÄKTA*prime* so that the front is facing forward.



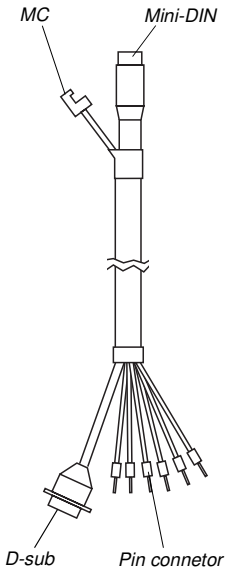
2.5 Connecting the recorder

The recorder supplied, REC 112, is connected to the system to print data obtained during a run. The recorder has two pens whose positions represent the levels of the two corresponding inputs. Start and stop of the recorder can be remotely controlled from the ÄKTA*prime* system.



CAUTION! The recorder must be connected to a grounded mains socket.

CAUTION! Ensure that the mains voltage selector is set to the mains voltage of the laboratory.

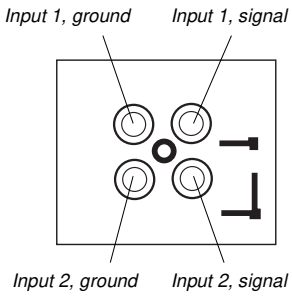


1 Check that the mains voltage selector is set to the mains voltage of the laboratory.

2 Connect the supplied signal cable to the ÄKTA*prime* system as follows:

- The mini-DIN connector to socket **Recorder.**
- The MC connector to socket **Rec On/off.**
  The left-hand pin in the socket is the ON/OFF-signal and the right-hand pin is signal ground.

3 Connect the pin connectors at the other end of the cable to the signal input plugs supplied with the recorder.



Note: *The signal cable is delivered with protective covers on each wire. Do not remove the protective covers from unused connections as this may disturb the measurement.*

4 Connect the plugs to the signal inputs on the recorder.
Pin designations for the signals (and colours on the corresponding cable wire) are as follows:

Pin no.	***Signal***[1]	***Range***
1 (Brown)	Channel 1	0–1 V
2 (Red)	Signal ground	0 V
3 (Orange)	Channel 2	0–1 V
4 (Yellow)	Signal ground	0 V
5 (Green)	Channel 3	0–1 V
6 (Black, thin)	Signal ground	0 V

[1] The channels have the following default parameter settings:
Channel 1 – UV absorbance
Channel 2 – %B
Channel 3 – Conductivity
These settings can be changed in the **Set Parameters** menu.

5 Connect the D-sub connector to the D-sub socket in the recorder.
Pin 12 is the ON/OFF-signal and pin 15 is signal ground.

6 Connect the mains cable to a properly grounded mains socket.

7 Switch on the mains power to the recorder.

2.6 System self-test

Start the system and run the system self-test as follows:

Selftest **Please wait...**

1 Switch on the system at the mains switch on the rear panel. The system now performs a self-test.

ÄKTAprime **V1.00**

2 First the system name and software version number is shown for a few seconds. Several messages are then shown during the self-test. If an error is detected, an error message is shown on the display.

Templates

3 The self-test takes about 30–40 seconds. When the start-up is completed with no errors, the display shows the **Templates** menu and is ready for use.

The system can be used immediately but the full specifications are not obtained until after 1 hour of lamp warm-up.

3 Operation

3.1 Getting started

This section is written for users who are not familiar with the ÄKTA*prime* system. Here you will learn the basics about the system.

This chapter also contains the following sections:

- Operating the user interface.
- Main menu overview.
- Preparing the system for a run.
- Running the system using application templates or method templates.
- Running the system manually.
- Printing the result.

Note: *To follow the instructions, it is not necessary to read the comments (written with smaller fonts) containing additional information.*

Pre-requisites

The system must be installed and functioning as described in chapter *2 Unpacking and installation*.

IMPORTANT! Before using ÄKTAprime, read all the safety information in section *1.3 Safety*.

Typographical conventions

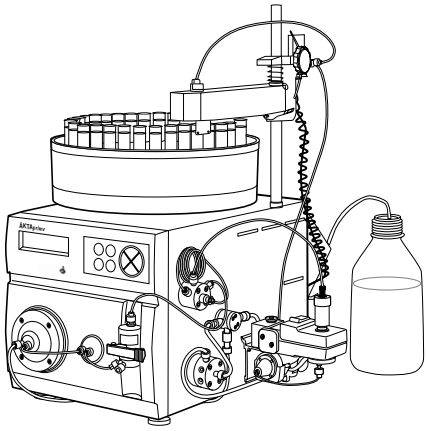
Keyboard options and menu selections are identified in the text by **bold** text.

The system and the software

ÄKTA*prime* is an automated liquid chromatography system for standard separation applications.

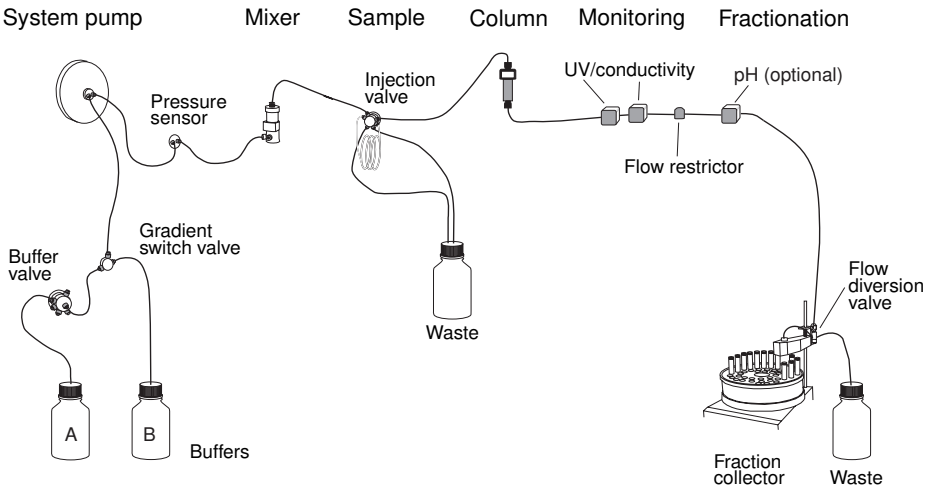
ÄKTA*prime* is a "one-box" system, containing a monitor for measuring UV, conductivity and pH, a sample pump and a fraction collector. Other components, such as mixer, injection valve, buffer valve and switch valves, are also integrated in the system.

All software is embedded in the system. The user interface consists of a keyboard with membrane keys and an alphanumeric LCD display.



Comment:

The flow path between the different components in the system is shown and described below.



1 Tubings are placed in the buffer bottles. The buffer valve and the gradient switch valve select the buffer to be used.

2 The system pump pumps the solution to the mixer via a pressure sensor.

3 The flow path continues from the mixer to the injection valve.

4 A sample loop is connected to the injection valve. The sample loop is filled manually by connecting a fill port to the injection valve and then using a syringe.

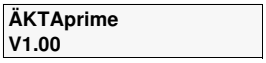
5 After the injection valve, the flow is directed to the column, and then to the UV flow cell in the optical unit, the conductivity flow cell and the flow restrictor located below the optical unit.

Note: *In standard configuration, the pH flow cell is not included. When the pH flow cell is mounted in the flow path, it is connected after the flow restrictor.*

6 The flow continues to the flow diversion valve mounted on the fraction collector. This valve is used for switching the outlet flow between waste and fraction collection.

3.2 On/off

1 Switch on the system at the mains switch on the rear panel. The system immediately performs a self-test.



2 During the self-test, several messages are shown on the display, e.g. system name and software version number. If an error is detected during the self-test, an error message is shown.

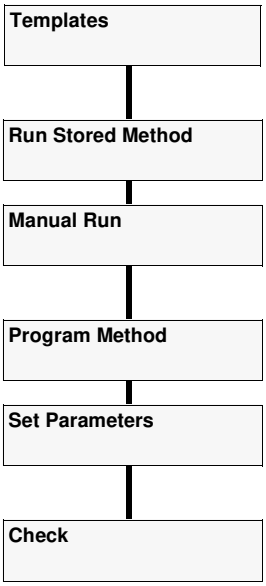
3 All parameters are set to factory default values.

4 The self-test takes about 30–40 seconds. When the start-up is completed, the display shows the **Templates** menu.

The system can be used immediately but the full specifications are not obtained until after 1 hour of lamp warm-up.

3.3 Main menu overview

The main menu level can be accessed from any sub menu by pressing **Esc** repeatedly.



Templates. This menu appears at start-up after the self-test. Used for running pre-made application templates and method templates. This is described in section *3.7 Starting a run*.

Run Stored Method. Used for running methods that are programmed by the user. Refer to section *3.11 Running a stored method*.

Manual Run. Used for running the system manually without using methods. This is described in detail in section *3.12 Running the system manually*.

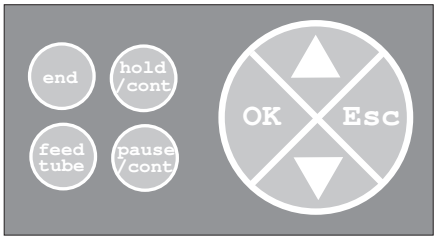
Program Method. Used for programming user-specific methods. This is described in chapter *4 Method programming*.

Set Parameters. Used to set parameters for measuring UV, conductivity, pH, temperature, and for running the pump and the mixer. This is described in *Reference information* section *C.2 Set Parameters menus*.

Check. Used for checking internal system parameters, such as serial number, pump run time and lamp intensity. This is described in *Reference information* section *C.1 Check menus*.

3.4 Operating the user interface

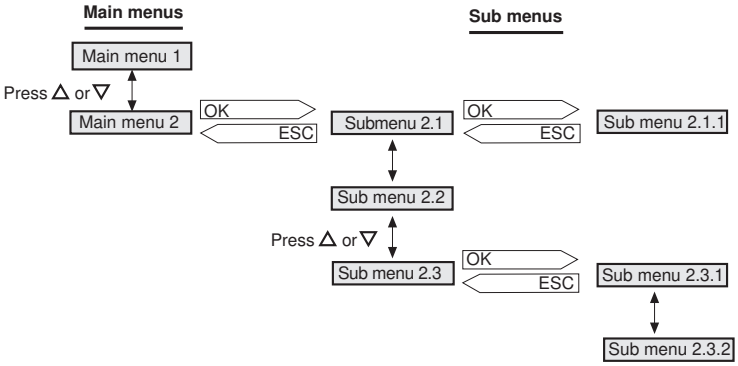
Menu navigation



△ ▽ Press △ or ▽ to select a specific menu.

OK Press **OK** to enter a sub menu.

Esc Press **Esc** once to move back one menu level, or repeatedly to return to the main menu level.



Control keys

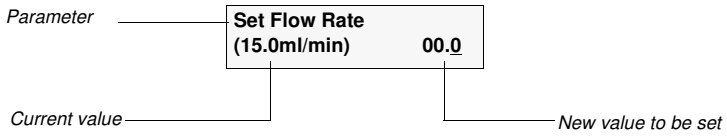
feed tube Press **feed tube** to advance the fraction collector one position. The tube feed is delayed according to the set value in the **Set Delay UV to Frac** menu.

end Press **end** to interrupt method operation before the method is completed.
Press **end** to stop manual operation.

hold /cont Press **hold /cont** to hold method time or volume and to hold the gradient at the current concentration. Pump and fraction collector continue uninterrupted.
Press **hold /cont** again to resume the normal method operation.

pause /cont Press **pause /cont** to pause all operation without ending the method. All functions, including pump and fraction collector, are stopped.
Press **pause /cont** again to restart the method operation.

Changing a parameter value



To change a parameter value:

1 Press **OK** to enter the set value mode.

2 Press △ or ▽ to change the set value.
A cursor below a text or numerical value shows what is affected when pressing the keys.

3 Press **OK** to verify the set value and exit the set value mode.
To cancel, press **Esc**.

3.5 Preparing the system for a run

Make sure the power to the system is switched on and that no error messages have been registered during the system self-test.

General system preparation

1 The correct tubing for the column you intend to use must be installed. See section 3.6 for an overview of columns with recommended tubing.

2 Immerse buffer inlet tubings A1 and B in liquids as follows:

- If the tubings are filled with ethanol: In deionized water.
- Otherwise: A1 in buffer A and B in buffer B.

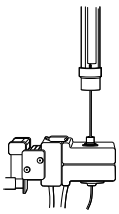
Note: *Only use degassed liquids to make sure that the liquid remains free from air bubbles.*

Note: *When using degassed ethanol, make sure that the concentration does not fall below the required value.*

3 Put the brown waste tubing from port NO on the flow diversion valve mounted on the fraction collector into a waste bottle. Check that the tubing from the flow restrictor outlet is connected to the flow diversion valve port marked IN.

4 Put the brown waste tubings from the ports 4 and 5 on the injection valve into a waste bottle.

5 Calibrate the pH monitor (optional) if required. Refer to section *B.3 Calibrating the pH electrode (optional)*.



6 Connect the column between port 1 of the injection valve and the UV flow cell. Use a suitable length of PEEK tubing in combination with unions and connectors supplied with the system.

7 Insert a sufficient number of tubes into the fraction collector and place the arm at the first tube.

8 Connect the sample loop between port 2 and port 6 on the injection valve. If a Superloop™ is needed, additional information is supplied in the instruction for the Superloop. If using the system pump for applying the sample, see section *3.6 Fluid handling components*.

9 Fill the inlet tubings with liquids by running the system pump manually. Pump the liquids at 30 ml/min. The injection valve must be in position WASTE. To fill inlet tubing B, enter 100% in the **Set Concentration %B** menu.

Note: *Do not use ethanol immediately before or after using a buffer with high salt concentration. Always flush an intermediate liquid, such as deionised water, in between.*

10 To remove any trapped air bubbles in the filled flowpath, purge the pump with liquids in the following order: 1. deionized water, 2. 20% ethanol, 3. deionized water and 4. buffer solution.

Manual Run

Purging can be done manually through inlet A1, while carefully immersing the tubing in the respective liquid. Set the injection valve to position WASTE. Run 30 ml of each liquid at 50 ml/min. Press the **pause/cont** button to start and stop the pump when changing liquid.

An automatic purging procedure that uses additional inlet tubings and the **System Wash Method** can also be used. This is described in section *8.12 Removing trapped air bubbles*.

11 Connect a union luer female/1/16" male or an injection fill port to port 3 on the injection valve. Apply the sample with a syringe.

Note: *More information regarding buffer solutions, columns, etc. for the respective application template is found on the cue cards supplied.*

Preparing the recorder

1 Set the **zero** keys to the **down** position.

2 Select a suitable low chart speed (0.5–2 mm/min) with the **chart speed** selector and the **mm/s–mm/min** key.

3 Set both **pens** to position **down**. Use the **adjust** knob to make a coarse zero adjustment to the right-hand zero on the chart.

4 Set **rec.** to position **on** and make a final zero adjustment.

5 Set **rec.** to position **off.** Use the forward **feed** key to align the short-nib pen with a grid line and press the **set** key (i.e. set start position).

6 Set the **zero** keys to the **up** position.

7 Select a suitable chart speed (2–20 mm/min) according to the length of the purification.

8 Select 1 V with the **range V/mV** selector.

9 The recorder is made ready to use by setting the **rec.** key to **on**. The chart paper starts rolling when the run starts.

More information about the recorder is provided in the *Recorder REC 112 User Manual* supplied.

For information on how to associate a measurement parameter to an output channel, and how to set the measurement range of a parameter, see section *B.4 Setting analogue outputs*.

Calibrations

The table below lists the type and frequency of calibrations that can be done on ÄKTA*prime*. Refer to section *B Calibration and analogue output settings* in *Reference information* for descriptions of how to perform these calibrations.

Component	***How often***
System pump	Whenever running conditions are changed, e.g. viscosity of sample or buffer, temperature, back-pressure, etc.
Conductivity flow cell	
Cell constant	Only necessary if specific conductivity with high accuracy is measured.
Temperature	Must be done when changing the flow cell.
Entering a new cell constant	Must be done when changing the flow cell.
Pressure offset	When required.
pH electrode (optional)	Every day

3.6 Fluid handling components

Columns and tubing

A wide range of pre-packed columns for techniques such as ion exchange, gel filtration, hydrophobic interaction and affinity chromatography are suitable for use with ÄKTA*prime*. A comprehensive list of the recommended pre-packed columns is given below.

On delivery, the system is equipped with 0.75 mm i.d. tubing from the pump to the outlet, and 1.0 mm i.d. waste tubings.

When running columns with a low maximum pressure and high flow rates, PEEK tubing with a larger inner diameter may be used instead to prevent increased back-pressure, which could cause the column to rupture.

Note: *If tubings are changed, the frac delay volume must be measured and changed.*

Recommended columns

The tables below list the recommended columns.

Ion Exchange Columns

Code no.	*Column name*
17-1153-01	HiTrap™ Q, 1 ml
17-1154-01	HiTrap Q, 5 ml
17-1151-01	HiTrap SP, 1 ml
17-1152-01	HiTrap SP, 5 ml
17-5092-01	HiPrep™ 16/10 Q XL
17-5093-01	HiPrep 16/10 SP XL
17-5091-01	HiPrep 16/10 CM
17-5090-01	HiPrep 16/10 DEAE
17-1064-01	HiLoad™ 16/10 Q
17-1066-01	HiLoad 26/10 Q
17-1137-01	HiLoad 16/10 SP
17-1138-01	HiLoad 26/10 SP
17-1177-01	RESOURCE™ Q, 1 ml
17-1179-01	RESOURCE Q, 6 ml
17-1178-01	RESOURCE S, 1 ml
17-1180-11	RESOURCE S, 6 ml

Size Exclusion (Gel filtration) Columns

Code no.	*Column name*
17-1165-01	HiPrep 16/60 Sephacryl S100 HR
17-1194-01	HiPrep 26/60 Sephacryl S100 HR
17-1166-01	HiPrep 16/60 Sephacryl S200 HR
17-1195-01	HiPrep 26/60 Sephacryl S200 HR
17-1167-01	HiPrep 16/60 Sephacryl S300 HR
17-1196-01	HiPrep 26/60 Sephacryl S300 HR
17-1139-01	HiLoad 16/60 Superdex 30 prep grade
17-1140-01	HiLoad 26/60 Superdex 30 prep grade
17-1068-01	HiLoad 16/60 Superdex 75 prep grade
17-1070-01	HiLoad 26/60 Superdex 75 prep grade
17-1069-01	HiLoad 16/60 Superdex 200 prep grade
17-1071-01	HiLoad 26/60 Superdex 200 prep grade

Hydrophobic Interaction Columns

Code no.	*Column name*
17-5095-01	HiPrep 16/60 Phenyl (high sub)
17-5094-01	HiPrep 16/60 Phenyl (low sub)
17-5097-01	HiPrep 16/60 Octyl
17-5096-01	HiPrep 16/60 Butyl
17-1085-01	HiLoad 16/60 Phenyl
17-1086-01	HiLoad 26/60 Phenyl
17-1084-01	RESOURCE ETH 1 ml
17-1085-01	RESOURCE ISO 1 ml
17-1086-01	RESOURCE PHE 1 ml

Chelating Columns

Code no.	*Column name*
17-0408-01	HiTrap Chelating 1 ml
17-0409-01	HiTrap Chelating 5 ml

Affinity Columns

Code no.	*Column name*
17-0402-01	HiTrap Protein A 1 ml (5 pcs)
17-0402-03	HiTrap Protein A 1 ml (2 pcs)
17-0403-01	HiTrap Protein A 5 ml
17-0404-01	HiTrap Protein G 1 ml (5 pcs)
17-0404-03	HiTrap Protein G 1 ml (2 pcs)

Code no.	Column name
17-0405-01	HiTrap Protein G 5 ml
17-0406-01	HiTrap Heparin, 1 ml
17-0407-01	HiTrap Heparin, 5 ml
17-5079-01	HiTrap rProtein A, 1 ml (5 pcs)
17-5079-02	HiTrap rProtein A, 1 ml (2 pcs)
17-5080-01	HiTrap rProtein A, 5 ml
17-0412-01	HiTrap Blue, 1 ml
17-0413-01	HiTrap Blue, 5 ml
17-0716-01	HiTrap NHS-activated, 1 ml
17-0717-01	HiTrap NHS-activated, 5 ml
17-5105-01	HiTrap Con A, 1 ml
17-5106-01	HiTrap Lentil Lectin, 1 ml
17-5108-01	HiTrap Peanut Lectin, 1 ml
17-5107-01	HiTrap Wheat Germ Lectin, 1 ml
17-5110-01	HiTrap IgM Purification, 1 ml
17-5111-01	HiTrap IgY Purification, 5 ml
17-5112-01	HiTrap Streptavidin, 1 ml
17-5130-01	GSTrap™, 1 ml (5 pcs)
17-5130-02	GSTrap, 1 ml (2 pcs)
17-5131-01	GSTrap, 5 ml

Buffer Exchange/Desalting Columns

Code no.	Column name
17-1408-01	HiTrap Desalting
17-5087-01	HiPrep 26/10 Desalting

Sample application overview

With ÄKTA*prime*, the sample can be applied in three different ways to suit the application.

The sample can be applied using:

- Sample loops, filled manually with a syringe.
- Superloop, filled manually with a syringe.
- The system pump for large sample volumes.

The following table shows which technique is recommended for different sample volumes.

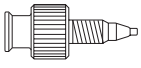
Sample application technique	***Volume to inject***
Sample loop, manual filling	25 µl - 5 ml
Superloop, manual filling	1 ml - 150 ml
System pump	>10 ml

The different sample techniques are described in the following sections.

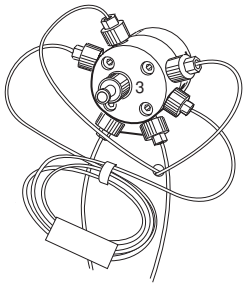
Manual filling of sample loops

Preparation

Prepare the injection valve as follows:

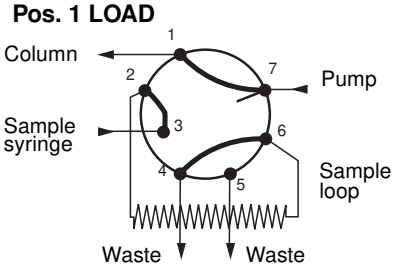


1 Connect the supplied luer female/1/16" male union connector to valve port 3.



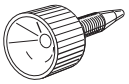
2 Ensure that a waste tubing is connected to port 4 of the injection valve.

3 Mount the sample loop between ports 2 and 6 of the injection valve.

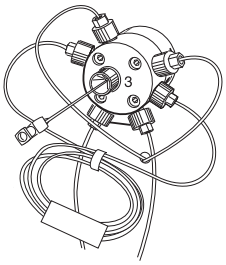


Note: *If the syringe is taken out before the sample is injected onto the column, self-drainage will occur and the loop will be emptied.*

An injection fill port is an alternative to the luer union connector. If used, prepare the injection valve as follows:



1 Loosely thread the injection fill port screw into valve port 3.



2 Insert an injection needle (0.7 mm o.d.) into the injection fill port.

3 Tighten the fill port until the nozzle has formed a seal around the needle's tip, i.e. when it feels as if you are penetrating a septum at the end of the injection fill port. The seal should allow easy insertion and removal of the needle.

4 Mount the syringe holder in the fill port.

5 Check the waste tubing and mount the sample loop as described for using a luer union connector.

Five sizes of sample loops are available:

Loop 100 µl, 25 MPa	18-1113-98
Loop 500 µl, 10 MPa	18-1113-99
Loop 1 ml, 10 MPa	18-1114-01
Loop 2 ml, 10 MPa	18-1114-02
Loop 5 ml	18-1140-53

Two techniques can be used for filling the sample loop; partial or complete filling.

Type of filling	*Volume to load*
Partial filling	max. 50% of the sample loop volume
Complete filling	2–5 times the sample loop volume

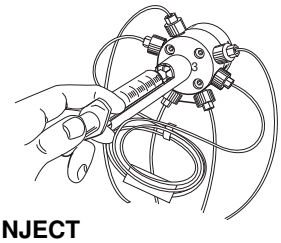
Partial filling

Partial filling is used when high recovery is required. The sample volume loaded should be, at maximum, 50% of the loop volume. The volumetric accuracy is that of the syringe. Partial filling allows the injected volume to be changed without changing the loop and does not waste sample. The sample loop must be completely filled with buffer before the sample can be loaded.

Partial filling is achieved as follows:

Note: *The flow must be off. For example, when running the system manually, press Pause.*

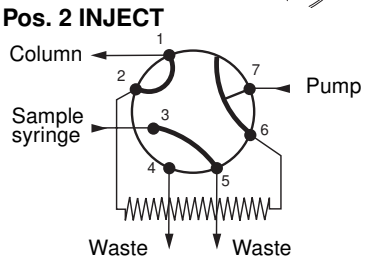
1 Set the injection valve to position LOAD.



2 Load the syringe with a large volume of buffer (5 times the loop volume).

3 Fill the sample loop carefully with buffer.

4 Set the injection valve to position INJECT.



Note: *If the syringe is taken out when the injection valve is in position LOAD, self-drainage will occur and air will enter the sample loop.*

5 Load the syringe with the required volume of sample.

Note: *No more than half (50%) a loop volume of sample should be loaded into the loop.*

6 Insert the syringe into the injection fill port on the injection valve. Set the injection valve to position LOAD.

Note: *Do not load the sample before the valve is in position LOAD.*

7 Gently load the syringe contents into the sample loop.

8 Leave the syringe in position. The sample will be injected onto the column when the valve is switched to INJECT in the method.

Note: *If the syringe is taken out before the sample is injected onto the column, self-drainage will occur and the loop will be emptied.*

Complete filling

In this method, an excess of sample is used to ensure that the sample loop is filled completely, which gives reproducible sample volumes. In preparative applications, the sample volume should be at least 2 times the volume of the sample loop. For analytical reproducibility, a sample volume 5 times the volume of the sample loop should be used. About 2 to 3 loop volumes of sample are required to achieve 95% of maximum loop volume. Five loop volumes ensures better precision.

With complete loop filling, the sample volume can only be changed by changing the loop size.

Complete filling is achieved as follows:

1 Set the injection valve to position LOAD.

2 Load the syringe with sample (2–5 times the loop volume).

3 Gently load the syringe contents into the loop.

4 Leave the syringe in position. The sample will be injected onto the column when the valve is switched to INJECT in the method.

Note: If the syringe is taken out before the sample is injected onto the column, self-drainage will occur and the loop will be emptied.

Emptying the sample loop

When emptying the sample loop, a buffer volume of at least 5 times the sample loop volume should be used to flush the loop and ensure that all sample is injected onto the column.

Applying samples with Superloop

Superloop permits the introduction of larger volumes of sample (1-150 ml) onto the column.

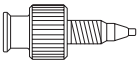
Superloop is an accessory available in three sizes:

Volume	*Max. allowed column pressure*	*Code no.*
10 ml	4 MPa	18-1113-81
50 ml	4 MPa	18-1113-82
150 ml	2 MPa	18-1023-85

All the sample is applied, which gives good reproducibility and high recovery. The sample is not diluted as the buffer pushing the movable seal is kept separate. The loaded sample can be injected all at once or in several smaller volumes, down to 1 ml portions, permitting automated repetition of sample injection. The Superloop is filled manually with a syringe.

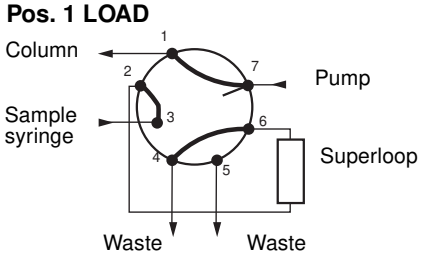
Preparation

Prepare the injection valve and connect Superloop as follows:



1 Connect the supplied luer female/1/16" male union connector to port 3 of the injection valve.

2 Ensure that tubing for the waste is connected to port 4 of the injection valve.



3 Ensure that Superloop is filled with liquid (see separate Superloop instruction).

4 Mount Superloop in a column holder as close to the injection valve as possible.

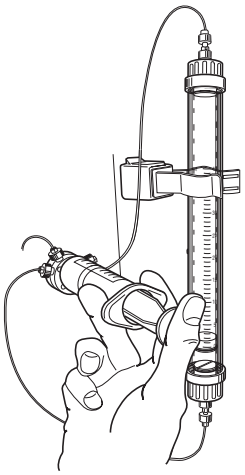
5 Connect the bottom tubing to injection valve port 2.

6 Connect the top tubing to injection valve port 6.

7 Ensure all connections are fingertight.

Filling Superloop

Fill the Superloop as follows:



1 Set the injection valve to position INJECT.

2 Start the system pump and let it run until the movable seal has reached the bottom of Superloop.

3 Stop the system pump and set the injection valve to position LOAD.

4 Load a large volume syringe with sample.

5 Gently load the syringe contents into the Superloop through port 3.

6 Leave the syringe in position. The loaded sample can be injected all at once or in several smaller volumes, down to 1 ml portions. The volume to inject is set in templates or in programmed methods in menu **Set Sample Inj. Vol.**

Set Sample Inj. Vol.	
(0.0 ml)	**0.0**

7 The sample is applied to the column when the injection valve is set to position INJECT. When the required volume has been injected, set the valve to LOAD. When using method templates, this is performed automatically.

Applying large samples with the system pump

Larger sample volumes can be applied through the system pump.

Note: *In isocratic techniques (e.g. size exclusion chromatography), band broadening will be large when applying sample with the system pump.*

Note: *If the system pump is used for sample application, the sample should be pre-filtered (minimum 0.45 µm) to remove contamination. Otherwise, the inlet filter may be clogged very quickly.*

Preparation

1 The sample must be particle-free and filtered through a 0.45 µm filter.

2 Connect the inlet tubing to port 8 on the buffer valve.

3 Place the other end of the inlet tubing in the bottle with the sample.

Note: *Make sure that the sample inlet tubing is filled with sample, and that no air bubbles are trapped in the pump. Refer to section 3.5 Preparing the system for a run.*

Applying the sample

1 Select a template in sub menu **Method Template** in menu **Templates**, or select a stored method where the buffer valve is used for sample application.

   In a stored method, the buffer valve should be set to position 8 and the injection valve to position LOAD when applying the sample.

2 In method templates, select sample application with system pump.

3 Set the required parameters and the sample volume.

4 Start the run.

Note: *When using the system pump for sample application, an extra 15 ml of buffer is used for washing after the sample application.*

Cleaning the pump

WARNING! NaOH is injurious to health. Avoid spillage.

When the pump has been used for sample application, cleaning the pump might be required. If so, pump a cleaning or sanitizing agent through the pump by running **System Wash Method**. The standard recommendation is to pump 1 M NaOH for 30 minutes and then wash out immediately with buffer.

Mixing gradients

Gradients

Gradients are mixed using two separate buffers; one connected to the buffer valve and the other connected to the buffer switch valve. The flow is routed through the system pump to the mixer.

Mixer

The mixer is supplied with a 2 ml mixer chamber. For optimal gradients at very high flow rates, a larger mixer chamber is required.

WARNING! When using hazardous chemicals, ensure that the mixer chamber has been flushed with distilled water before removing the chamber.

Other mixer chambers with 0.6, 5 and 12 ml mixer volumes are available as accessories.

When using eluents that are more difficult to mix, such as isopropanol and water, a larger mixer volume will give better mixing.

Note: *If the pH (optional) and conductivity readings indicate uneven mixing of your buffers (unstable readings), change to a larger mixing chamber.*

Collecting fractions

Fractions are collected in tubes in the fraction collector. It is possible to fractionate in two different ways:

- Eluate fractionation
- Peak fractionation

Eluate fractionation

Eluate fractionation means that fixed fractions (fixed volume, time or number of drops) are collected during elution within a set interval of time or volume. The fraction properties are preset in the application templates and the method templates. Otherwise, they are set in the menus **Set Fraction Base** and **Set Fraction Size** when setting the process parameters. Press **OK** to enter the respective menu. Set the fraction base and the fraction size, then press **OK**. 0 means no fractionation.

Set Fraction Base
(ml) min ml drp

Set Fraction Size
(1.00 ml)

Peak fractionation

Peak fractionation allows you to collect fixed fractions (fixed volume, time or number of drops) during elution within a set interval of time or volume. In addition, it also allows you to collect peaks during the eluation. In this case, the slope of the curve dictates when the actual fractionation should start and end.

Set Peak Collect
(no)

Set Slope
(0.00 mAU/min) 0.00

The properties for controlling the start and end points are set in the menu **Set Peak Collect** when setting the process parameters.
no means no peak fractionation. Press **OK** to enter the menu. Set the slope in the **Set Slope** menu and press **OK**.

Setting the slope is described in section *C.2 Set Parameters menus* in *Reference information*.

3.7 Starting a run

Final checks

Before starting any method, we recommend that you make certain checks to ensure that problems are not encountered once the run has been started.

1 Check that the inlet tubings are immersed in the correct bottles for the method you are selecting.

2 Check that there is sufficient eluent available.

3 Check that the waste bottle is not full and will accept the volume diverted to it during the run.

4 Check that the pump has been purged (i.e. no air in the inlet tubing). If not, purge the pump by running **System Wash Method** in the **Application Template** menu under **Templates**. The application templates already include the tubing priming.

5 Check that the correct wavelength is set on the optical unit and that the correct UV flow cell is installed.

6 Calibrate the pH electrode if required (optional). Refer to section *B.3 Calibrating the pH electrode (optional)*.

7 Check that the fraction collector has sufficient tubes fitted.

8 Check that the correct column has been fitted and equilibrated (if not included in the method).

9 Check that the chart recorder is set correctly.

Selecting an application template

ÄKTA*prime* is run by either using a pre-made template or method, or by running the system manually.

The following four running options are available:

- Application templates
  Templates for running the most frequent purifications. These templates only require the sample volume as input; all other process parameters are preset.
  This section shows how to run an application template.

- Method templates
  Templates for common purification techniques; ion exchange, hydrophobic interaction, affinity and gel filtration. These templates require more input from the operator, such as flow rate and elution volume.
  Section *3.10 Running a method template* describes how to perform a run using a method template.

- Stored methods
  These methods are programmed, line-by-line, and stored by the operator. When creating a stored method, all process parameters must be programmed. A stored method can also be based on a method template.
  Section *3.11 Running a stored method* describes how to run a stored method.

- Manual run
  By running the system manually the operator chooses not to use a pre-programmed template or method. The process parameters are set before the run but they can not be stored for future use.
  Section *3.12 Running the system manually* describes how to run the system manually.

This section describes how to run an application template. Alternatively, follow the instructions on the cue card supplied to run an application template:

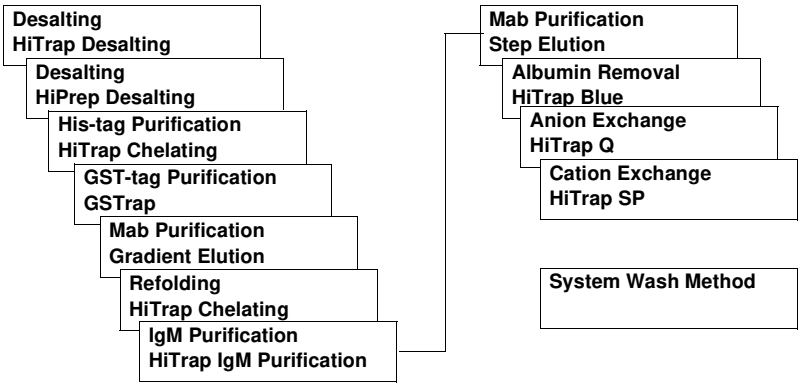
Templates

1 In the main menu, choose menu **Templates** and press **OK**.

Application template

2 Choose menu **Application Template** and press **OK**.

3 Choose an application template and press **OK**.



Sample appl. volume
0.0

4 In the **Sample appl. volume** menu, set the sample volume with the up and down buttons. Press **OK**.

Note: *The sample volume entered should include sample wash out volume if needed.*

Press OK to start run

5 To start the run, press **OK** at the **Press OK to start run** prompt.

For more information about application templates, see chapter *5 Template description.*

3.8 During a run

Viewing progress

Running display

The progress of the run and the status of important process parameters can be viewed directly on the front panel display. Four display alternatives with run data are available. Select the desired running display by pressing △ or ▽.

M RUN	**10.0 ml**
20.0 ml/min	**1.10 MPa**

Running display 1 shows method number or type (**M** = manual run, **AT** = application template, **MT** = method template), running mode indication, elapsed method volume or time, current flow rate and pressure.

The available running modes are:

Run	The system runs with the set flow rate.
End	The system is not running.
Pause	The pump is stopped but the set flow rate and the gradient values are retained.
Hold	The pump continues to run but the gradient is held at the current value.

0.00002AU	**pH 8.50**
20%B	**22.90mS/cm**

Running display 2 shows UV absorbance value, pH, concentration of buffer B and actual conductivity value in mS/cm or µS/cm.

Cond 78.8%Tc	**22.4°C**
Tube:01	**Frac 5.0 ml**

Running display 3 shows the conductivity as a percentage of the maximum conductivity setting, current temperature, tube number and fraction size.

Waste V: (waste)	
BV(1)	**IV:(waste)**

Running display 4 shows the position of the waste valve, the buffer valve and the injection valve.

Printing progress

The process parameters can be printed directly during the run. Three analogue output channels are available; two of them can be connected to the REC 112 recorder for printing the curves.

The default setting of the channels is as follows:

Channel	*Parameter*
1	UV absorbance
2	Theoretical gradient (B%)
3	Conductivity

Pressure, temperature and pH are also available.

The channel setting is changed in the **Set Parameters** menu, sub menu **Setup Analogue Out,** before setting the run.

It is also possible to connect a computer to the **RS-232** socket at the rear panel for exporting run data and a method print-out. Data is sent through pin 3 and received through pin 2. Ground is on pin 5.

Changing parameters

During a run, some of the process parameters can be changed. They can be changed at any time during the run, with the exception of the gradient. The gradient can only be changed if no gradient is running or if the system is paused (press the **Pause/Cont** button) or held (press the **Hold/Cont** button).

To change a parameter, follow the instruction below. The new setting takes effect immediately.

Changing the concentration of buffer B

1 Select menu **Set Concentration %B**. The current setting is displayed. Press **OK**.

2 Set the new concentration and press **OK**.

Changing the flow rate

1 Select menu **Set Flow Rate**. The current setting is displayed. Press **OK**.

2 Set the new flow rate and press **OK**.

Changing the fraction size

1 Select menu **Set Fraction Size**. The current setting is displayed. Press **OK**.

2 Set the new fraction size and press **OK**.

Setting the buffer valve position

1 Select menu **Set Buffer Valve Pos**. The current setting is displayed. Press **OK**.

2 Set the new position and press **OK**. Refer to the number printed on the buffer valve.

Setting the injection valve position

1 Select menu **Set Inject Valve Pos**. The current setting is displayed. Press **OK**.

2 Set the new position and press **OK**.

Waste – the flow is diverted to waste (ports 4 and 5).
Load – the sample loop is loaded (between ports 2 and 6) when injecting the sample through port 3.
Inject – the sample loop is emptied through port 1 and the flow directed to the column.

Autozero on the recorder

Autozero

Select menu **Autozero**. Press **OK** to set the recorder output signal to zero.

Setting an event mark

Event Mark

Select menu **Event Mark**. Press **OK** to set an event mark on the chart.

When running the system manually, the options for changing the parameters during a run are different. Refer to section *3.12 Running the system manually*.

Interrupting a run

There are three ways to interrupt a run:

End method (yes) yes no

- Pressing the **end** button interrupts the run with the prompt **End method?**. Entering **y** and then pressing **OK** terminates the run. Entering **n** and then pressing **OK** resumes the run.
- Pressing the **pause/cont.** button stops the pump but the set flow rate and the gradient values are retained. Press **pause/cont.** again to resume the run.
- Pressing the **hold/cont.** button holds the gradient at the current value and the pump continues to run. Press **hold/cont.** again to resume the gradient formation.

Completing a run

Method Complete Press OK to continue

1 When the run is finished, the display shows **Method Complete**. Press **OK**.

Memory Print Out (no) yes no

2 The post-run printing display is shown. Press **yes** for making a print-out (see section *3.9 Post-run printing*). Otherwise, select **no**.

The run is now completed.

3.9 Post-run printing

Selected run data is usually printed directly during the run. However, if a re-print of the result is desired, this can be done as a post-run print-out.

Make sure the recorder is properly connected to the rear panel.

The system has three analogue output channels for printing the run data curves after the run. You can choose to print out UV absorbance, pH, conductivity, theoretical gradient (%B), temperature or pressure.

Memory Print Out?
(yes) yes no

When the run is completed, or if it has been aborted, the prompt **Memory Print Out?** is displayed.

1 To print out the run data, select **yes**.
Otherwise, select **no**.

Set Rec Out 1 (UV)
UV pH Cond %B Tmp Pr

2 At the **Set Rec Out 1** menu, select the parameter to be printed on channel 1. Press **OK.**

Set Rec Out 2 (UV)
UV pH Cond %B Tmp Pr

3 At the **Set Rec Out 2** menu, select the parameter to be printed on channel 2. Press **OK.**

Set Rec Out 3 (UV)
UV pH Cond %B Tmp Pr

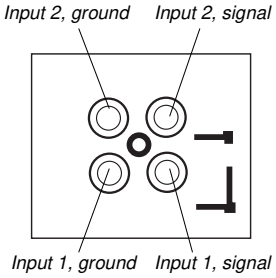
4 At the **Set Rec Out 3** menu, select the parameter to be printed on channel 3. Press **OK.**

Autoscaling
(no) yes no

5 If auto-scaling of the UV-curve is required, select **yes**.
Otherwise, select **no**.

6 Connect the desired cable wires to the recorder signal input.

Cable wire no.	*ÄKTAprime signal*
1 (Brown)	Channel 1
2 (Red)	Signal ground
3 (Orange)	Channel 2
4 (Yellow)	Signal ground
5 (Green)	Channel 3
6 (Black, thin)	Signal ground



7 Press the **set** key on the recorder to define the start position for the print-out.

8 Press **OK** to print the curves.

The system stores the run data from the latest run. Therefore, post-run print-out can be done either at the end of the run as described, or before the next run in the **Set Parameters** menu:

1 Set the **rec.** key on the recorder to **off**.

2 Press the **home** key to get the paper back to the start position.

3 Set the **rec.** key to **on** to enable the print-out.

4 Set the **pen** key to position **down**.

Set Parameters

5 Select main menu **Set Parameters** and press **OK**.

Memory Print Out

6 Select menu **Memory Print Out** and press **OK**.

Print out to
Recorder **Computer**

7 At the **Print out to** menu, select **Recorder** and press **OK**.

8 Select the desired parameters to be printed as in steps 2–5.

The print-out will now overlay the previous curves.

9 Repeat the procedure above to select other parameters and print out the curves.

3.10 Running a method template

The ÄKTA*prime* system contains four *method templates* based on the most common purification techniques. When using a method template, some parameters are set by the operator when preparing the run. Before starting the run, the operator has the option to save the settings in a method. This allows the operator to edit the method later and to reuse it.

Go through the procedure below to run a method template.

Selecting method template

1 Perform the general preparation of the system according to the description in chapter *3.5 Preparing the system for a run.*

Templates

2 Select main menu **Templates** and press **OK**.

Method Template

3 Select sub menu **Method Template** and press **OK.**

4 Select the desired template and press **OK.**

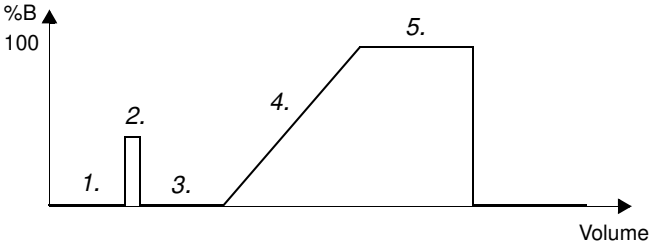
Gelfiltration/
Buffer Exchange

Ion Exchange/
Gradient elution

HIC
Gradient elution

Affinity
Step Gradient

Setting the parameters



The figure above shows the theoretical concentration of buffer B.
The numbers correspond to parameters to be set before starting the run.

Sample inject by
InjV Pump

1 Select sample injection through the injection valve or through the system pump. Refer to section *3.6 Fluid handling components* for more information about sample application.

Set Pressure Limit
(1.00 MPa) 1.00

2 Set the pressure limit and press **OK**. Set the limit to the maximum back-pressure for the column used + 0.2 MPa (back-pressure contribution from flow restrictor). Refer to the column documentation for maximum back-pressure.

Set Flow Rate	
(--.- ml/min)	0.00

3 Set the flow rate and press **OK**.

Set Fraction Size	
(0.0 ml)	0.0

4 Set the fraction size and press **OK**.

Set Equilibr. Volume	
(0.0 ml)	0.0

5 Set the equilibration volume (*1*. in the figure) and press **OK**.

Set Sample Inj. Volume	
(0.0 ml)	0.0

6 Set the sample volume (*2*.) to be injected and press **OK**.

Set Wash 1 Volume	
(0.0 ml)	0.0

7 Set the wash 1 volume (*3*.) and press **OK**.
This setting does NOT apply to the Gel filtration method template.

Set Elution. Volume	
(0.0 ml)	0.0

8 Set the elution volume (*4*.) and press **OK**.

Set Wash 2 Volume	
(0.0 ml)	0.0

9 Set the wash 2 volume (*5*.) and press **OK**.
This setting does NOT apply to the Gel filtration method template.

Method ready?		
(yes)	yes	no

10 Select **yes** at the **Method ready?** prompt and press **OK**.

Storing the method

Save Method		
(yes)	yes	no

1 Select **yes** if storing the method, then press **OK**.
Otherwise, select **no** and press **OK**.

Free Methods		25
Sel. Method	(Free)	16

Free Methods		25
Sel. Method	(Used)	16

2 To store the method, select a method number and press **OK**.
Free means that the selected number is free for storing a new method.
Used means that the number is already used.
Select a free method number and press **OK**. Alternatively, press **OK** to clear the number in the **Clear Method** menu.

Starting the run

Press OK to start run

1 Press **OK** at the **Press OK to start run** prompt to start the run.

2 See section *3.8 During a run* for a description of viewing and printing run data, and changing parameters during the run.

Finishing the run

Method Complete
Press OK to continue

1 Press **OK** at the **Method Complete** prompt to finish the run.

To abort the run before it is finished, press **End**. Confirm the following message by selecting **yes**, then press **OK**.

2 When the run is finished, the curves obtained can be printed on the chart recorder. This is described in section *3.9 Post-run printing*.

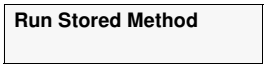
3.11 Running a stored method

The ÄKTA*prime* system can store up to 40 programmed methods. The methods are made either by using a method template or by programming line-by-line. Programming a method is described in chapter *4 Method programming*.

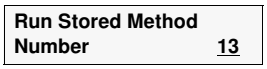
Go through the procedure below to run a stored method.

Selecting a stored method

1 Perform the general preparation of the system according to the description in chapter *3.5 Preparing the system for a run*.



2 Select main menu **Run Stored Method** and press **OK**.



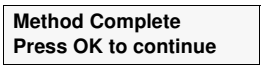
3 Select the method number and press **OK**.

Starting the run

1 Press **OK** at the **Press OK to start run** prompt to start the run.

2 During the run, important parameter values are shown on the display and are also printed on the recorder. Some of the parameter settings can be changed during the run. See section *3.8 During a run* for a description of viewing and changing parameters during the run.

Finishing the run



1 Press **OK** at the **Method Complete** prompt to finish the run.

To abort the run before it is finished, press **End**. Confirm the following message by selecting **yes**, then press **OK**.

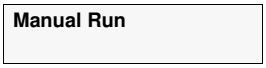
2 When the run is finished, the curves obtained can be printed on the chart recorder. This is described in section *3.9 Post-run printing*.

3.12 Running the system manually

To run the ÄKTA*prime* system manually, without using a pre-programmed method or a template, follow the procedure described in the sections below.

Preparing a manual run

1 Perform the general system preparation (refer to section *3.5 Preparing the system for a run*).



2 In the main menu, select menu **Manual Run** and press **OK**.

Setting the parameters

Use the arrow keys to go through the menu options and set the parameters as required. The settings take effect as soon as the instruction is confirmed by pressing **OK**.

Setting the method base

1 Select menu **Set Method Base**. The current setting is displayed. Press **OK**.

2 Select time (**min**) or volume (**ml**) and press **OK**.

Setting the concentration

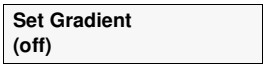
Set the start concentration of buffer B as follows:

1 Select menu **Set Concentration %B**. The current setting is displayed. Press **OK**.

2 Set the desired concentration and press **OK**.

Setting a gradient

To create a gradient from the start, enter the target concentration of buffer B and the duration of the gradient in volume or time (depending on the method base).



1 Select menu **Set Gradient** (default setting: **off**). Press **OK**.

2 Set the length (volume or time) for the target concentration of buffer B to be reached. Press **OK**.

3 Set the target concentration. Press **OK**.

The result will be a gradient starting with the concentration set in menu **Set Concentration %B** and finishing with the target concentration.

Setting the flow rate

1 Select menu **Set Flow Rate**. The current setting is displayed. Press **OK**.

Set Flow Rate
(0.1 ml/min) 0.8

2 Set the flow rate and press **OK**.

Setting the fraction base

1 Select menu **Set Fraction Base**. The current setting is displayed. Press **OK**.

Set Fraction Base
(ml) min ml drp

2 Choose time (**min**), volume (**ml**) or drops (**drp**). Press **OK**.

Setting the fraction size

1 Select menu **Set Fraction Size**. The current setting is displayed. Press **OK**.

Set Fraction Size
(00.0 ml) 0.2

2 Set the fraction size and press **OK**.

Setting the pressure limit

1 Select menu **Set Pressure Limit**. The current setting is displayed. Press **OK**.

Set Pressure Limit
(1.00 MPa) 1.00

2 Set the pressure limit and press **OK**.
Set the limit to the maximum back-pressure for the column used + 0.2 MPa (back-pressure contribution from flow restrictor). Refer to the column documentation for maximum back-pressure.

Setting the buffer valve position

1 Select menu **Set Buffer Valve Pos**. The current setting is displayed. Press **OK**.

Set Buffer Valve Pos
(Pos 1) 1

2 Set the position and press **OK**. Refer to the number printed on the buffer valve.

Setting the injection valve position

1 Select menu **Set Inject Valve Pos**. The current setting is displayed. Press **OK**.

Set Inject Valve Pos
(Load) Waste Load Inject

2 Set the position and press **OK**.

Waste – the flow is diverted to waste (ports 4 and 5).
Load – the sample loop is loaded (between ports 2 and 6) when injecting the sample through port 3.
Inject – the sample loop is emptied through port 1 and the flow directed to the column.

Starting the run

Press OK to start run

1 Press **OK** at the **Press OK to start run** prompt to start the run.

During the run

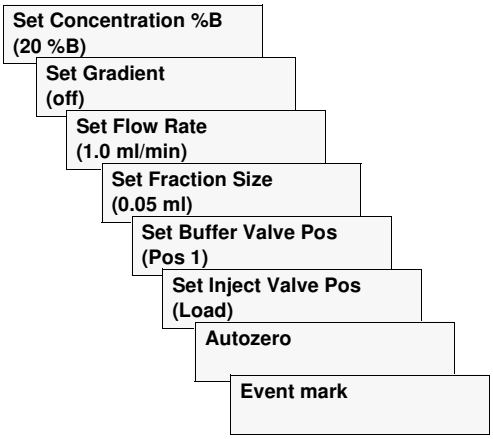
Viewing parameters

During the run, important parameter values are shown on the display and are also printed on the recorder. See section *3.8 During a run* for a description of viewing parameters during the run.

Changing parameters

The most important parameter values can be changed at any time during a manual run, with the exception of the gradient. The gradient can only be changed if no gradient is running or if the system is paused (press the **Pause/Cont** button) or held (press the **Hold/Cont** button).

The following parameters can be changed during the run. In addition, the recorder functions **Autozero** and **Event mark** are also available.



To change a parameter, see the instruction in the previous section *Preparing a manual run*. The new setting takes effect immediately.

To autozero the recorder or to set an event mark on the chart, select the desired option, then press **OK**.

Finishing the run

Method Complete
Press OK to continue

1 Press **OK** at the **Method Complete** prompt to finish the run.

To abort the run before it is finished, press **End**. Confirm the following message by selecting **yes**, then press **OK**.

2 When the run is finished, the curves obtained can be printed on the recorder. This is described in section *3.9 Post-run printing*.

3.13 Completion of a run and storage

All valves return to default position (i.e. position 1) after a run.

Between runs

If a buffer containing salt has been run, it is very important to wash the system and the column with distilled water, especially if organic solvent, e.g. ethanol, is to be used in the next run. Run the **System Wash Method** or run distilled water manually through the system.

Storage

CAUTION! Never leave the pH electrode in the flow cell for any period of time when the system is not used, since this may cause the glass membrane of the electrode to dry out. Dismount the pH electrode from the flow cell and fit the end cover filled with a 1:1 mixture of pH 4 buffer and 2 M KNO_3.
Do NOT store in water only.

CAUTION! Do not allow solutions which contain dissolved salts, proteins or other solid solutes to dry out in the UV flow cell.
Do not allow particles to enter the UV flow cell as damage to the flow cell may occur.

Overnight

The system, except the pH electrode (if applicable), can be left filled with a buffer overnight. For storage of the pH electrode, see the separate instruction below.

Weekend and long term storage

If you are not using the system for a few days or longer, wash the system with distilled water. Remove the column and the pH electrode (optional). Replace the column by a bypass capillary and fit the dummy pH electrode (if applicable). Then wash the system with 20% ethanol and store it in 20% ethanol (not the pH electrode, see separate instruction below). Rinse all tubing and all flow paths used.

The UV flow cell can also be stored dry by flushing as above with distilled water and then 20% ethanol through the flow cell. Replace the red protective caps. Never use compressed air as this may contain droplets of oil.

Storage of the pH electrode

The pH electrode should always be stored in a 1:1 mixture of pH 4 buffer and 2 M KNO_3 when not in use.

Electrode regeneration: If the electrode has dried out, immerse the lower end of the electrode overnight in a buffer with a 1:1 mixture of pH 4 buffer and 2 M KNO_3.

3.14 Restart after power failure

If the power to the system is interrupted, it automatically restarts when power is restored and displays the main operating menu. All set values and the data from the latest run are retained, and the lamp is switched on.

3.15 Cold room operation

Cold room operation is sometimes necessary to keep the biomolecule(s) of interest stable.

Preparation

1 Place the separation unit in the cold room and let it stabilize for at least 12 hours.

2 Tighten all connections and pump water through the system to check for leaks.

3 Tighten any leaking connector.

Running

1 Ensure that the temperature of the buffers has reached the ambient temperature.

2 Calibrate the pH electrode (optional).

3 Check the pH of the buffers.

Removal from cold room

1 Loosen all connections to prevent them sticking when the system returns to room temperature.

2 Allow the separation unit to stabilize at room temperature for at least 12 hours.

3 Tighten all connections and pump water through the system to check for leaks.

4 Tighten any leaking connector.

4 Method programming

4.1 General

The operator can create fully customized methods for purification. The methods are made either by programming line-by-line or by using a method template.

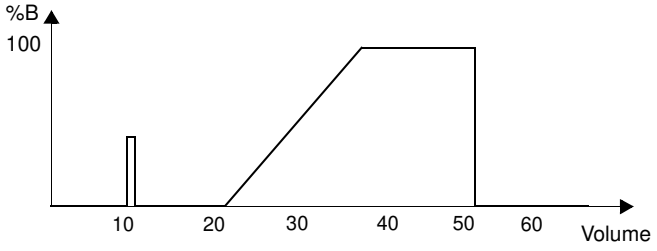
A method consists of a series of *breakpoints* which define changes in one or more parameters. The methods are programmed on a time or a volume base. The system can store up to 600 breakpoints in totally 40 user-defined methods for future use.

It is important to plan the method properly before starting the programming. Begin with illustrating the run as the progress of the theoretical concentration of buffer B during the run. Then define all breakpoints and the actions at the breakpoints that are required to achieve this progress.

4.2 Programming line-by-line

The example below illustrates a simple method for a gradient elution with a linear gradient from 0 to 100%. The sample is loaded manually through the injection valve. Fraction collecting starts at the beginning of the elution.

The table shows the breakpoints for the same method. Values actively entered for each breakpoint are shown in bold. S represents the sample volume.



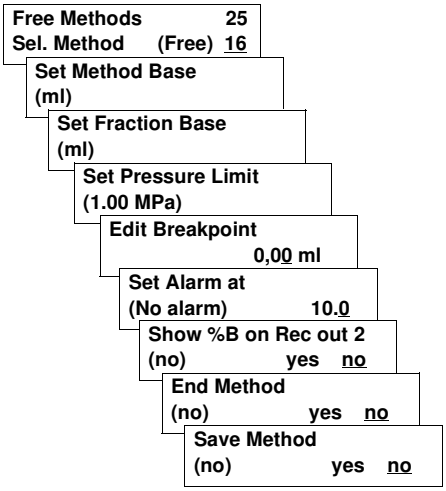
Volume	*Conc %B*	*Flow*	*Fract.*	*Buffer V*	*Inject V*	*Comment*
0	0	**1**	0	pos 1	LOAD	Equilibration
10	0	1	0	pos 1	**INJECT**	Sample applic.
10+S	0	1	0	pos 1	**LOAD**	Elution delay/wash
20+S	0	1	**1**	pos 1	LOAD	Elution, start fraction, start gradient
35+S	**100**	1	1	pos 1	LOAD	Column wash
49.9+S	100	1	0	pos 1	LOAD	End column wash, end fractions
50+S	**0**	40	**0**	pos 1	WASTE	Priming
70+S	0	**1**	0	pos 1	**LOAD**	Re-equilibration
80+S	0	1	0	pos 1	LOAD	End method

To program a new method line-by-line, follow the instruction below.

Program Method

1 Select main menu **Program Method** and press **OK**.

The menu contains the sub menus shown below. Move through the menus using the arrow buttons.



Selecting a method number

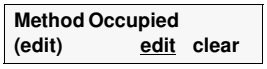
1 Select a number for the new method.

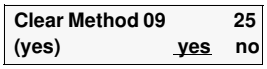
Free Methods 25
Sel. Method (Free) 16

If the display shows **Free**, the selected number is free for storing a new method. Press **OK** and continue with the parameter settings.

Free Methods 25
Sel. Method (Used) 16

If the display shows **Used**, a method already occupies that number. Select a free number and press **OK**.
To clear a stored method, press **OK** only.





2 To clear the stored method in the **Method Occupied** menu, select **clear** and press **OK**.
Then select **yes** in the **Clear Method** menu and press **OK**.

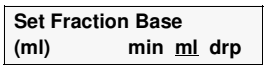
General parameter set-up

Setting the method base

1 Select menu **Set Method Base** and press **OK**.

2 Select time (**min**) or volume (**ml**) and press **OK**.

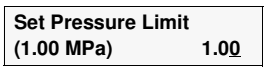
Setting the fraction base



1 Select menu **Set Fraction Base** and press **OK**.

2 Choose time (**min**), volume (**ml**) or drops (**drp**). Press **OK**.

Setting the pressure limit



1 Select menu **Set Pressure Limit** and press **OK**.

2 Set the pressure limit and press **OK**. Set the limit to the maximum back-pressure for the column used + 0.2 MPa (back-pressure contribution from flow restrictor). Refer to the column documentation for maximum back-pressure.

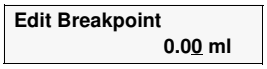
Setting breakpoints

The breakpoints are set on a time or volume base, depending on the Method Base setting in the previous section. The first breakpoint must be at time/volume 0.00.

If several breakpoints have been set, parameters can be changed in them by selecting the desired breakpoint with the arrow buttons.

In a new method, the default value in the first breakpoint is 0 for all parameters (%B, flow rate, etc.), Buffer valve (1), Injection valve (load) and no peak collection. All breakpoints after the first one will inherit the parameter values of the previous breakpoint.

Selecting a breakpoint



1 Press OK to enter the breakpoint selection mode.

2 Select the desired time/volume of the breakpoint with the arrow buttons and press **OK**.

To create a new breakpoint, go through all breakpoints. After the last one, the breakpoint value changes to **NEW**. Pressing **OK** here creates a new breakpoint with value 0.00. This value can be changed with the arrow buttons.

Setting the concentration

Set the concentration of buffer B as follows:

Set Concentration %B
(20 %B) 30

1 Select menu **Set Concentration %B** and press **OK**.

2 Set the desired concentration and press **OK**.

To create a linear gradient, set two breakpoints with different values for concentration of B. This creates a linear gradient from the first to the second value over the interval between the breakpoints.

To create a step gradient, set two breakpoints separated by 0.1 ml or min with different values for the buffer B concentration. This creates an immediate change in the B concentration between the breakpoints; a step gradient.

Setting the flow rate

Set Flow Rate
(0.1 ml/min) 0.8

1 Select menu **Set Flow Rate** and press **OK**.

2 Set the flow rate (cannot be 0.0) and press **OK**.

Setting the fraction size

Set Fraction Size
(00.0 ml) 0.2

1 Select menu **Set Fraction Size** and press **OK**.

2 Set the fraction size and press **OK**.

Setting the buffer valve position

Set Buffer Valve Pos
(Pos 1) 1

1 Select menu **Set Buffer Valve Pos** and press **OK**.

2 Set the position and press **OK**. Refer to the number printed on the buffer valve.

Setting the injection valve position

Set Inject Valve Pos
(Load) Waste Load Inject

1 Select menu **Set Inject Valve Pos** and press **OK**.

2 Set the position and press **OK**.

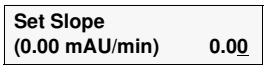
Waste – the flow is diverted to waste (ports 4 and 5).
Load – the sample loop is loaded (between ports 2 and 6) when injecting the sample through port 3.
Inject – the sample loop is emptied through port 1 and the flow directed to the column.

Setting peak collection

The fraction size must be set >0 to activate peak collection. If fraction size is set to zero, fractions will not be collected at peaks.

The threshold slope of the UV curve, i.e. when the system is to detect a peak, should be entered as mAU/min. The fraction collector will change tubes whenever the slope exceeds the set value. The peak end is determined automatically by the system.

1 Select menu **Set Peak Collect** and press **OK**.

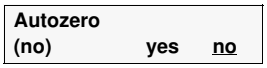


2 In the **Set Slope** menu, set the slope and press **OK**.

Use a previous chromatogram from an identical run to determine the slope. We recommend starting with a slope of about 10% of the peak height in mAu/min (with a time averaging of 2.6 s). Perform a blank run with the chosen setting to check that tube changes do not occur as a result of baseline disturbances.

Setting UV signal autozero

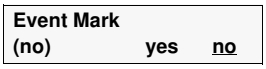
1 Select menu **Autozero** and press **OK**.



2 Select **yes** and press **OK** to set the output signal to zero.
At the next breakpoint, the setting is reset to **no**.

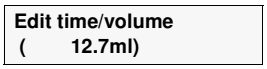
Setting an event mark

1 Select menu **Event Mark** and press **OK**.

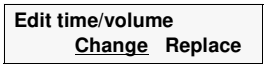


2 Select **yes** and press **OK** to set an event mark on the chart.
Event Mark is reset to **no** at next breakpoint.

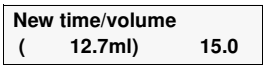
Editing time/volume of a breakpoint



1 Select menu **Edit time/volume** and press **OK**.

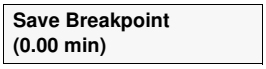


2 Select **Change** or **Replace** and press **OK** to edit the time/volume of the breakpoint.
Change will also change the time/volume of all the following breakpoints accordingly.
Replace will not change the time/volume of the other breakpoints.



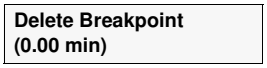
3 Edit the time/volume and press **OK**.

Saving the breakpoint



To save the breakpoint, select menu **Save Breakpoint** and press **OK**.

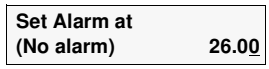
Deleting a breakpoint



To delete an existing breakpoint, select menu **Delete Breakpoint** and press **OK**.

Setting an alarm

If an alarm should sound during or after the run:



1 Go to the **Set Alarm at** menu.

2 Enter the desired time or volume elapsed from the method start, then press **OK**. For example, entering 26 ml will sound an alarm when 26 ml has been pumped from the method start.
Entering zero deactivates the alarm.

Printing the method

Print out the programmed method (concentration of B curve) on recorder channel 2 as follows:

1 Go to the **Show %B on Rec out 2** menu.

2 Select **yes** and press **OK**. The recorder now prints out the theoretical %B curve.

Ending the method

The method ends at the last breakpoint. If a period of constant parameters is required at the end of the method, enter a final breakpoint with the same parameters as the last one.

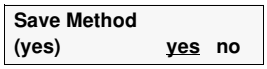
1 Go the **End Method** menu.

2 Select **yes** and press **OK**.

3 Select a final breakpoint with the same parameters.

Saving the method

When all breakpoints are set, save the method as follows:



1 Go to the **Save Method** menu.

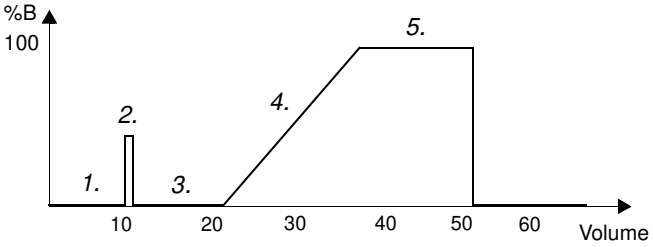
2 Select **yes** and press **OK**.

4.3 Programming using method templates

The method templates provide an alternative procedure for creating new methods for future use. In the method templates, the operator sets the length of the interval between each breakpoint. When programming line-by-line, each breakpoint is set.

The illustration below shows an example method for gradient elution with a linear gradient from 0 to 100%. The sample is applied manually. Fraction collecting starts at the beginning of the elution. The numbers represent the parameters to be set.

The table shows the parameters at each interval. S represents the sample volume.



Volume	*Conc %B*	*Flow*	*Fract.*	*Buffer V*	*Inject V*	*Parameter*
10	0	F	0	pos 1	LOAD	1. Equilibration volume
S	0	F	0	pos 1	INJECT	2. Sample volume.
10	0	F	0	pos 1	LOAD	3. Wash 1 volume
15	100	F	1	pos 1	LOAD	4. Elution volume
15	100	F	1	pos 1	LOAD	5. Wash 2 volume
20	*0*	*F*	*0*	*pos 1*	*LOAD*	*Re-equilibration (hidden in template)*

Follow the instructions below to create a method.

Selecting method template

Templates

1 Select main menu **Templates** and press **OK**.

Method Template

2 Select sub menu **Method Template** and press **OK**.

3 Select the desired template and press **OK**.

Gelfiltration/ Buffer Exchange
Ion Exchange/ Gradient elution
HIC Gradient elution
Affinity Step Gradient

The method templates are described in chapter *5 Template description*.

4 Go through the parameters using the arrow buttons and set the values as desired.

Setting the parameters

Sample inject by
InjV Pump

1 Select sample injection through the injection valve or through the system pump. Refer to section *3.6 Fluid handling components* for more information about sample application.

Set Pressure Limit
(1.00 MPa) 1.00

2 Set the pressure limit and press **OK**. Set the limit to the maximum back-pressure for the column used + 0.2 MPa (back-pressure contribution from flow restrictor). Refer to the column documentation for maximum back-pressure.

Set Flow Rate
(--.- ml/min) 0.00

3 Set the flow rate and press **OK**.

Set Fraction Size
(0.0 ml) 0.0

4 Set the fraction size and press **OK**.

Set Equilibr. Volume
(0.0 ml) 0.0

5 Set the equilibration volume (*1.* in the figure) and press **OK**.

Set Sample Inj. Volume
(0.0 ml) 0.0

6 Set the sample volume (*2.*) to be injected and press **OK**.

Set Wash 1 Volume
(0.0 ml) 0.0

7 Set the wash 1 volume (*3.*) and press **OK**.
This setting does NOT apply to the Gel filtration method template.

Note: *15 ml of buffer is automatically added to Wash 1 when using the system pump for the sample application.*

Set Elution. Volume
(0.0 ml) 0.0

8 Set the elution volume (*4.*) and press **OK**.

Set Wash 2 Volume
(0.0 ml) 0.0

9 Set the wash 2 volume (*5.*) and press **OK**.
This setting does NOT apply to the Gel filtration method template.

Method ready?
(yes) yes no

10 Select **yes** at the **Method ready?** prompt and press **OK**.

Storing the method

Save Method
(yes) yes no

1 To store the method, select **yes** and press **OK**.

Free Methods 25
Sel. Method (Free) 16

Free Methods 25
Sel. Method (Used) 16

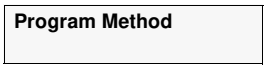
2 Select a method number and press **OK**.
Free means that the selected number is free for storing a new method.
Used means that the number is already in use.
Select a free method number and press **OK**. Alternatively, press **OK** to clear the number in the **Clear Method** menu.

The programming is now finished and the method ready for use.

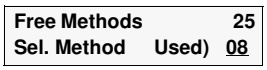
4.4 Editing a stored method

To edit an existing method, follow the instruction below. Refer to section *4.2 Programming line-by-line* for more detailed information about setting the parameters.

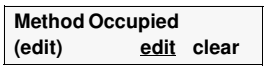
Selecting method



1 Select main menu **Program Method** and press **OK**.



2 Select the number of the method and press **OK**.



3 Select **yes** and press **OK**.

Use the arrow buttons to go through the sub menus and change the parameters as desired (see also section *4.2 Programming line-by-line*).

Editing an existing breakpoint

1 Go to the **Edit Breakpoint** menu.

2 Use the down button to scroll through the existing breakpoints.

3 Press **OK** at the desired breakpoint to enter the parameter menus.

4 Edit the parameters as required. All values are default the previously stored values.

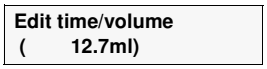
5 Save the new parameter values by pressing **OK**.

Editing time/volume of a breakpoint

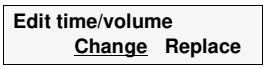
1 Go to the **Edit Breakpoint** menu.

2 Use the down button to scroll through the existing breakpoints.

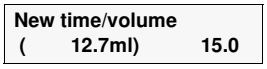
3 Press **OK** at the desired breakpoint to enter the parameter menus.



4 Select menu **Edit time/volume** and press **OK**.

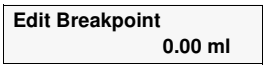


5 Select **Change** or **Replace** and press **OK** to edit the time/volume of the breakpoint.
Change will also change the time/volume of all the following breakpoints accordingly.
Replace will not change the time/volume of the other breakpoints.



6 Edit the time/volume and press **OK**.

Inserting a breakpoint



1 Go to the **Edit Breakpoint** menu.

2 Use the down button to scroll through all existing breakpoints After the last breakpoint, the breakpoint value changes to **NEW**.

3 Press **OK** at breakpoint **NEW** to create a new breakpoint. This breakpoint will have the value **0.00**.

4 Set the breakpoint value with the arrow buttons and press **OK**.

5 Edit the parameters as required.

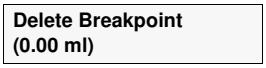
6 Select the **Save Breakpoint** menu. Save the new breakpoint by pressing **OK**.

Deleting a breakpoint

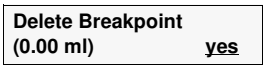
1 Go to the **Edit Breakpoint** menu.

2 Use the down button to scroll through the existing breakpoints.

3 Press **OK** at the desired breakpoint to enter the parameter menus.



4 Select the **Delete Breakpoint** menu and press **OK**.



5 Select **yes** and press **OK** to delete the breakpoint.

Printing the method

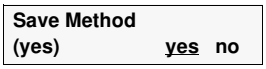
Print out the modified method (gradient curve) on recorder channel 2 as follows:

1 Go to the **Show %B on Rec out 2** menu.

2 Select **yes** and press **OK**. The recorder now prints out the gradient curve.

Saving the method

When all breakpoints are set, save the method as follows.



1 Go to the **Save Method** menu.

2 Select **yes** and press **OK**.

5 Template description

5.1 General

ÄKTA*prime* contains a collection of pre-constructed templates for common chromatographic applications.

The templates are grouped into two categories:

- Application templates.
  These templates only require the sample volume as input, then they are ready to use.
- Method templates.
  These templates require more input from the operator, such as flow rate, elution volume and equilibration volume. Methods that are made from the method templates can also be stored in the system for future use.

5.2 Application templates

General

The system is supplied with templates for the following applications:

- Desalting.
- Purification of His-tagged proteins.
- Purification of GST-tagged proteins.
- Purification of monoclonal antibodies.
- IgM purification.
- Removal of albumin.
- Protein renaturation on column.
- Anion exchange.
- Cation exchange.

The system also contains a template for washing and priming the system, System Wash Method.

In the application templates, all process parameters are pre-programmed and can not be changed. The system only needs the sample volume as input.

Find an application template as follows:

Templates

1 In the main menu, choose menu **Templates**, and press **OK**.

Application template

2 Choose menu **Application Template**, and press **OK**.

3 Select the desired template with the up and down buttons.

In all application templates, sample application is made by using a syringe and a sample loop.

For more information on how to run an application template, refer to section *3.7 Starting a run*.

The application templates are described below. The templates are illustrated by the buffer gradient during the run. The table shows how the parameter values change accordingly. Parameter S represents the sample volume.

Information about which buffer solutions to use is provided on the cue cards supplied.

HiTrap desalting

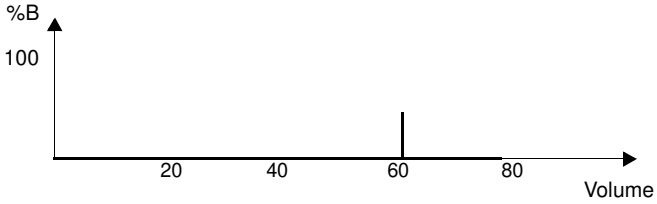
The HiTrap desalting application template is used for desalting a sample solution.

Desalting
HiTrap Desalting

To access the template, select **Desalting**/**HiTrap Desalting** and press **OK**.

Column: HiTrap 5 ml Desalting

Total run time: approx. 9 min + sample application time



Volume	*Conc %B*	*Flow*	*Fract.*	*Buffer V*	*Inject V*	*Comment*
0	0	40	0	pos 1	WASTE	Priming A1
35	0	5	0	pos 1	LOAD	Equilibration
60						Autozero
60	0	5	0	pos 1	INJECT	Sample application
60+S	0	5	1	pos 1	LOAD	Elution
75+S						End method

HiPrep desalting

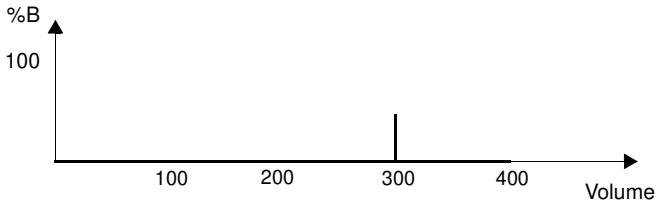
The HiPrep desalting application template is used for desalting a sample solution.

Desalting
HiPrep Desalting

To access the template, select **Desalting/HiPrep Desalting** and press **OK**.

Column: HiPrep 26/10 Desalting

Total run time: approx. 18 min + sample application time



Volume	*Conc %B*	*Flow*	*Fract.*	*Buffer V*	*Inject V*	*Comment*
0	0	40	0	pos 1	WASTE	Priming A1
35	0	20	0	pos 1	LOAD	Equilibration
300						Autozero
300	0	20	0	pos 1	INJECT	Sample application
300+S	0	20	5	pos 1	LOAD	Elution
400+S						End method

His-tag purification

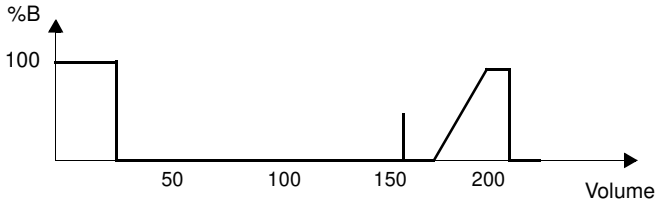
The His-tag purification application template is used for purification of His-tagged proteins.

His-tag Purification HiTrap Chelating

To access the template, select **His-tag Purification/HiTrap Chelating** and press **OK**.

Column: HiTrap Chelating 1 ml

Total run time: approx. 77 min + sample application time



Volume	*Conc %B*	*Flow*	*Fract.*	*Buffer V*	*Inject V*	*Comment*
0	100	40	0	pos 2	WASTE	Priming B
25	100	40	0	pos 2	WASTE	End priming B
25+0.1	0	40	0	pos 2	WASTE	Priming A2
60	0	1	0	pos 2	LOAD	Water wash
65	0	40	0	pos 3	WASTE	Priming A3
100	0	1	0	pos 3	LOAD	Ni^{+} application
101	0	40	0	pos 2	WASTE	Priming A2
116	0	1	0	pos 2	LOAD	Water wash
121	0	40	0	pos 1	WASTE	Priming A1
156	0	1	0	pos 1	LOAD	Equilibration
166						Autozero
166	0	1	0	pos 1	INJECT	Sample application
166+S	0	1	0	pos 1	LOAD	Buffer wash
176+S	0	1	1	pos 1	LOAD	Elution
196+S	100	1	1	pos 1	LOAD	Elution wash out
213+S	100	1	0	pos 1	LOAD	End wash
213.1+S	0	40	0	pos 2	WASTE	Priming A2
228+S	0	1	0	pos 2	LOAD	Re-equilibration
233+S						End method

GST-tag purification

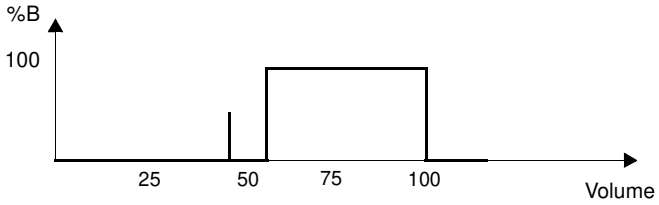
The GST-tag purification application template is used for purification of GST-tagged proteins.

GST-tag Purification GSTrap

To access the template, select **GST-tag Purification/GSTrap** and press **OK**.

Column: GSTrap 1 ml

Total run time: approx. 37 min + sample application time



Volume	*Conc %B*	*Flow*	*Fract.*	*Buffer V*	*Inject V*	*Comment*
0	0	40	0	pos 1	WASTE	Priming A1
35	0	1	0	pos 1	LOAD	Equilibration
45						Autozero
45	0	1	0	pos 1	INJECT	Sample application
45+S	0	1	0	pos 1	LOAD	Wash
55+S	0	1	0	pos 1	LOAD	End wash
55.1+S	100	40	0	pos 1	WASTE	Priming B
90+S	100	1	1	pos 1	LOAD	Elution
100+S	100	1	0	pos 1	LOAD	End elution
100.1+S	0	40	0	pos 1	WASTE	Priming A1
115+S	0	1	0	pos 1	LOAD	Re-equilibration
120+S						End method

Mab purification (step elution)

The Mab purification (step elution) application template is used for purification of monoclonal antibodies by step elution.

Mab Purification Step elution

To access the template, select **Mab Purification/Step elution** and press **OK**.

Column: HiTrap Protein G 1 ml (alt. HiTrap Protein A or HiTrap rProtein A 1 ml)

Total run time: approx. 37 min + sample application time

The buffer gradient and the parameter table are the same as for GST-tag Purification.

Albumin removal

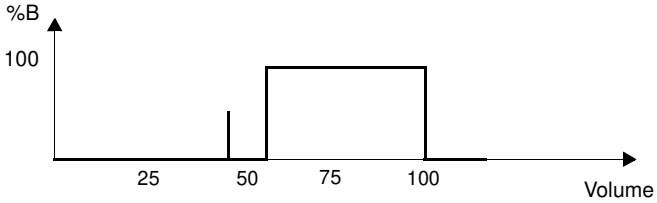
The Albumin removal application template is used for removing albumin.

Albumin Removal HiTrap Blue

To access the template, select **Albumin Removal/HiTrap Blue** and press **OK**.

Column: HiTrap Blue 1 ml

Total run time: approx. 37 min + sample application time



Volume	*Conc %B*	*Flow*	*Fract.*	*Buffer V*	*Inject V*	*Comment*
0	0	40	0	pos 1	WASTE	Priming A1
35	0	1	0	pos 1	LOAD	Equilibration
45						Autozero
45	0	1	1	pos 1	INJECT	Sample application
45+S	0	1	1	pos 1	LOAD	Elution
55+S	0	1	0	pos 1	LOAD	End elution
55.1+S	100	40	0	pos 1	WASTE	Priming B
90+S	100	1	1	pos 1	LOAD	Albumin wash out
100+S	100	1	0	pos 1	LOAD	End wash out
100.1+S	0	40	0	pos 1	WASTE	Priming A1
115+S	0	1	0	pos 1	LOAD	Re-equilibration
120+S						End method

Mab purification (gradient elution)

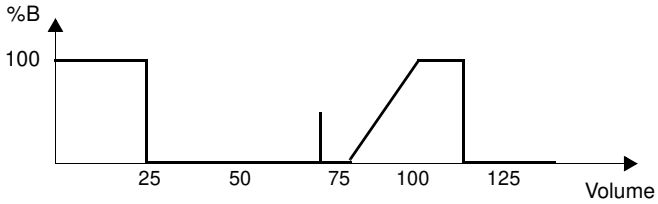
The Mab purification (gradient elution) application template is used for purification of monoclonal antibodies using a pH gradient

Mab Purification Gradient elution

To access the template, select **Mab Purification/Gradient elution** and press **OK**.

Column: HiTrap Protein A 1 ml (alt. HiTrap rProtein A or HiTrap Protein G 1 ml)

Total run time: approx. 63 min + sample application time



Volume	*Conc %B*	*Flow*	*Fract.*	*Buffer V*	*Inject V*	*Comment*
0	100	40	0	pos 1	WASTE	Priming B
25	100	40	0	pos 1	WASTE	End priming B
25.1	0	40	0	pos 1	WASTE	Priming A1
60	0	1	0	pos 1	LOAD	Equilibration
70						Autozero
70	0	1	0	pos 1	INJECT	Sample application
70+S	0	1	0	pos 1	LOAD	Elution delay
80+S	0	1	1	pos 1	LOAD	Elution
100+S	100	1	1	pos 1	LOAD	Elution wash out
117+S	100	1	0	pos 1	LOAD	End wash out
117.1+S	0	40	0	pos 1	WASTE	Priming A1
132+S	0	1	0	pos 1	LOAD	Re-equilibration
137+S						End method

Anion exchange

The Anion exchange application template is used for separation of molecules that have negative charge.

Anion Exchange HiTrap Q

To access the template, select **Anion Exchange/HiTrap Q** and press **OK**.

Column: HiTrap Q 1 ml

Total run time: approx. 63 min + sample application time

The buffer gradient and the parameter table are the same as for Mab purification (gradient elution).

Cation exchange

The Cation exchange application template is used for separation of molecules that have positive charge.

Cation Exchange
HiTrap SP

To access the template, select **Cation Exchange**/**HiTrap SP** and press **OK**.

Column: HiTrap SP 1 ml

Total run time: approx. 63 min + sample application time

The buffer gradient and the parameter table are the same as for Mab purification (gradient elution).

IgM purification

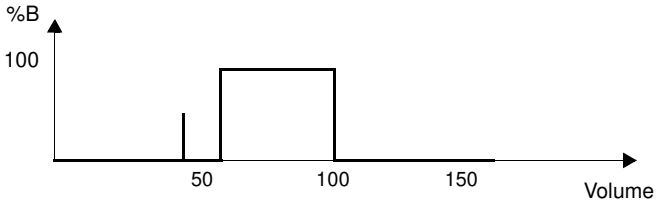
The IgM purification application template is used for purification of monoclonal antibody IgM.

IgM Purification
HiTrap IgM Purification

To access the template, select **IgM Purification**/**HiTrap IgM Purification** and press **OK**.

Column: HiTrap IgM Purification 1 ml

Total run time: approx. 48 min + sample application time



Volume	*Conc %B*	*Flow*	*Fract.*	*Buffer V*	*Inject V*	*Comment*
0	0	40	0	pos 1	WASTE	Priming A1
35	0	1	0	pos 1	LOAD	Equilibration
45						Autozero
45	0	1	0	pos 1	INJECT	Sample application
45+S	0	1	0	pos 1	LOAD	Wash
55+S	0	1	0	pos 1	LOAD	End wash
55.1+S	100	40	0	pos 1	WASTE	Priming B
90+S	100	1	1	pos 1	LOAD	Elution 1
100+S	100	1	0	pos 1	LOAD	End elution 1
100.1+S	0	40	0	pos 2	WASTE	Priming A2
135+S	0	1	1	pos 2	LOAD	Elution 2
145+S	0	40	0	pos 1	WASTE	Priming A1
160+S	0	1	0	pos 1	LOAD	Re-equilibration
165+S						End Method

Refolding

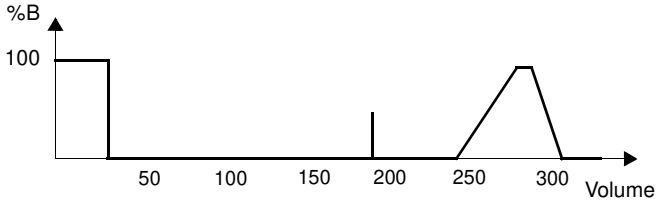
The Refolding application template is used for protein renaturation on column.

Refolding
HiTrap Chelating

To access the template, select **Refolding/HiTrap Chelating** and press **OK**.

Column: HiTrap Chelating 1 ml

Total run time: approx. 112 min + sample application time



Volume	*Conc %B*	*Flow*	*Fract.*	*Buffer V*	*Inject V*	*Comment*
0	100	40	0	pos 5	WASTE	Priming B
25	100	40	0	pos 5	WASTE	End priming B
25.1	0	40	0	pos 5	WASTE	Priming A5
50	0	40	0	pos 2	WASTE	Priming A2
85	0	1	0	pos 2	LOAD	Water wash
90	0	40	0	pos 3	WASTE	Priming A3
125	0	1	0	pos 3	LOAD	Ni^{+} application
126	0	40	0	pos 2	WASTE	Priming A2
141	0	1	0	pos 2	LOAD	Water wash
146	0	40	0	pos 1	WASTE	Priming A1
181	0	1	0	pos 1	LOAD	Equilibration
191						Autozero
191	0	1	0	pos 1	INJECT	Sample application
191+S	0	1	0	pos 1	LOAD	Buffer wash
201+S	0	40	0	pos 1	LOAD	Priming A4
236+S	0	1	0	pos 1	LOAD	Buffer wash
246+S	0	0.5	0	pos 1	LOAD	Refolding
276+S	100	0.5	0	pos 1	LOAD	End refolding
286+S	100	1	1	pos 2	WASTE	Elution
306+S	0	1	1	pos 2	LOAD	Re-equilibration
323+S						End method

System wash

The System wash template is used for rinsing and priming the tubings and the components in the flow path.

System Wash Method

1 To access the template, select **System Wash Method** and press **OK**.

Select Buffer V. Pos
B, A: 2, 3, –, –, –, –, –, OK

2 Select the buffer inlets to be washed and press **OK**.

Note: *Inlet A1 is pre-selected and will always be washed.*

Total run time: depends on the number of buffer inlets selected.

The table below shows how the tubings are washed.

Volume	*Conc %B*	*Flow*	*Fract.*	*Buffer V*	*Inject V*	*Comment*
0	100	50	0	pos 8	WASTE	Wash B
24,9	100	50	0	pos 8	WASTE	End wash B
50	0	50	0	pos 8	WASTE	Wash A8
75	0	50	0	pos 7	WASTE	Wash A7
100	0	50	0	pos 6	WASTE	Wash A6
125	0	50	0	pos 5	WASTE	Wash A5
150	0	50	0	pos 4	WASTE	Wash A4
175	0	50	0	pos 3	WASTE	Wash A3
200	0	50	0	pos 2	WASTE	Wash A2
225	0	50	0	pos 1	WASTE	Wash A1
250	0	50	0	pos 1	WASTE	Air wash out
275	0	1	0	pos 1	LOAD	Wash Inject v. load
276	0	1	2	pos 1	LOAD	Re-equilibration
278						End Method

5.3 Method templates

General

The system is supplied with templates for the four most common purification techniques:

- Gel filtration/buffer exchange.
- Ion exchange.
- Hydrophobic interaction chromatography.
- Affinity.

Find a method template as follows:

Templates

1 In the main menu, choose menu **Templates** and press **OK**.

Method template

2 Choose menu **Method Template** and press **OK**.

When using the method templates, the system needs some parameters to be entered. Besides pressure limit, flow rate and fraction size, the volumes at the main phases during the run must be set.

The operator also has to select sample injection using the sample pump or via the sample port. If volume changes due to the selection are required, this is handled automatically within the templates.

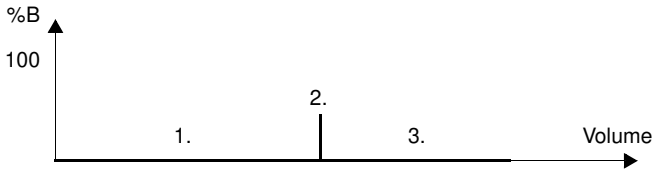
For more information on how to run a method template, refer to section *3.10 Running a method template*.

The method templates are described below. The templates are illustrated by the buffer gradient during the run. The table shows how the parameters to be entered correspond to the phases during the run.

Gel filtration/buffer exchange

Gelfiltration/ Buffer Exchange

To access the template, select **Gelfiltration/Buffer Exchange** and press **OK.**

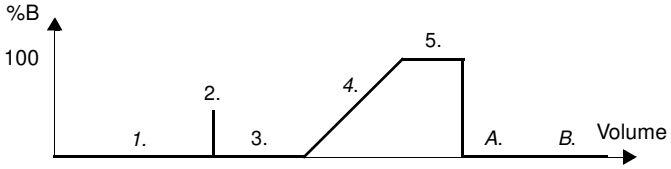


Number	*Parameter*
1	Equilibration volume
2	Sample application volume
3	Elution volume

Ion exchange

Ion Exchange
Gradient elution

To access the template, select **Ion Exchange**/**Gradient elution** and press **OK**.



Number	*Parameter*
1	Equilibration volume
2	Sample application volume
3	Wash 1 volume *Hidden: If using the system pump for sample application, 15 ml of buffer is added to Wash 1.*
4	Elution volume
5	Wash 2 volume
A	*Hidden: Priming with buffer A (20 ml, 40 ml/min)*
B	*Hidden: Re-equilibration (= equilibration volume)*

HIC (hydrophobic interaction chromatography)

HIC
Gradient elution

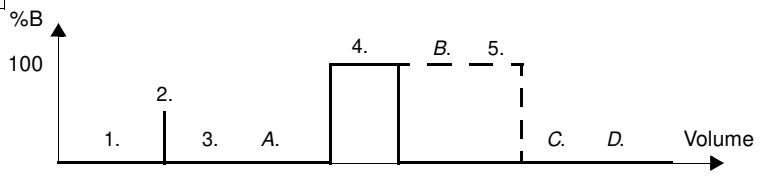
To access the template, select **HIC**/**Gradient elution** and press **OK**.

The HIC method template has the buffer gradient and parameter settings table as the Ion exchange method template.

Affinity

Affinity
Step Gradient

To access the template, select **Affinity**/**Step Gradient** and press **OK**.



Number	*Parameter*
1	Equilibration volume
2	Sample application volume
3	Wash 1 volume *Hidden: If using the system pump for sample application, 15 ml of buffer is added to Wash 1.*
A	*Hidden: Priming with elution buffer A (15 ml, 40 ml/min)*
4	Elution volume
B	*Hidden: Priming with Wash 2 buffer (15 ml, 40 ml/min), if Wash 2*
5	Wash 2 volume (if applicable)
C	*Hidden: Priming with buffer A (20 ml, 40 ml/min)*
D	*Hidden: Re-equilibration (= equilibration volume)*

6 Handling components

6.1 General

Components in ÄKTA*prime* sometime need to be adjusted or changed to adapt the system to a specific need. For instance, the UV sensitivity can be changed by fitting another UV flow cell, the appropriate of the optical filter can be altered, or the rack in the fraction collector changed according to the tube size used.

This chapter describes how to perform the modifications that are required, and how to re-install the components.

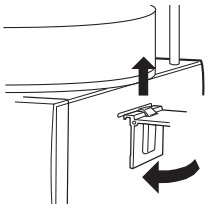
The description covers the following components:

- The optical unit, including flow cells and filters.
- The conductivity cell.
- The fraction collector.
- The pH flow cell and electrode (optional).

6.2 Installing the optical unit

Connecting the optical unit holder

Hook the holder into the slot on the right hand side of the system. Secure it by pushing up the slide clamp.



Changing UV flow cell

A preparative (2 mm) flow cell is included in the system. An analytical (5 mm) flow cell is available as an accessory. The flow cell can be changed when required, for example from 2 mm to 5 mm to increase the sensitivity, or from 5 mm to 2 mm to decrease the sensitivity.

Change the flow cell as follows:

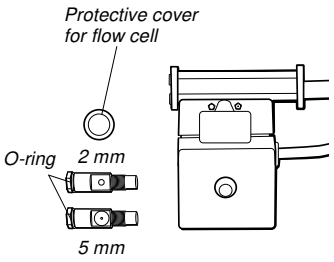
1 Disconnect the inlet and the outlet capillaries from the flow cell.

Note: *Avoid spillage.*

2 Loosen the flow cell by turning the locking nut and remove it.

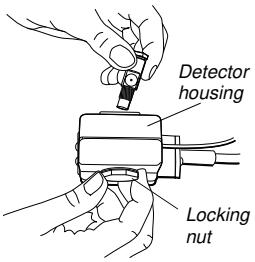
3 Remove the protective cover from the old flow cell and transfer it to the new flow cell.

4 Insert the new flow cell into the detector housing from above.



Note: *The flow cell can only be placed in one correct position.*

5 Secure the flow cell by turning the locking nut until it reaches its stop position.



Note: *If the locking nut is not tightened sufficiently, the monitor will function poorly (e.g. drifting base-line).*

6 Place the protective cover around the flow cell to protect the electronics inside the optical unit from liquid spillage.

Note: *Ensure that the Hg lamp position and the filter are selected according to the wavelength to be used. This is described in the Changing the lamp assembly (optional) section below.*

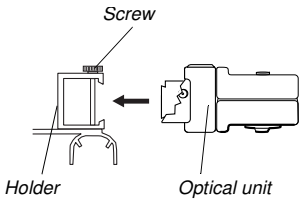
Connecting the optical unit to the system

If the optical unit has been disconnected from the system, connect it as follows:

1 Place the optical unit in the holder.

2 Secure it by tightening the screw in the holder.

3 Connect the lamp cable to the socket **UV Lamp** on the rear panel of the module.

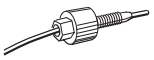


4 Connect the signal cable to the socket **UV** on the rear panel of the module.

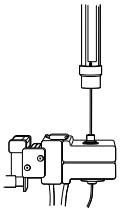
Connection to the column

1 Fix the optical unit directly under the column if possible.

Note: *Always position the optical unit with the filter wheel cover facing upwards.*



2 Connect the column outlet tubing directly onto the top of the optical unit using a fingertight connector and screw to finger-tightness.



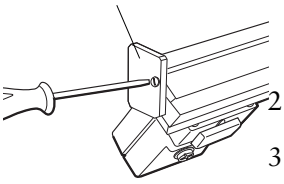
3 Connect the optical unit outlet tubing onto the opposite hole in the flow cell. Use fingertight connectors.

If no outlet tubing exists, cut a piece of PEEK tubing (i.d. 0.75 mm, o.d. 1/16”). The length should be 170 mm.

4 Connect the other end of the tubing to the conductivity flow cell.

Changing the lamp assembly (optional)

WARNING! The system uses high intensity ultra-violet light. Do not remove the UV lamp while the system is running. Before replacing a UV lamp, ensure that the lamp cable is disconnected from the rear of the system to prevent injury to the eyes. If the mercury lamp is broken, make sure that all mercury is removed and disposed according to national and local environmental regulations.

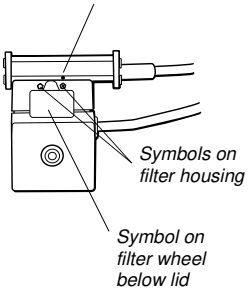


1 Use a screwdriver to detach the end plate by removing one and loosening the other of the two holding screws on the lamp housing to be removed.

2 Slide the lamp housing off the filter housing.

3 Detach the end plate, as in step 1 above, from the lamp housing to be fitted to the optical unit.

4 Slide the lamp housing onto the filter housing. The lamp and signal cables should be on the same side. As you slide the lamp housing into position, depress the two pressure pads on the filter housing in sequence to facilitate the installation.



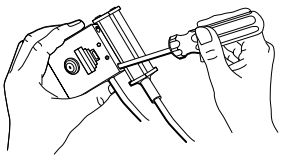
5 Refit the lamp housing end plate.

6 Slide the lamp housing firmly into place. There will be a faint click when the housing is positioned correctly. The Hg lamp housing can take up two positions, one for 280 nm, marked by ◻ on the filter housing, and the other marked by for ◙ all other wavelengths. The Zn lamp housing has only one position.

7 Set the wavelength to be used by selecting lamp position (indicated by a dot on the lamp housing) in combination with the appropriate filter, i.e. the dot on the lamp housing should be adjacent to the symbol on the filter housing corresponding to the symbol on the filter wheel for the filter to be used. A click will indicate that the filter is in position.

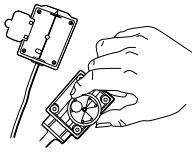
Changing the filter (optional)

The Hg optics with 254 and 280 nm filters and the Zn optics with the 214 nm filter are delivered with filters installed. If other filters are to be used, install them as follows:



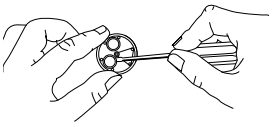
1 If the Zn lamp is attached, remove the lamp housing as described in section *Changing the lamp assembly (optional)*.

2 Remove the four screws in the filter housing. Separate the filter housing from the detector housing.

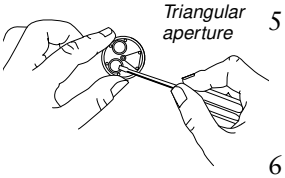


3 Carefully remove the filter wheel from the filter housing.

4 If necessary, remove the filter(s) from the filter wheel by pressing it (them) out, e.g. with a small screwdriver.



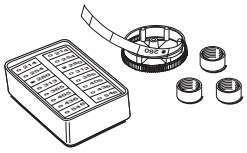
Note: *Filters are sensitive optical components. Never touch the optical surfaces or expose them to temperatures above 60 °C. Clean them with dry lens cleaning tissue and store them, when not in use, in the box in which they were supplied. Heavy contamination may be removed by using a lens tissue dipped in ethanol.*



5 Insert the filter(s) of choice into the filter wheel (maximum 3 filters) with the correct orientation (the mirror side facing upwards) and position over one of the three triangular apertures. The filters snap in by pressing them quite firmly. Do not touch the filter surface.

6 Remove the circular plastic band showing the wavelength(s).

7 Remove labels from the band if necessary.



8 Place the correct labels on the band with the label designation facing outwards. Ensure that the label position corresponds to the filter position, i.e. the label should be placed opposite the filter.

9 Reassemble the circular plastic band with the filter wheel peg fitting into the band notch.

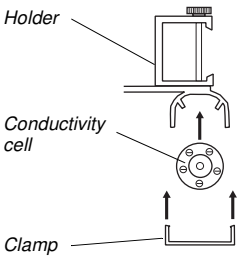
10 Check that all filters are clean. Insert the filter wheel back into the filter housing.

Note: *The filter wheel can be placed only in one correct position.*

11 Reassemble the filter housing with the detector housing by fastening the four screws.

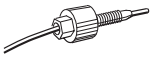
6.3 Installing the conductivity cell

1 Place the conductivity cell in its holder, or in a suitable location, as close to the optical unit/column as possible. The cell can be placed up to 1.5 m from the system housing. Secure the cell with the clamp.



Note: *When the conductivity flow cell is used in conjunction with the pH electrode, place the conductivity flow cell and select its flow direction so that the screw head end of the flow cell faces the flow restrictor.*

2 Connect the conductivity cell to the socket **Conductivity Flow Cell** on the rear panel of the system.



3 Connect the tubing with fingertight connectors.

6.4 Installing the fraction collector

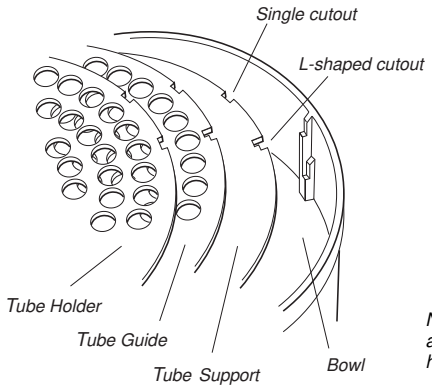
Assembling the tube rack

There are three types of tube racks:

Rack	*Max. no. tubes*	*Tube diam.*	*Tube length*
12 mm	175	12 mm	50-180 mm
18 mm	95	10-18 mm	50-180 mm
30 mm	40	30 mm	30-180 mm

The 12 and 30 mm racks are available as accessories. Also available for use with the 12 mm tube rack is a double-ended Eppendorf tube holder (18-8522-01). One end holds 1.5 ml Eppendorf tubes, the other holds 0.5 ml tubes.

ÄKTA*prime* is delivered with the 18 mm rack mounted. Each rack is made up of a combination of a bowl, tube supports, tube guide and tube holder.



Note that the tube guide has both single and L-shaped cutouts, while the tube holder has only single cutouts.

When assembling a rack, different cutouts are used for the various items depending on the length of the tubes. The cutouts are summarized in the tables below.

Tube racks, 12 and 18 mm

Item	*50-85 mm tubes*	*85-100 mm tubes*
Tube support	L-shaped cutout	Not required
Tube guide	Single cutout	L-shaped cutout
Tube holder	Single cutout	Single cutout

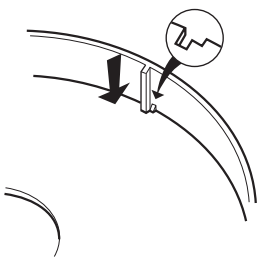
Tube racks, 30 mm

Item	*30-50 mm tubes**	*50-85 mm tubes*	*85-100 mm tubes*
Tube support	Single cutout	L-shaped cutout	Not required
Tube guide	Single cutout	Single cutout	L-shaped cutout
Tube holder	Single cutout	Single cutout	Single cutout

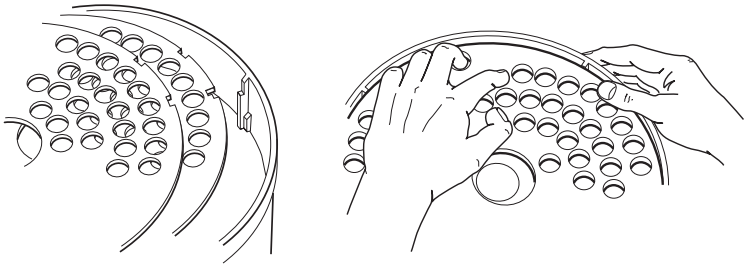
**For 30-50 mm tubes, insert the tube guide from the 18 mm rack using the single cutout before inserting the tube support for the 30 mm rack.*

1 Insert the tube support, if required, in the bowl. The circular marks on the support should face down.

2 Insert the tube guide with the tube numbers facing upwards. The guide should rest about 1 cm above the support.



3 Insert the tube holder, checking that position 1 is directly above position 1 in the tube guide. Push the flexible bowl out at each rib and snap the holder under the top of the rib.

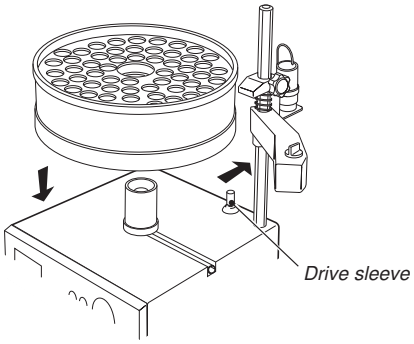


Do not force the holder into place as this may damage the lip. The surface of the holder should be level.

Mounting the tube rack

1 Gently move the delivery arm out to the second stop.

2 Place the rack over the central spindle and pull the spring loaded drive sleeve out so that the rack comes to rest.

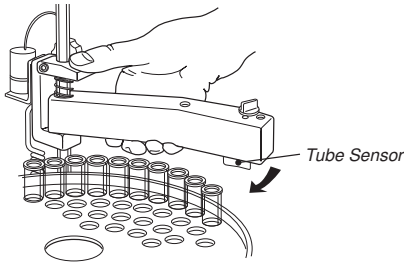


Inserting the collection tubes

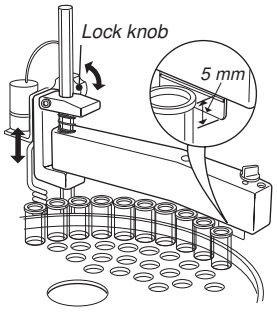
Insert the sufficient collection tubes into the rack, starting in position 1, pushing each one down as far as it will go. All the tubes must be of the same length and diameter, and there should be no empty spaces in the sequence.

Adjusting the delivery arm

1 Lower the arm and allow it to move so that the tube sensor touches the collection tubes of the outer track.

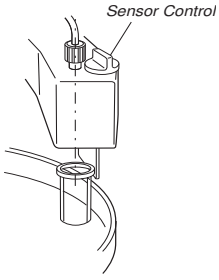


2 Adjust the arm bracket so that the bottom of the tube sensor is about 5 mm below the top of the tubes. The tubes should always be below the horizontal mark on the tube sensor



3 Lock the arm bracket at this height with the lock knob.

4 Rotate the rack counter clockwise by hand, until the rear half of the tube sensor rests against tube 1. When the fraction collector is started, the bowl moves to the correct position to collect the first fraction in tube 1.



5 Check that the sensor is in the correct position for the tube size. The eluent tubing should be over the centre of the collection tube. Use the red sensor control to position the tube holder.

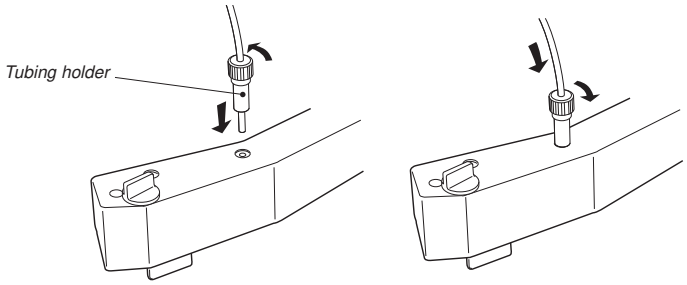
Connecting tubing

Select the tubing with the required inner diameter 0.75 mm. To change the tubing, follow steps 1-5.

1 Fit a 41 cm long tubing by lifting out the tubing holder from the delivery arm, loosening the nut and then inserting the tubing.

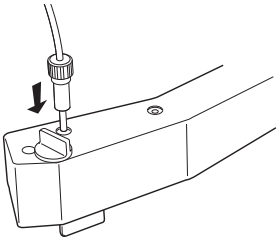
WARNING! When using hazardous chemicals, avoid spillage during fraction collection and when the delivery arm is moved out.

Note: *The tubing must be long enough to ensure free movement of the delivery arm.*



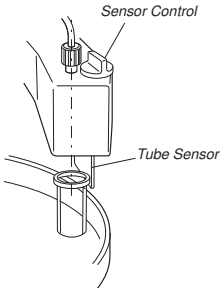
2 Place the tubing holder over the length guide (small hole) in the delivery arm, push the tubing down to the bottom of the guide and tighten the nut. This ensures that the correct length of tubing is exposed.

3 Re-install the tubing holder into the delivery arm.



4 Set the red sensor control to position the tubing over the centre of the collection tube.

5 Connect the other end of the tubing to port NC of the flow diversion valve.

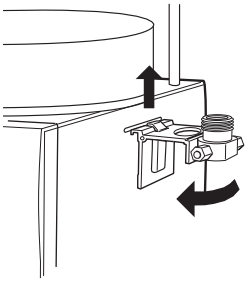


Flow diversion valve

The flow diversion valve mounted on the fraction collector is used as follows:

- Port IN
  Connected to the flow restrictor.
- Port NO
  Connected to waste.
- Port NC
  Connected to the tubing holder fitted on the delivery arm on the fraction collector.

6.5 Installing the pH flow cell and electrode (optional)

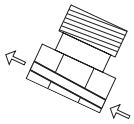


Mounting the flow cell holder

In the ÄKTA*prime* system, the pH electrode is optional.

1 Hook the flow cell holder on the right hand side of the housing. Secure it with the slide clamp.

If the flow cell holder is not used, the flow cell must still be installed at an angle of 30° from the vertical with the outlet placed higher than the inlet to prevent air bubbles being trapped in the cell.



The flow direction is marked on the flow cell.

2 Connect the tubing with finger-tight connectors.

Inserting the pH electrode

Note: *Handle the pH electrode with care.*

CAUTION! The tip of the pH electrode consists of a thin glass membrane. Protect it from breakage, contamination and drying out or the electrode will be destroyed. Always store the electrode with the end cover filled with a 1:1 mixture of pH 4 buffer and 2 M KNO_3. **Do NOT store in water only.**

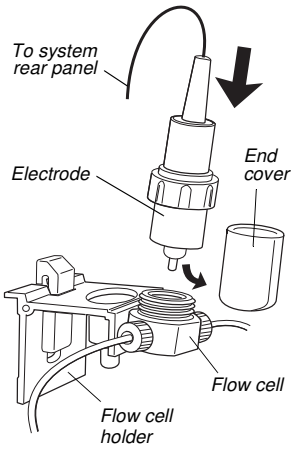
1 Unpack the pH electrode. Ensure that it is not broken or dry.

2 Before using the electrode, remove the electrode end cover and immerse the glass bulb in buffer for 30 minutes.

3 Carefully insert the electrode in the flow cell. Tighten the nut by hand to secure the electrode.

Note: *If the flow cell is full of liquid, it is not possible to insert the electrode. If so, loosen the inlet connection while inserting the electrode to allow the liquid to run out from the flow cell. Remember to re-tighten the connector.*

Note: *If the electrode is not fully inserted, the system will leak and a dead volume will occur in the holder.*



4 Connect the pH electrode cable to the socket **pH-Probe** on the rear of the system.

7 Maintenance

7.1 Periodic maintenance

Regular maintenance will help to keep your ÄKTA*prime* running smoothly. Follow the recommendations in this chapter to keep the system in good working order.

Do not allow spilt liquid to dry on the instrument. Wipe the surface regularly with a damp cloth. Let the system dry completely before using it.

WARNING! Always disconnect the power supply before attempting to replace any item on the system during maintenance.

WARNING! If there is a risk that large volumes of spilt liquid have penetrated the casing of the instrument and come into contact with the electrical components, immediately switch off the system and contact an authorised service technician.

WARNING! When using hazardous chemicals, ensure that the entire system has been flushed thoroughly with distilled water before maintenance.

WARNING! NaOH is injurious to health. Avoid spillage.

WARNING! Only spare parts that are approved or supplied by Amersham Biosciences may be used for maintaining or servicing the system.

WARNING! Use ONLY tubings supplied by Amersham Biosciences to ensure that the pressure specifications of the tubings are fulfilled.

WARNING! If the system is turned, the external capillaries and other tubing may become entangled in nearby objects and be pulled from their connections causing leakage.

Interval	*Action*
Every day	
System	• Inspect the complete system for eluent leakage. • The system can be left filled with buffer overnight. If you are not using the separation unit for a few days, wash the flow path with degassed distilled water. Remove the column and the pH electrode (optional). Replace the column by a bypass capillary and remove the pH electrode (if applicable). Then wash the system with 20% ethanol and store it in 20% ethanol. Make sure that all tubing and all flow paths used are rinsed.
System pump	• Check for leakage. If there are signs of liquid leaking from the pump, check the tubing connections. • If there are signs of erratic or pressure pulsation, wash the system with 96% ethanol and then with distilled water.
pH electrode (optional)	• Calibrate the pH electrode according to the section *Calibrating the pH electrode (optional)*.
Every week	
Inlet filters	• Check the inlet filters visually and replace them if necessary.
Every month	
Monitor	• Check the monitor according to section 7.4.

Interval	*Action*
Flow restrictor	• Check that the flow restrictor generates the following back-pressure: 0.2 ± 0.05 MPa. Check the back-pressure as follows: 1 Disconnect the flow restrictor 2 Connect a capillary to port 1 of the injection valve. 3 Run the pump manually at 10 ml/min with water. Note the back-pressure on the running display. 4 Connect the flow restrictor to the open end of the capillary. 5 Run the pump at 10 ml/min with water. Note the back-pressure on the running display. 6 Calculate the back-pressure generated by the flow restrictor. Replace it if it is not within limit.
Every 6 months or more often if required	
Monitor	• Clean the UV flow cell according to section 7.9 or 7.10. • Change the pH electrode. Refer to section 7.14.
Fraction collector	• Check the drive sleeve on the tube rack. Replace if worn. • Check the number of tube shifts according to section 7.6.
Superloop	• Check that the top, bottom and moveable seal O-rings are in good condition. Replace if necessary. Also check that the bottom end pieces are clean and undamaged.
Mixer	• Check that the mixer chamber is clean and without damage. Check the tubing connectors. Replace if required. Refer to section 7.16.
Yearly	
Injection valve and buffer valve	• Check for external or internal leakage. Replace channel plate and distribution plate when required. Refer to section 7.8.

Interval	*Action*
Every 2 years	
Mixer	• Replace the complete mixing chamber. Refer to section 7.16.
Superloop	• Replace O-rings. Refer to section 4 in the Superloop Instruction.
When required	
Monitor	• Clean the conductivity flow cell according to section 7.11. • Clean the pH electrode flow cell according to section 7.13.

7.2 Cleaning the system

To bypass the column, use a piece of 0.75 mm i.d. PEEK tubing supplied with the system. If the column is to be left in the flow path, make sure that the maximum allowed flow and pressure are not exceeded.

For column cleaning procedures and storage instructions, please refer to the respective instructions supplied with the column.

At the end of the day

The system can be left filled with a buffer overnight.

If the system will be used with other buffers next day, rinse the system with degassed distilled water using **System Wash Method** as follows:

1 Submerge the inlet tubings in distilled water.

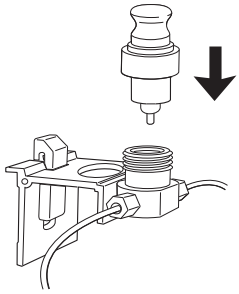
2 In the **Templates** menu, select **Application Template** and then **System Wash Method**.

3 Select used valve ports and press **OK** to start the method.

Leaving the system for a few days

CAUTION! Never leave the pH electrode in the electrode holder for any period of time when the system is not used, since this may cause the glass membrane of the electrode to dry out. Remove the pH electrode from the flow cell and fit the end cover filled with a 1:1 mixture of pH 4 buffer and 2 M KNO_3.
Do NOT store in water only.

Run **System Wash Method** with distilled water. Repeat with 20% ethanol (not the pH electrode, see separate instructions below).



pH electrode (optional)

The pH electrode should always be stored in a 1:1 mixture of pH 4 buffer and 2 M KNO_3 when not in use. When the pH electrode is removed from the flow cell, a dummy electrode can be inserted in the flow path.

Monthly cleaning

WARNING! NaOH is injurious to health. Avoid spillage.

Clean the system every month or when problems such as ghost peaks occur. The system is cleaned as follows:

1 Disconnect the column and replace it with a suitable capillary.

2 Place all the inlet tubings in 1 M NaOH.

3 Run the **System Wash Method** method for all inlet tubings.

4 Flush the whole system with 1 M NaOH for 20 minutes (1 ml/min).

5 Immediately repeat steps 3 and 4 with distilled water to rinse the system of NaOH.

Other cleaning considerations

After repeated separation cycles, contaminating material may progressively build up in the system and on the columns. This material may not be removed by the cleaning step described above. The nature and degree of contamination depends on the sample and the chromatographic conditions employed.

7.3 Moving the system

CAUTION! Never lift the system by the components mounted on the system chassis.

WARNING! If the system is turned, the external capillaries and other tubing may become entangled in nearby objects and be pulled from their connections causing leakage.

Before moving the system, ensure that all cables and capillaries connected to peripheral equipment and liquid containers are disconnected.

Lift the system by placing your fingers in the gap between the base and the work bench surface, grasping firmly and lifting.

7.4 Checking the UV monitor

Checking lamp intensity

Check Lamp Intensity
R 215.5 S 214.7mV

1 Select menu **Check** and press **OK**.

2 Select menu **Check Lamp Intensity**.

If:
R<300mV for 254 nm,
R<150mV for 280 nm, or
R<150mV for 214 nm,
replace the lamp according to section *7.15 Changing the UV lamp*, or contact Amersham Biosciences for lamp replacement.

Checking lamp run time

Check Lamp Run Time
Hg 2300h Zn 340h

1 Select menu **Check** and press **OK**.

2 Select menu **Check Lamp Run Time**.

- The lifetime of a Hg lamp at 254 nm is typically 7000 hours in room temperature (in coldroom, typically 2000 h).
- The lifetime of a Hg lamp at 280 nm is typically 3500 hours in room temperature.
- The lifetime of a Zn lamp is typically 2000 hours in room temperature.

When necessary, replace the lamp according to section *7.15 Changing the UV lamp*, or contact Amersham Biosciences for lamp replacement.

Checking autozero

The internal absorbance value for autozero can be checked to test the consistency of buffers.

1 Select menu **Check** and press **OK**.

Check Autozero
AZ 0.0001 AU

2 Select menu **Check Autozero**. The autozero absorbance value for the wavelength used is shown.

7.5 Checking the pump

Checking pump run time

1 Select menu **Check** and press **OK**.

Check Pump Run Time
00014h

2 Select menu **Check Pump Run Time**.

The lifetime of the pumping parts is typically 1000 hours in room temperature.

When necessary, contact Amersham Biosciences for replacement.

Checking pumped volume

1 Select menu **Check** and press **OK**.

Check Pumped volume
194529452ml

2 Select menu **Check Pumped Volume**.

When required, contact Amersham Biosciences for replacement.

7.6 Checking the fraction collector

Checking tube shifts

Check Tube Shifts
17564

1 Select menu **Check** and press **OK**.

2 Select menu **Check Tube Shifts**.

When required, contact Amersham Biosciences for replacement.

7.7 Checking the rotary valves

Check Valve Shifts
BV:17564 IV:28143

1 Select menu **Check** and press **OK**.

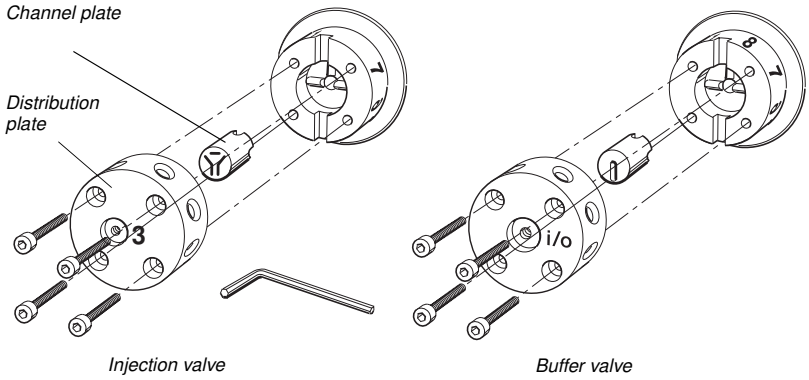
2 Select menu **Check Valve Shifts**.

The value after **BV** shows the number of buffer valve shifts. The value after **IV** the number of injection valve shifts. One shift means the shifting between two adjacent positions. The lifetime of the valves is >50 000 shifts.

When necessary, replace the sealings according to section *7.8 Changing the channel plate and distribution plate on rotary valves*, or contact Amersham Biosciences for sealing replacement.

7.8 Changing the channel plate and distribution plate on rotary valves

A replacement kit for each valve is available. Refer to *Reference information*.



1 Ensure that the valve is in position 1 (see figure) and then disconnect all tubings.

2 Remove the four screws on the front using a 3 mm Allen key. Loosen each one equally in turn so that the distribution plate comes off parallel to the valve body.

3 Slide the screws out.

4 Remove the distribution plate containing the ports.

5 Remove the old channel plate and insert the new one.

6 Remount a new distribution plate so that the text **3** (injection valve) or **i/o** (buffer valve) is horizontal and to the right of the central tubing connection. Using the Allen key, tighten the four screws in turn, a little at a time, until the distribution plate is fixed to the valve body.

7.9 Cleaning the UV flow cell in-place

WARNING! NaOH is injurious to health. Avoid spillage.

Pump a cleaning or sanitizing agent through the flow cell. The standard recommendation is to pump 1 M NaOH for 30 minutes and then wash out immediately with buffer.

7.10 Cleaning the UV flow cell off-line

A clean flow cell is essential for ensuring the correct operation of the UV monitor.

CAUTION! Do not allow solutions that contain dissolved salts, proteins or other solid solutes to dry out in the flow cell. Do not allow particles to enter the flow cell as damage to the flow cell may occur.

1 Connect a syringe to the inlet of the flow cell and squirt distilled water through the cell in small amounts. Then fill the syringe with a 10% surface active detergent solution like Decon 90, Deconex 11, RBS 25 or equivalent, and continue to squirt five more times.

2 Leave the detergent solution in the flow cell for at least 20 minutes.

3 Pump the remaining detergent solution through the flow cell.

4 Rinse the syringe and then flush the flow cell with distilled water (10 ml).

7.11 Cleaning the conductivity flow cell off-line

WARNING! NaOH is injurious to health. Avoid spillage.

If the conductivity measurements are not comparable to previous results, the electrodes in the flow cell may be contaminated and require cleaning. To clean the flow cell:

1 Pump 15 ml of 1 M NaOH at 1 ml/min through the flow cell either by using the system pump or a syringe.

2 Leave for 15 minutes.

3 Rinse thoroughly with degassed distilled water.

Note: *If the flow cell is totally blocked, the blockage can be removed using a needle or a wire with a diameter less than 0.8 mm.*

7.12 Changing the conductivity cell

The conductivity flow cell can be changed when required. Make sure the system is switched off before connecting/disconnecting the cell from the rear of the system.

If the cell is replaced with a new flow cell, the system must be calibrated with the new cell constant written on the flow cell package. See section *B.2 Calibrating conductivity*. If the cell constant is not known, it can be determined (see also section *B.2 Calibrating conductivity*).

7.13 Cleaning the pH electrode (optional)

Note: *The pH electrode has a limited lifetime and should be replaced every six months, or when the response time is slow or the slope is out of range (<80%).*

WARNING! NaOH is injurious to health. Avoid spillage.

Use one of the following procedures to clean the electrode to improve the response:

- **Salt deposits**: Dissolve the deposit by immersing the electrode, first in 0.1 M HCl, then in 0.1 M NaOH, and again in 0.1 M HCl. Each immersion is for a period of 5 minutes. Rinse the electrode tip in distilled water.
- **Oil or grease films**: Wash the electrode tip in liquid detergent and water. If the film is known to be soluble in a particular organic solvent, wash with this solvent. Rinse the electrode tip in distilled water.
- **Protein deposits**: Dissolve the deposit by immersing the electrode in a 1% pepsin solution in 0.1 M HCl for five minutes, followed by thorough rinsing with distilled water.

It these procedures fail to rejuvenate the electrode, the problem is most likely a clogged liquid junction. Use the following procedure:

1 Heat a 1 M KNO_3 solution to 60-80 °C.

2 Place the electrode tip in the heated KNO_3 solution.

3 Allow the electrode to cool while immersed in the KNO_3 solution before re-testing.

If these steps fail to improve the electrode response, replace the electrode.

7.14 Changing the pH electrode (optional)

See section *6.5 Installing the pH flow cell and electrode (optional)*.

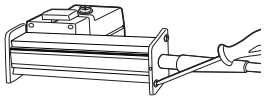
7.15 Changing the UV lamp

- The lifetime of a Hg lamp at 254 nm is typically 7000 hours in room temperature (in coldroom, typically 2000h).
- The lifetime of a Hg lamp at 280 nm is typically 3500 hours in room temperature.

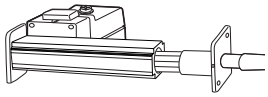
Note: The lifetime of a Zn lamp is typically 2000 hours in room temperature.

WARNING! The system uses high intensity ultra-violet light. Do not remove the UV lamp while the system is running. Before changing a UV lamp, ensure that the lamp cable is disconnected from the system to prevent injury to eyes. If the mercury lamp is broken, make sure that all mercury is removed and disposed of according to national and local environmental regulations.

Unscrewing the lamp



Removing/inserting the lamp



1 Remove the two screws on the lamp housing end plate that is attached to the power plate.

2 Carefully slide the lamp out of the lamp housing.

3 Without touching the lamp glasses, insert the new lamp into the lamp housing and secure the end plate with the two screws.

4 Select menu **Set Parameters** and press **OK**.

5 Select menu **Setup and Calibration** and press **OK**.

6 Select menu **Setup UV** and press **OK**.

7 Select menu **Set Lamp Run Time** and press **OK**.

8 Set the **Lamp Run Time** counter to zero. Press **OK** to acknowledge.

9 Press **Esc** three times to return to the main menu level.

7.16 Replacing the mixer chamber

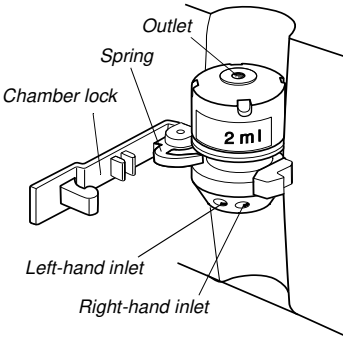
WARNING! When using hazardous chemicals, ensure that the entire system has been flushed thoroughly with distilled water before maintenance.

1 Make sure the pump is stopped.

2 Place the buffer bottles lower than the mixer to prevent draining, and then remove the inlet and outlet tubing.

3 Open the chamber lock holding the mixer chamber. A spring is securing the chamber in position when the lock is opened.

4 Pull out the mixer chamber gently.



5 Move the stop plug to the right-hand inlet of the new mixer chamber.

6 Insert the new mixer chamber and close the lock.

7 Replace the inlet and outlet tubing.

8 Trouble-shooting

8.1 Faults and actions

This section lists faults observed with specific monitor measurements and the specific components. The faults are listed as follows:

If the suggested actions do not correct the fault, call Amersham Biosciences.

ÄKTAprime
V 1.00

When contacting Amersham Biosciences for support, state the program version of the system, which is shown for a few seconds during start-up.

WARNING! The system should not be opened by the user. It contains high voltage circuits that can deliver a lethal electric shock.

8.2 System

Fault	*Action*
No text on the front display	1 Check that the mains cable is connected and that the power is switched on.

8.3 UV curve

Fault	*Action*
Noisy UV-signal, signal drift or instability	1 Select menu **Check Autozero** to check the autozero absorbance value. If the value is between 1.5 and 2, there may be air bubbles in the flow cell, or the wrong buffer system is in use. 2 Wrong filter for the lamp is being used. Check that the lamp is in the proper position and that the correct filter is used. 3 The buffer may be impure. Check if the signal is still noisy with water. 4 There may be air in the flow cell. Check that the flow restrictor generates a back-pressure of 0.2 ±0.05 MPa. Replace it if this is not within the limits. 5 If there is a lot of air in the water, degas the buffer before use. 6 Check the connections of the optical unit. 7 Clean the UV flow cell, see sections 7.9 and 7.10. 8 Locking nut in optical unit not properly tightened. Turn the locking nut to the stop position. 9 Air bubbles trapped in the pump. Refer to section 8.12.
Ghost peaks	1 Check that there is no air in the eluent. 2 Clean the system in accordance with section 7.2. 3 Clean the column in accordance with the column instructions. 4 Check that the mixer is functioning properly and that the correct chamber volume is being used. 5 Unless you are using a low pressure column, try using a flow restrictor FR-904 instead of FR-902. This generates a higher back-pressure (0.4 MPa instead of 0.2 MPa).
Low sensitivity	1 Aging lamp. Check the lamp and replace if necessary. 2 Wrong lamp position. Check that the lamp position and the wavelength used (filter position) fit together.
Error in external chart recorder	1 Check the recorder according to the manufacturer's instructions.

8.4 Conductivity curve

Fault	*Action*
Incorrect or unstable reading	1 Check that the conductivity flow cell cable is connected properly to the rear of the system. 2 Check that the system pump operates properly. 3 If temperature compensation is being used, check that the temperature sensor is calibrated, and that the correct compensation factor is used. 4 Check that the column is equilibrated. If necessary, clean the column. 5 Check the operation of the mixer.
Baseline drift or noisy signal	1 There may be air in the flow cell. Use a flow restrictor after the flow cell and check that the flow restrictor gives a back-pressure of 0.2 ±0.05 MPa. 2 Check for leaking tubing connections. 3 Check that the column is equilibrated. If necessary, clean the column. 4 Check the operation of the mixer and the system pump. 5 Clean the flow cell according to the procedures in sections 7.11.
Conductivity measurement with the same buffer appears to change over time	1 Clean the flow cell according to the procedures in sections 7.11. 2 The ambient temperature may have changed. Use a temperature compensation factor.
Waves on the gradient	1 Check that the system pump and the valves are operating properly and are programmed correctly. 2 Change to a larger mixing volume if necessary. 3 Check the operation of the mixer.
Absolute conductivity value is wrong	1 Turn the flow cell so the end with screws faces the pH flow cell. 2 Recalibrate the conductivity cell. 3 Calibration solution, 1.00 M NaCl, not correctly prepared. Prepare a new calibration solution and recalibrate the conductivity cell.
Ghost peaks appear in the gradient profile	1 A charged sample has been detected (e.g. protein). 2 Air bubbles are passing through the flow cell. Check for loose tubing connections. If necessary, use a flow restrictor after the conductivity cell.

Fault	*Action*
Non-linear gradients or slow response to %B changes	1 Check that the tubing has been washed properly and that the pump is operating. 2 Change to a smaller mixer volume.

8.5 pH curve (optional)

Fault	*Action*
Incorrect/unstable pH reading	1 Check that the electrode cable is connected properly to rear of the system. 2 Check that the system pump operates properly. 3 Check that the electrode is correctly inserted in the flow cell and, if necessary, hand-tighten the nut. 4 If air in the flow cell is suspected, tap the flow cell carefully or tilt it to remove the air. Alternatively, flush the flow cell with buffer at 20 ml/min for 30 s. Use a flow restrictor after the pH electrode. 5 Check that the pH electrode is not broken. 6 Check that the pH electrode is calibrated. 7 Check the slope. If it is outside the range 80–105% or if the asymmetry potential deviates more than ±60 mV from 0 mV, clean the pH electrode. Recalibrate. If the problem persists, replace the pH electrode. 8 Clean the pH electrode if required (see section 7.13). 9 Compare the response of the pH electrode with that of another pH electrode. If the response differs greatly, the electrode may require cleaning or replacement. 10 There may be interference from static fields. Connect the pH flow cell to the rear of the system using a standard laboratory 4 mm “banana plug” cable. 11 Check that the pH electrode has been calibrated at the correct temperature. 12 In organic solvents such as ethanol, methanol and acetonitrile, stable pH measurements are not possible since dehydration of the membrane will occur. We recommend that the pH electrode is not used in applications using organic solvents. 13 Clogged liquid junction. Refer to section 7.13.
No response to pH changes	1 Check that the electrode cable is connected properly to rear of the system. 2 The electrode membrane may be cracked. If so, replace the electrode.

Fault	*Action*
Small response to pH changes	1 Clean the pH electrode according to section 7.13 and recalibrate. 2 If the problem persists, replace the pH electrode.
Slow pH response or calibration impossible	1 Check the electrode glass membrane. If it is contaminated, clean the electrode according to the instructions in section 7.13. 2 If the membrane has dried out, the electrode may be restored by soaking it in buffer overnight. 3 Clogged liquid junction. Refer to section 7.13.
pH values vary with varied back-pressure	1 Replace the pH electrode.

8.6 Pressure curve

Fault	*Action*
Pressure limit exceeded, inaccurate reading	1 Calibrate the pressure monitor. 2 Check that inlet or outlet tubings are not clogged. Replace if necessary.
Erratic flow, noisy baseline signal, irregular pressure trace	
Possible causes are:	
Air bubbles passing through or trapped in the pump	1 Check that there is sufficient eluent in the reservoirs. 2 Check all connections for leakage. 3 Use degassed buffers only. 4 Remove any air bubbles according to section 8.12.
Blockage or partial blockage of the flow path	1 Flush the flow path to clear the blockage. 2 If necessary, replace the tubing. 3 Check the inlet tubing filter.

8.7 System pump

Fault	*Action*
Erratic flow	1 Calibrate the flow rate. 2 Check the inlet and outlet tubings. 3 Remove any air bubbles according to section 8.12.

8.8 Mixer

Fault	*Action*
Leakage	1 Check the tubing connections. Retighten or replace if necessary. 2 Check the mixer chamber. Replace if liquid has penetrated the mixer chamber walls and sealings.

8.9 Fraction collector

Fault	*Action*
No tube change	1 Press the **feed tube** key. If the motor does not start and an error appears, call Amersham Biosciences. 2 Push the delivery arm out to a safety stop. Press the **feed tube** key. If the motor starts, press the tube sensor together within 2 seconds . The motor should stop without an error code reported. If an errors appears, check the connection in the arm. If this is correct, the sensor or sensor connection are faulty. Call Amersham Biosciences.
Tubes skipped	1 The spring tension may be insufficient. Perform the actions described in section 8.13.
Drop synch. is not functioning	1 The drop sensor photocell located above the tube sensor is dirty. Clean the photocell with a damp cloth.

8.10 Buffer valve and injection valve

Fault	*Action*
The valve is switching to wrong position	The valve parts may have been incorrectly reassembled after replacement. 1 Check that the distribution plate marking **i/o** (buffer valve) or **3** (injection valve) is horizontal.

Fault	*Action*
External leakage	1 Check the tubing connections. Tighten or replace if required.
Internal leakage	Internal leakage can be detected at the small hole on the underside of the valve body. 1 Internal parts may be worn. Change channel plate and distribution plate according to section 7.8.
High back-pressure	1 Perform cleaning-in-place by flushing the system with detergent. 1 Change channel plate and distribution plate according to section 7.8.

8.11 Error messages

If the suggested actions do not correct the fault, call Amersham Biosciences.

Messages	*Action*
34 Start up failed Retry/Call service	1 Perform a new start-up. The preceding message may tell more about the cause 2 If not, call service.
35 WARNING wrong averaging time set	1 Wrong value for averaging time set. See section *Set Parameters menus* in *Reference information.*
50 Electrical error Call for service ↓ **57 Electrical error Call for service** **75 Electrical error Call for service**	1 Call for service.
60 Tube switch always active	1 Check that the fraction collector is not stuck. 2 Check the tube indicator. 3 If the problem remains, call service.
61 No more tube is available	1 Put more tubes in the fraction collector.
62 Check that the tube position is OK	1 Check that a tube in the fraction collector touches the tube indicator. 2 Check the cable to the tube indicator. 3 If the problem remains, call service.

Messages	*Action*
64 ERROR No drops check sensor	1 Check the cable to the tube indicator. 2 Check that it is dripping. If it flows continuously, reduce the flow or turn off the Drop sync function. 3 If the problem remains, call service.
65 ERROR Pump failure	1 Restart the system. 2 If the problem remains, call service.
66 Too short time between feeds	1 Reduce the flow or increase the fraction size.
67 ERROR Injection valve failure	1 Call service.
68 ERROR Buffer valve failure	1 Call service.
69 ERROR Stop grad. set HOLD or PAUSE	1 Set the system in HOLD or PAUSE, or stop the gradient (%B).
70 Lamp disconnected If not, call service	1 Connect the lamp or call service.
71 WARNING low light intensity	1 Check the cables to the optical unit. 2 Check that lamp and filter position correspond. 3 Change lamp. If the problem remains, call service.
72 Change lamp or call service **76 Change lamp or call service**	1 If used in cold room, additional warm-up might be needed. 2 If the problem remains, change the lamp. 3 If the problem remains, call service.
73 WARNING Too much straylight leaks in	1 Check that the filter wheel cover is closed. 2 Check that non-transparent tubings are used at the UV flow cell inlet and outlet. 3 Check that the optical unit is not exposed to direct sunlight. 4 If the problem remains, call service.
77 WARNING Autozero out of range	1 Autozero is not allowed on a level above 2 AU. 2 Check buffers. 3 Clean UV flow cell.
78 ERROR Over pressure	1 Re-program the method.
79 ERROR Method corrupt in eeprom	1 Check which method is corrupted (erased). 2 If the problem remains, call service.

Messages	*Action*
80 ERROR Reading from eeprom	1 Call service
81 ERROR Writing to eeprom	1 Call service.
82 ERROR Parameter fail in method	1 Re-program the method.
83 WARNING temp_cal will be changed **84 WARNING cond_cal will be changed**	1 Press OK to accept change. 2 Press ESC to skip the change.
85 WARNING conscale (0-100%)<0.1mS	1 The difference between 0% and 100% must be at least 0.1 mS/cm. 2 Increase the span between zero and full scale setting. See section *Set Parameters menus* in *Reference information*.
86 WARNING cond_cell bad/not connected	1 Check that the conductivity cell is connected. 2 Recalibrate temperature. 3 If the problem remains, replace the conductivity cell.
87 WARNING pH -probe bad/not connected	1 Check the pH electrode connection. 2 Clean the pH electrode. 3 If the problem remains, change the pH electrode.
88 Electrical error Call for service	1 Factory calibration for pH electrode is lost. The monitor can still be used but may not meet the specifications for pH measurements. 2 Call service.
89 Electrical error Call for service	1 Factory calibration for conductivity is lost. The monitor can still be used but may not meet the specifications for conductivity measurements. 2 Call service.
90 ATTENTION set<=0mV first **91 WARNING bad pH ad value**	1 Only visible to service personnel.
92 WARNING electrode slope <70 or >110% **93 pH _cal failed check electrode**	1 Electrode slope is out of range. Check buffers and recalibrate. 2 Clean the pH electrode and recalibrate 3 If the message remains, call service.
94 WARNING <1pH unit between cal_buff 1&2	1 The difference between the pH of the buffers used during calibration must be at least 1 pH unit.

Messages	Action
95 Temp cal failed check cond cell	1 Check that the conductivity cell is connected. Recalibrate. 2 The measured temperature value differs from the reference value by more than ±5°C, or the actual temperature is lower than -8°C. Recalibrate.
97 WARNING pH scale (0-100%)<pH unit	1 The difference between the zero level and full scale must be at least 1 pH unit. Increase the span between zero and full scale settings. See section *Set Parameters menus* in *Reference information*.
98 Cal failed. Cell constant not 0.1-300	1 Conductivity cell constant is out of range. 2 Wrong solution used during calibration. Use 1.00 M NaCl and recalibrate. 3 Air in conductivity cell during calibration. Flush the flow cell with calibration solution and recalibrate. 4 Dirty conductivity cell. Clean the flow cell and recalibrate. 5 If the problem remains, change the conductivity cell.
99 ERROR Out of method memory	1 Maximum number of breakpoints in memory is 600. Delete a method to get more memory.
ERROR key	1 A key was pressed during self-test, or is faulty. 2 Switch off the system. 3 Switch on the system.
ERROR Number 102-104 **ERROR Number 109-113** **ERROR Number 119-121**	1 Switch off the system. 2 Check all connections. 3 Switch on the system.
Exc x/y in ab.c **Exc DIV/0 in ab.c** **Exc instr in ab.c** **Exc address in ab.c**	1 Switch off the system. 2 Check all connections. 3 Switch on the system.

8.12 Removing trapped air bubbles

Remove trapped air bubbles in the flow path by purging the pump with liquids. Use the liquids in the following order: 1. deionized water, 2. 20% ethanol, 3. deionized water and 4. buffer solution.

Note: *All liquids used must be degassed.*

Note: *When using degassed ethanol, make sure that the concentration does not fall below the required value.*

Purging can be done manually through inlet A1, while carefully immersing the tubing in the respective liquid. Set the injection valve to position WASTE. Run 30 ml of each liquid at 50 ml/min. Press the **pause/cont** button to start and stop the pump when changing liquid.

An automatic purging procedure that uses additional inlet tubings is described below:

1 Connect an inlet tubing to buffer valve port 4. Immerse the tubing in a vessel filled with deionized water.

2 Connect an inlet tubing to buffer valve port 3. Immerse the tubing in a vessel filled with 20% ethanol.

3 Connect an inlet tubing to buffer valve port 2. Immerse the tubing in a vessel filled with deionized water.

4 Check that the inlet tubing to buffer valve port 1 is immersed in a buffer solution.

5 Fill the empty inlet tubings manually.

6 In **Templates**, select the **System Wash Method** under **Application template**.

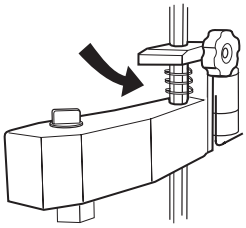
7 Select ports 2, 3 and 4 to be washed. Port 1 is pre-selected. Deselect port B.

8 Press **OK** to start the run.

The pump first draws the liquids in the following order: deionized water, ethanol, deionized water, and finally the buffer solution. Consequently, when the run is finished, the system is filled with the buffer solution connected to buffer valve port 1.

8.13 Adjusting the spring tension of the delivery arm

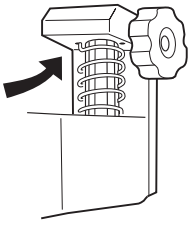
Incorrect spring tension can cause the fraction collector to skip tubes. The effect is greater as the arm moves towards the centre.



Spring tension is temperature sensitive. Low temperature reduces the spring tension so it may be necessary to re-adjust the tension if the collector is used in a cold room.

1 Remove the arm bracket from the stand.

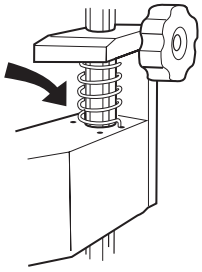
2 Dismantle the delivery arm from the bracket.



3 The top of the spring is fastened in one of two holes in the top of the arm bracket.

Looking at the arm bracket from the front of the unit, moving the spring from the right hand to left hand hole increases the tension, and conversely moving from the left hand to the right hand hole decreases the tension.

Hold the spring near the top and pull or prise it down and out of the top hole. Insert the spring in the other hole.



4 The bottom of the spring is fastened in one of four holes, equally spaced 1/4 turn apart. To adjust the bottom of the spring, hold it near the lower end and lift or prise the bottom of the spring out of the hole.

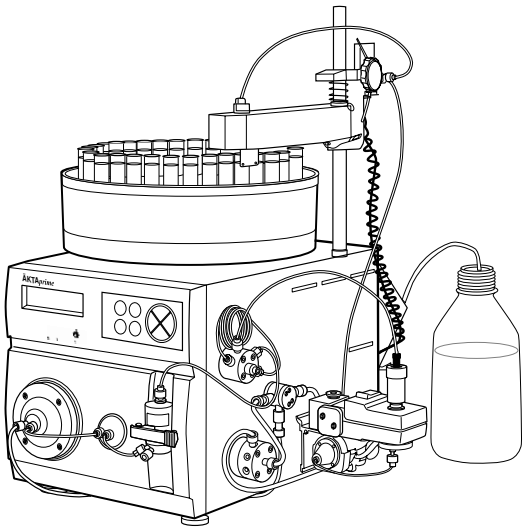
To increase the tension move the spring counter-clockwise.

To decrease the tension move the spring clockwise.

Reference information

A System description

A.1 The system



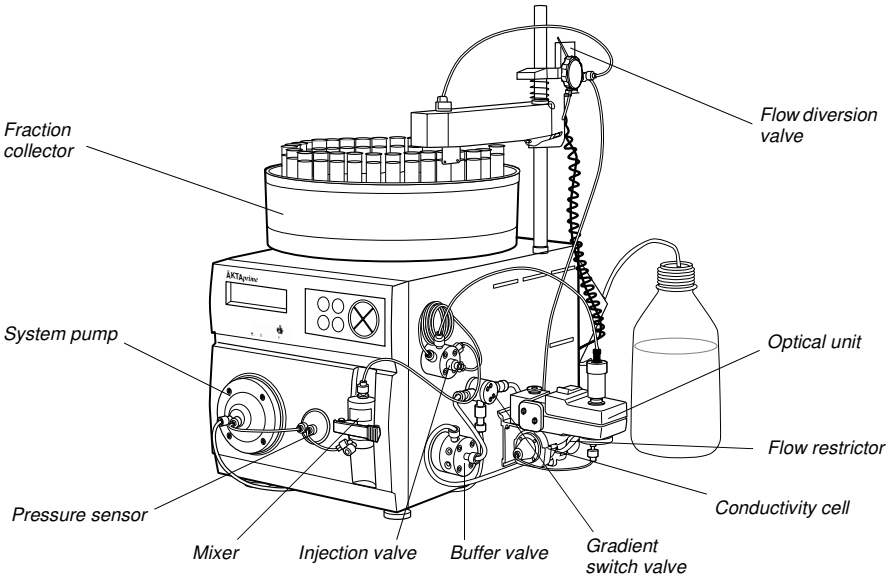
ÄKTA*prime* is a compact separation unit comprising components for fluid handling and for measuring UV-absorption, conductivity and pH (optional).

This section gives a brief description of the system and its components. It also describes optional components that may be connected to the system.

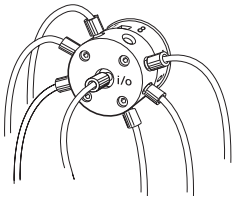
A built-in power control board supplies the components with power, and a controller handles the communication between the components via an internal high speed network.

A.2 Components

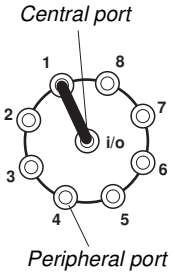
The following illustration shows the location of the components in the system.



A.2.1 Buffer valve



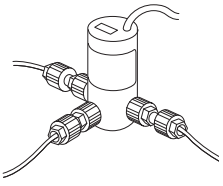
This is a rotary valve which has 8 positions. The valve has a 360° rotating channel plate. As the plate is turned by the motor, the central port on the front is connected to one of the peripheral ports 1–8, allowing a clear liquid path. The valve switching is controlled by the system by reading the actual position of the channel plate.



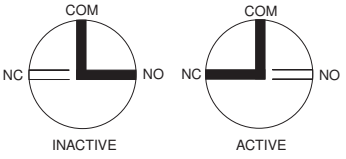
The buffer valve is used on the low pressure side in the flow path (before the pump). It it used for switching between sample and buffer solutions.

The switching parts are made of PEEK, which ensures long mechanical and chemical lifetime.

A.2.2 Switch valve

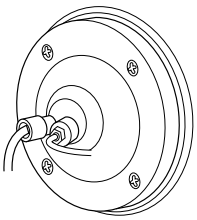


This is a 3-port/2-way valve, supplied with 24 V DC. It has one permanently open port marked COM and two ports marked NC (normally closed) and NO (normally open). Port COM may be used as an inlet or an outlet port.



There are two switch valves in the system; one is used for gradient formation and the other one for flow diversion during fractionation.

A.2.3 System pump

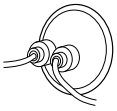


This is a pump with a single input and a single output. It contains three internal liquid chambers for delivering a smooth flow. The pump delivers up to 50 ml/min and the maximum pressure is 1.0 MPa.

Each of the three chambers has a flexible membrane actuated by a piston. When a membrane is retracted, liquid is drawn into the chamber, and when the membrane is pressed in, the liquid is forced out from the chamber. The stepper motor assembly that drives the pistons creates a pumping action on the membranes that is phase-shifted 120°. Consequently, the three chambers will draw and expel liquid in a sequential order, which results in a smooth and continuous liquid delivery.

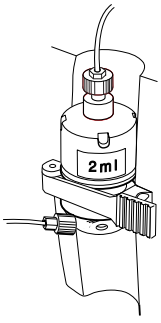
The wetted parts in the system pump are made of PEEK, Kalrez, Simriz, ceramic and polyethylene.

A.2.4 Pressure sensor



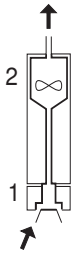
The pressure in the system is continuously measured by the pressure sensor located next to the system pump. The pressure is shown on the display during the run. For the protection of the column used, a maximum pressure limit can be set.

A.2.5 Mixer



This is a dynamic, single chamber mixer with interchangeable mixer chambers. The system is delivered with a 2 ml chamber.

The eluents are mixed in two steps:



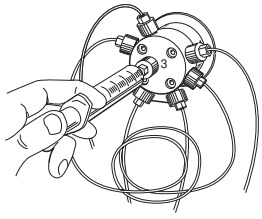
1 Premixing in a static mixer with a small volume (22 µl).

2 Dynamic mixing in a chamber with a rotating stirrer.

A mixer motor inside the system spins a magnet at 600 rpm, which causes the stirrer in the mixing chamber to rotate.

A.2.6 Injection valve

A seven port motorized rotary valve is used as sample injection valve. It has a valve body with a rotating central core, the channel plate. As the channel plate is rotated by the motor, different ports are connected.



The valve has three different operating positions which make it possible to:

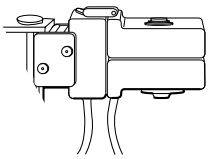
- Load a sample loop without disturbing column equilibration.
- Wash the sample loop while the column is in operation.
- Wash the pump for eluent exchange without disturbing the column.

The geometry of the valves ensures that the flow path is completely swept so that solvent or sample "memory effect" is virtually non-existing. The switching parts are made of PEEK, which ensures both long mechanical and chemical lifetime.

A.2.7 Monitor

This is a high precision on-line monitor for handling measurement data from the UV optical unit, the conductivity cell and the pH electrode (optional). In combination with the flow cells, the monitor offers fixed wavelengths of 214 nm (Zn-lamp, optional), 254 and 280 nm (Hg-lamp), fast response, high accuracy and reproducibility, and low dead volumes.

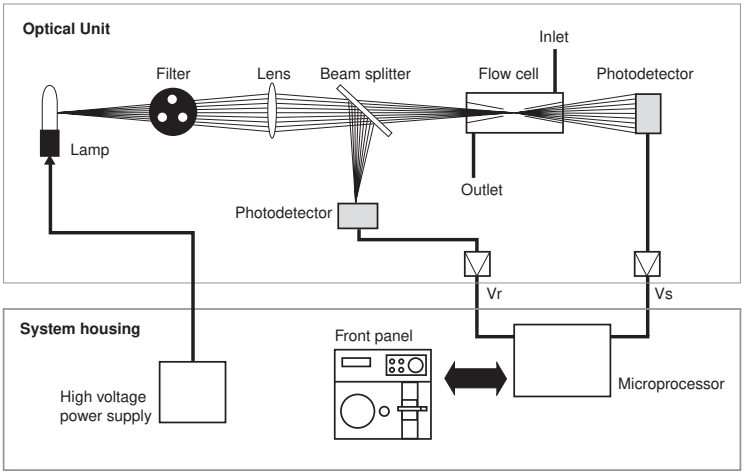
A.2.8 UV optical unit



The UV optical unit houses the lamp (Zn or Hg), the wavelength filter and the UV flow cell. There are two flow cells available; optical path length 2 mm or 5 mm (optional). The type of flow cell used depends on the sample amount applied and the size of the column.

UV flow cells with 2 and 5 mm path lengths

The light beam from the lamp is directed through a double conical or a straight flow-through cuvette (6 µl or 2 µl illuminated volume). The photodetector current is fed to the signal processing circuitry inside the system.



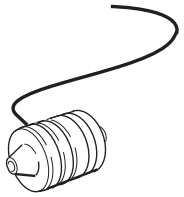
The reference signal comes from the same point in the lamp as the signal measuring the sample, thus assuring a stable baseline by eliminating the effects of variations in lamp intensity.

The Hg lamp emits light only at certain wavelengths. It does not emit light at 280 nm, so for this wavelength, the light is converted at a fluorescent surface before it passes the filter. On the lamp housing, there is a special exit for 280 nm light, which means that the lamp position needs to be changed when working with this wavelength.

For 214 nm wavelength, a Zn lamp is used. This lamp is larger than the Hg lamp and is therefore mounted in a larger lamp housing.

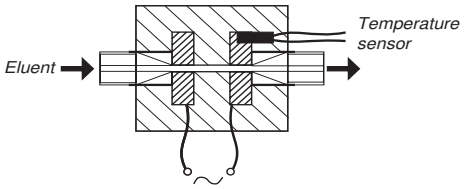
The lamp connectors are keyed to inform the monitor software which lamp type is connected.

A.2.9 Conductivity flow cell



The flow cell has two cylindrical titanium electrodes positioned in the flow path of the cell. An alternating voltage is applied between the electrodes and the resulting current is measured and used to calculate the conductivity of the eluent. The monitor controls the AC frequency and increases it with increasing conductivity between 50 Hz and 50 kHz giving maximum linearity and true conductivity values.

The conductivity is automatically calculated by multiplying the measured conductance by the flow cell's cell constant. The cell constant is pre-calibrated on delivery but can be measured with a separate calibration procedure. This procedure is described in *Reference information* section *B.2*.



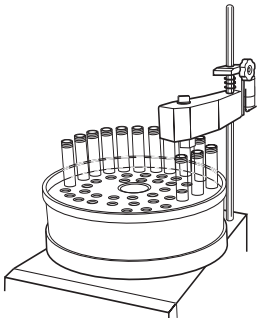
One of the electrodes has a small temperature sensor for measuring the temperature of the eluent in the flow cell. Temperature variations influence the conductivity and, in some applications when highly precise conductivity values are required, it is possible to program a temperature compensation factor that recalculates the conductivity to a set reference temperature.

A.2.10 Flow restrictor FR-902

The flow restrictor generates a steady back-pressure to prevent air bubbles being formed after the column in the flow cells. FR-902 is set at the factory to 0.2 MPa.

A.2.11 Fraction collector

The fraction collector can be used for both small scale and preparative scale purifications. It collects up to 175 fractions in 12 mm diameter tubes, up to 95 fractions in 10–18 mm diameter tubes, and up to 40 fractions in 30 mm diameter tubes.



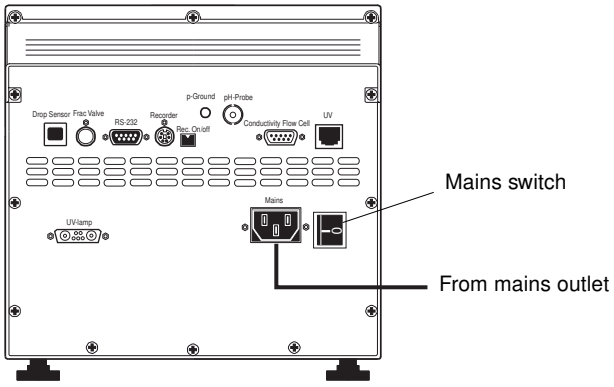
The fraction collector allows fixed volume fractionation or automatic peak fractionation. Fraction marks make it easy to identify fractions and peaks.

Fast tube change minimizes spills between tubes, eliminating it entirely below flow rates of 5 ml/min. Drop synchronization eliminates sample loss during tube change.

A.3 Electrical connections

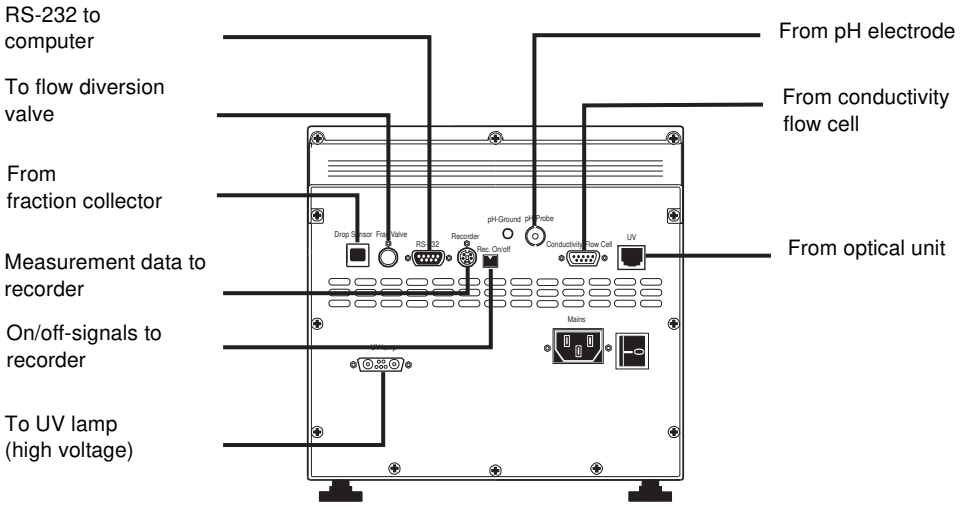
All electrical connections for ÄKTA*prime* are located at the rear of the system.

A.3.1 Mains cable



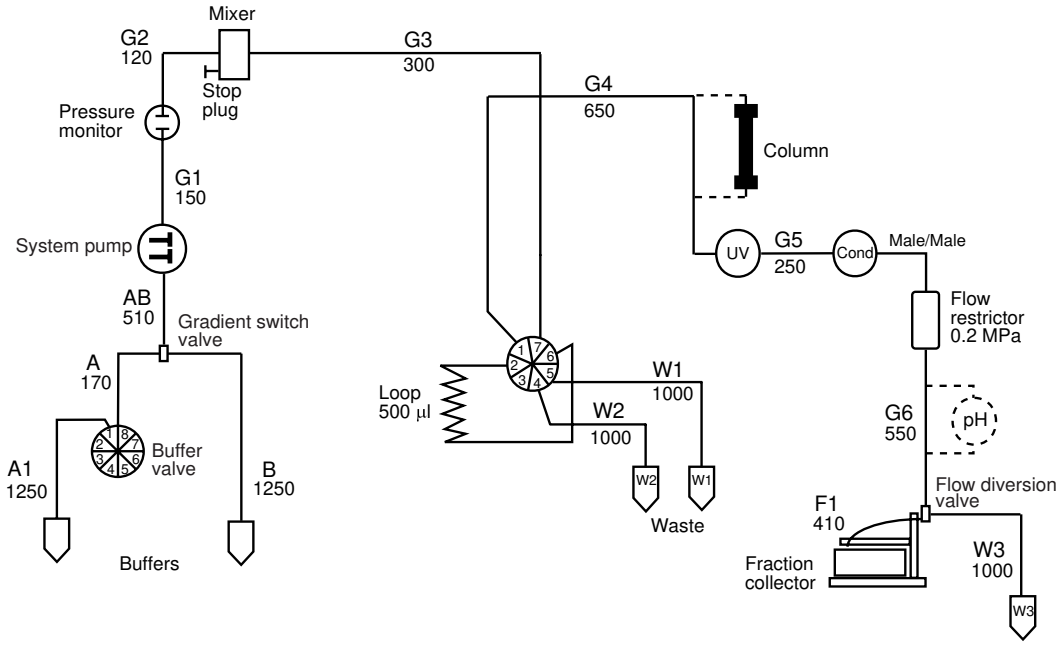
One mains input is required for the system.

A.3.2 Communication cables



A.4 Fluid handling path

The following flow diagram shows positions of the components in the ÄKTA*prime* system. Refer to the flow diagram for their locations in the fluid handling path.

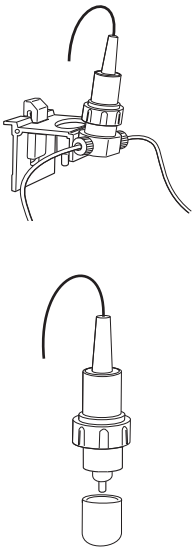


All capillaries are fitted at the factory. The figure states the length in millimetres of the pre-fabricated capillaries.

The table below shows the different tubings fitted at the factory on ÄKTA*prime*.

Tubing i.d.	*Tubing o.d.*	*Material*	*Colour*	*Max. pressure*	*Volume of 10 cm*	*Connected*
2.9 mm (A1, A, B)	3/16”	Teflon	Clear	3.4 MPa	660.5 µl	All inlet tubings from buffer vessels to gradient switch valve.
1.6 mm (AB)	1/8”	Teflon	Clear	2 MPa	201.1 µl	From gradient switch valve to system pump.
1.0 mm (W1–W3)	1/16”	PEEK	Brown	4.1 MPa	78.5 µl	Waste tubing.
0.75 mm (G1–G6, F1)	1/16”	PEEK	Green	2.6 MPa	44.2 µl	From system pump to fraction collector.
Union, m/m	1/16”	PEEK	Black (with orange tubing)	25 MPa	–	Between conductivity flow cell and flow restrictor.
Stop plug	1/16”	PEEK	Black	–	–	Mixer inlet.

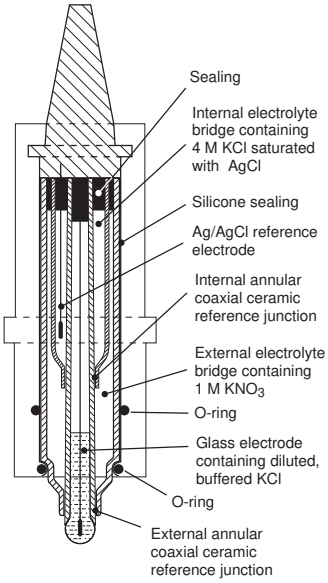
A.5 Optional components



A.5.1 pH flow cell with electrode

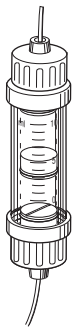
The pH electrode is of the sealed combination double junction type. It contains a sealed Ag/AgCl reference which cannot be refilled, an internal electrolyte bridge of 4 M KCl saturated with Ag/AgCl, an outer electrolyte bridge of 1 M KNO_3, an annular ceramic reference junction and a low profile pH membrane. The pH electrode is delivered in a transparent cover.

The flow cell is made of titanium. It should not be used with any other pH electrode.



A.5.2 Superloop

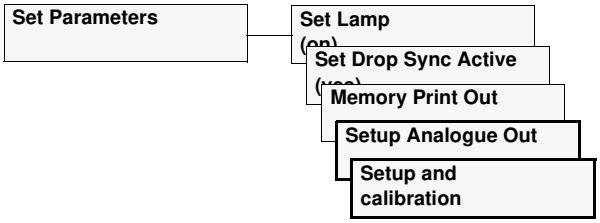
Superloop allows introduction of larger sample volumes into a pressurized fluid system. It is used together with the injection valve and replaces a simple sample loop.



Superloop consists of a movable seal in a glass tube. The seal divides the tube into two separate chambers. Depending on the flow direction, the seal moves towards either end piece of the glass tube. Superloop is available in three sizes (10, 50 and 150 ml) allowing application of 1–10, 1–50 and 1–150 ml samples respectively.

B Calibration and analogue output settings

All calibrations and analogue settings are made in the **Set Parameters** menu.



B.1 Calibrating the system pump

B.1.1 Calibrating the flow rate

Calibrate the pump whenever the running conditions are changed, e.g. viscosity of sample or buffer, temperature or back-pressure.

1 Make sure no bubbles are trapped in the flow path.

2 Immerse the inlet tubing A1 in a vessel filled with degassed buffer.

3 Place the waste tubing from port 5 on the injection valve in an empty vessel (the flow will automatically be diverted to port 5 during the calibration).

Start Pump Calibr. **800 pulses**

4 Select **Start Pump Calibration** in the **Setup and calibration** menu. Press **OK**.

Set Flow Rate **(10 ml/min) 2.0**

5 Enter the intended flow rate as calibration flow rate in the **Set Flow Rate** menu and press **OK**.

6 Measure the volume of the water collected in the vessel.

Enter Collected **Volume (ml) 2.05**

7 Enter the measured volume in ml in the **Enter Collected Volume** menu and press **OK**.

8 At the **Pump Calibrated OK** menu press **OK**.

B.1.2 Calibrating the pressure offset

The pump should be calibrated when required.

1 Make sure that the pressure sensor is exposed to atmospheric pressure only, i.e. no back-pressure.

Change Press Offset **(1005 mV)**

2 Select the **Change Press Offset** menu in the **Setup and calibration** menu. Press **OK**.

Set zero pressure **to calib. Press OK**

3 At the **Set zero pressure to calib**. menu press **OK**.
The calibration only takes a few seconds.

4 Press **OK** at the **Calibrating Offset Done!** menu.

B.2 Calibrating conductivity

The cell constant for the particular flow cell is written on the flow cell packaging. Refer to section *C.2.11 Setup adjust cell constant* for how to enter the cell constant.

Adjustment of the cell constant is only necessary when the monitor is to be used to determine conductivity with high accuracy. The procedure is described in section *C.2.10 Setup adjust conductivity*.

B.3 Calibrating the pH electrode (optional)

A good laboratory routine is to calibrate the pH measurement once a day, when the electrode is replaced or if the ambient temperature changes. The pH electrode is calibrated using standard buffer solutions in a two point calibration. The two buffer solutions can have any pH value as long as the difference between them is at least 1 pH unit. The calibration procedure can be done with the pH electrode either fitted in or removed from the flow cell.

B.3.1 Calibrating with the electrode outside the flow cell

When calibrating the electrode out of the flow cell and changing from one buffer to another, rinse the electrode tip with distilled water and dab it carefully with a soft tissue to absorb the remaining water. Do NOT wipe the electrode as this may charge it and give unstable readings.

The steps below describe the procedure used with the electrode removed from the flow cell.

1 Remove the pH electrode from the flow cell and immerse the electrode in the first standard buffer solution (normally pH 7.0).

Set Parameters

2 From the main menu, select menu **Set Parameters** by pressing the up or the down button. Press **OK**.

Setup and calibration

3 Select menu **Setup and calibration**. Press **OK**.

Setup pH

4 Select menu **Setup pH** and press **OK**.

Calibrate pH (7.00 - 12.00)

5 Select menu **Calibrate pH**. Current calibration values are displayed (buffer 1 - buffer 2).
Buffer 1 = fixed lower calibrated pH value. Range=0.00-14.00
Buffer 2 = fixed higher calibrated pH value. Range=0.00-14.00

Note: *The values for buffer 1 and 2 must differ by at least 1 pH unit.*

Calibrate pH Buffer 1

6 Press **OK** to access the settings menu. The order of calibration, buffer 1 or buffer 2, is optional. Press **OK** to start with buffer 1, or press the down button to start with buffer 2. In this example, we start with buffer 1.

Calibrate pH Buffer 1
(7.00) Please wait!

7 This text disappears when the reading is stable and the following text is then shown:

Calibrate pH Buffer 1
(7.00) 7.00

8 Adjust the pH value in the display with the up and down buttons so that it corresponds to the known pH value of the first buffer solution. Press **OK**.

Calibrate pH Buffer 2

9 At the buffer 2 calibrating menu, rinse the electrode tip with distilled water and then immerse the electrode in the second buffer solution (e.g. pH 4.0 or 9.0). Then press **OK**.

Calibrate pH Buffer 2
(9.00) Please wait!

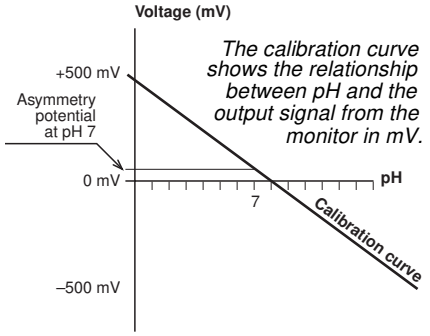
10 The text opposite disappears when the reading is stable and the text below is then shown.

Calibrate pH Buffer 2
(9.00) 9.00

11 Adjust the pH value in the display with the up and down buttons so that it corresponds to the known pH value of the second buffer solution. Press **OK**.

Calibrated Electrode
Slope 98.5% 9,5 mV

12 After the calibration with buffer 2, the system automatically enters the **Calibrated Electrode** menu. This menu shows the slope of the calibration curve, where 100% corresponds to 59.16 mV per pH step at 25°C. The asymmetry potential at pH 7 is shown as a mV value. Press **Esc** repeatedly to return to the **Set Parameters** menu.



13 Before use, rinse the electrode using distilled water.

A new electrode typically has a slope of 95–102% and an asymmetry potential within ±30 mV. As the electrode ages, the slope decreases and the asymmetry potential increases.

As a rule, when an electrode has an asymmetry potential outside ±60 mV and a slope lower than 80%, and no improvement can be made by cleaning, the electrode should be changed.

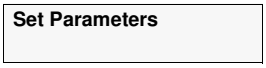
An electrode is still usable at lower slopes and higher asymmetry potentials but the response will be slower and the accuracy diminished.

B.3.2 Calibrating with the electrode in the flow cell

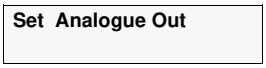
When calibrating with the electrode fitted in the flow cell, follow the above procedure but let at least 30–35 ml (with 2 ml mixer) of standard buffer solution be pumped through the system to stabilize pH. Leave the pump running while calibrating. Switch to the second standard buffer solution and repeat the procedure.

B.4 Setting analogue outputs

The system has three analogue output channels for connection to a chart recorder or a computer. This menu is used to select the measurement parameter that by default should be associated to each channel. The menu is also used for setting measurement parameters (zero and full range values) for **UV**, **Cond**, **pH** and **Press** on the analogue output channels.



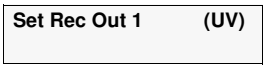
1 Select the **Set Parameters** menu in the main menu and press **OK**.



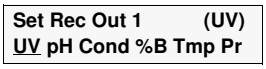
2 Select the **Setup Analogue Out** menu and press **OK** to enter the settings menu.

B.4.1 Setting parameters for the channels

UV, pH, conductivity, concentration of buffer B, temperature and pressure are measurement parameters that can be associated to the analogue output channels.



1 When entering the **Setup Analogue Out** menu, the setting for channel 1 is displayed first. Press the up and down buttons to display the settings for channels 2 and 3.

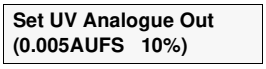


2 Select the desired channel and press **OK**. In this example, channel 1 is selected.

3 Select the desired parameter and press **OK**.

*Note: The analogue output level for **Tmp** has a fixed set value; 0 °C corresponds to 0 V and 50 °C corresponds to 1.0 V.*

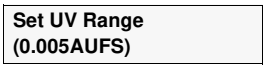
B.4.2 Setting the UV analogue output



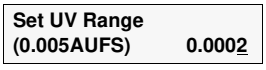
1 Select the **Set UV Analogue Out** menu in the **Setup Analogue Out** menu by using the up and down buttons. Current analogue settings are displayed (zero and full range values). Allowed full range values are 0.0001, 0.0002, 0.0005, 0.001, 0.002, 0.005, 0.01, 0.02, 0.05, 0.1, 0.2, 0.5, 1.0, 2.0 and 5.0. Zero level is set as a percentage of full scale.

2 Press **OK** to access the settings menu. The current setting is displayed. Press **OK**.

3 Set the desired zero level value. Press **OK** to acknowledge.



4 Press the down button to access the settings menu. The current setting is displayed. Press **OK**.



5 Set the desired full range value. Press **OK** to acknowledge.

B.4.3 Setting the Cond analogue output

Set Cond Analogue Out (00.00-50.00mS/cm)	1 Select the **Set Cond Analogue Out** menu in the **Setup Analogue Out** menu by using the up and down buttons. Current analogue settings are displayed (zero and full scale values).
Set Cond Zero Level (0.00mS/cm)	2 Press **OK** to access the settings menu. The current setting is displayed. Press **OK**.
Set Cond Zero Level (0.00mS/cm) 0.00	3 Set the desired zero level value. The range is 0.000–999.9 mS/cm. Press **OK** to acknowledge.
Set Cond Full Scale (50.00mS/cm)	4 Press the down button to access the next settings menu. The current setting is displayed. Press **OK**.
Set Cond Full Scale (50.00mS/cm) 50.00	5 Set the desired full scale value. The range is 0.000–999.9 mS/cm. Press **OK** to acknowledge.
	6 Press **Esc** to return to the **Set UV Analogue Out** menu.

B.4.4 Setting the pH analogue output

Note: *The pH values for zero level and full scale must differ by at least 1 pH unit.*

Note: *The zero level and full scale values can be calibrated in any order.*

Set pH Analogue Out (pH 0.00-14.00)	1 Select the **Set pH Analogue Out** menu in the **Setup Analogue Out** menu by using the up and down buttons. Current analogue settings are displayed (zero and full scale values).
Set pH Zero Level (pH 0.00)	2 Press **OK** to access the settings menu. The current setting is displayed. Press **OK**.
Set pH Zero Level (pH 0.00) 00.00	3 Set the desired zero level value. The range is pH -0.50–14.30. Press **OK** to acknowledge.
Set pH Full Scale (pH 14.00)	4 Press the down button to access the next settings menu. The current setting is displayed. Press **OK**.
Set pH Full Scale (pH 14.00) 14.00	5 Set the desired full scale value. The range is pH -0.50–14.30. Press **OK** to acknowledge.
	6 Press **Esc** to return to the **Set UV Analogue Out** menu.

B.4.5 Setting the Press analogue output

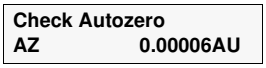
Set Press. Analogue Out (1.00 MPa)	1 Select the **Set Press Analogue Out** menu in the **Setup Analogue Out** menu by using the up and down buttons. The current analogue setting is displayed (full scale value).
Set Press. Analogue Out (1.00 MPa) 1.00	2 Press **OK** to access the settings menu. Set the desired full scale value. The range is 0.00–1.00 MPa. Press **OK** to acknowledge.

C Menus

C.1 Check menus

C.1.1 Checking autozero level

The module internal absorbance value for autozero can be checked to test the consistency of buffers.



1 Select menu **Check** and press **OK**.

2 Select sub menu **Check Autozero**. The autozero absorbance value for the used wavelength is shown.

C.1.2 Checking lamp run time

The lamp run time can be checked to determine the need for lamp replacement. Run times for both Hg and Zn lamps are monitored.

1 Select menu **Check** and press **OK**.

2 Select sub menu **Check Lamp Run Time**.

C.1.3 Checking lamp intensity

The lamp intensity can be checked to determine the status of the lamp used.

1 Select menu **Check** and press **OK**.

2 Select sub menu **Check Lamp Intensity**.

C.1.4 Checking pump run time

The pump run time can be checked to determine the need for maintenance.

1 Select menu **Check** and press **OK**.

2 Select sub menu **Check Pump Run Time**.

C.1.5 Checking pumped volume

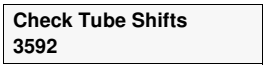
The volume delivered by the pump can be checked to determine the need for maintenance.

1 Select menu **Check** and press **OK**.

2 Select sub menu **Check Pumped Volume**.

C.1.6 Checking tube shifts

The number of tube shifts done by the fraction collector can be checked to determine the need for maintenance.



1 Select menu **Check** and press **OK**.

2 Select sub menu **Check Tube Shifts**.

C.1.7 Checking valve shifts

The number of shifts done by the buffer valve and the injection valve can be checked to determine the need for maintenance.

Check Valve Shifts **BV: 642 IV: 348**

1 Select menu **Check** and press **OK**.

2 Select sub menu **Check Valve Shifts**.

C.1.8 Checking the recorder

The function of a connected chart recorder can be tested.

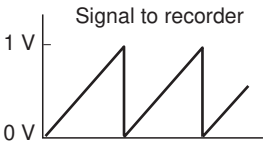
1 Select menu **Check** and press **OK**.

Check Recorder

2 Select sub menu **Check Recorder**.

3 Press **OK** to start the test.

The test will ramp the signal on each channel up to 1 V and then immediately decrease the signal back to 0 V. The test is run continuously. Compare the diagram of the chart recorder with the figure.



4 Stop the test by pressing **OK** or **Esc**.

C.1.9 Checking service mode

Service information relevant to the module can be checked. Information may not be available in all menus.

1 Select main menu **Check** and press **OK**.

Check Service Mode

2 Select menu **Check Service Mode** and press **OK**.

Telephone Service: **012345678901**

3 The service telephone number is displayed. Press **OK**.

Contract number: **01234567801**

4 The service contract number is displayed. Press **OK**.

Serial Number: **0123456 YM 012345**

5 The module serial number is displayed. Press **OK**.

ÄKTAprime **V1.00**

6 The system name and software version are displayed. Press **OK**.

Date of maintenance: **DD MMMM YYYY**

7 The date of the last service is displayed. Press **OK**.

Buzzer Test

8 A test of the system buzzer is performed. Press **OK**.

C.2 Set Parameters menus

C.2.1 Switching the lamp on/off

We recommend the lamp be switched off to conserve lamp operating time when no measurement is being made. A warm-up time of 60 minutes is required to achieve full specifications. However, in most cases, a warm-up time of 15 minutes is sufficient.

1 Select main menu **Set Parameters** and press **OK**.

2 Select sub menu **Lamp** and press **OK** to access the setting menu.

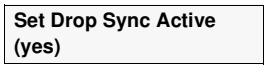
3 Switch the lamp on/off with the up and down buttons, and then press **OK**.

C.2.2 Setting drop synchronization

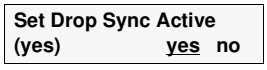
If drop synchronization is active, tube changes will only occur directly after a drop is registered by the drop counter to minimize spillage between tubes. Drop synchronization operates in all fraction collection modes; time, volume and drop.

Drop synchronization is only possible at flow rates up to 5 ml/min. If the flow limit is exceeded, an error message is displayed.

1 Select main menu **Set Parameters** and press **OK**.



2 Select sub menu **Set Drop Sync Active.** The current setting is shown. The setting will apply to all subsequent manual and method controlled operation until a new value is set.
Press **OK** to change the setting.



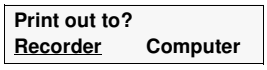
3 Select the desired setting and press **OK**.

C.2.3 Memory print out

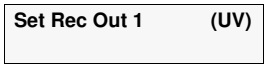
Measurement data from the last run can be printed to a recorder or a computer. Three channels are available for printing the curves that correspond to UV-absorbance, pH, conductivity, concentration of the B-buffer, temperature and pressure.

1 Select main menu **Set Parameters** and press **OK**.

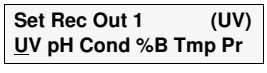
2 Select sub menu **Memory Print Out** and press **OK**.



3 Select printing to a recorder or a computer and press **OK**.

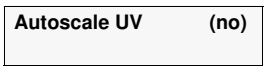


4 The current setting for channel 1 is displayed. Press **OK** to access the setting menu.

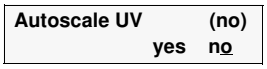


5 Select the parameter to be printed on channel 1 and press **OK**.

6 Repeat steps 4 and 5 for channels 2 and 3.



7 If the UV-curve is selected, the current setting for auto-scaling of the UV-curve is shown. Press **OK** to access the setting menu.



8 Select the desired setting and press **OK**.

The three selected curves are now printed.

C.2.4 Setting analogue outputs

This is described in section *B.4 Setting analogue outputs* in this chapter.

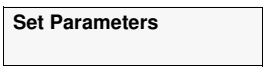
C.2.5 Setup calibrate pH

This is described in section *B.3 Calibrating the pH electrode (optional)* in this chapter.

C.2.6 Setup pH temperature compensation

The relationship between pH and the output signal from the pH electrode is temperature dependent. For accurate measurements during temperature changes, the pH measurement can be temperature compensated. In normal applications, when the temperatures of the buffers and calibration buffers are identical, temperature compensation is not necessary.

When using temperature compensation, it is important that the temperature of the pH electrode is the same as that of the conductivity flow cell since that is where the temperature is measured.



1 From the main menu, select sub menu **Set Parameters** and press **OK**.

2 Select sub menu **Setup and calibration**. Press **OK**.

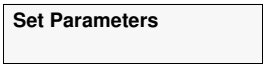
3 Select sub menu **Setup pH** and press **OK**.

4 Select sub menu **Set pH Temp Comp**. The current setting for showing pH is displayed. If **on** is shown, **Tc** is displayed in the running display. If **off** is shown (default), **Tc** is not displayed. Press **OK** to change the setting.

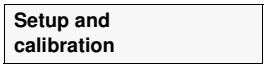
5 Select the desired setting and press **OK**.

C.2.7 Setup show pH

Normally, the pH is displayed in the running window. If not required, the pH display can be set to off.



1 From the main menu, select sub menu **Set Parameters** and press **OK**.



2 Select sub menu **Setup and calibration**. Press **OK**.

Setup pH	3 Select sub menu **Setup pH** and press **OK**.
Set Show pH (on)	4 Select sub menu **Set Show pH**. The current setting for showing pH is displayed. If **on** is shown, current pH is displayed in the running display. If **off** is shown, no pH is displayed in the running display. Press **OK** to change the setting.
Set Show pH (off) on off	5 Select the desired setting and press **OK**.

C.2.8 Setup conductivity temperature compensation

Set Parameters	1 From the main menu, select menu **Set Parameters** and press **OK**.
Setup and calibration	2 Select sub menu **Setup and calibration** and press **OK**.
Setup Cond	3 Select sub menu **Setup Cond** and press **OK**.
Set Cond Temp Comp (0.0%)	4 Select sub menu **Set Cond Temp Comp** and press **OK**. The current temperature compensation factor is shown. 0.0% means that the compensation is off (default setting). The range is 0.0–9.9%.
Set Cond Temp Comp (0.0%) 0.0	5 The current compensation factor is displayed as default. Adjust the compensation factor setting as necessary and press **OK**.

C.2.9 Setup conductivity reference temperature

Set Parameters	1 From the main menu, select menu **Set Parameters** and press **OK**.
Setup and calibration	2 Select sub menu **Setup and calibration** and press **OK**.
Setup Cond	3 Select sub menu **Setup Cond** and press **OK**.
Set Cond Ref Temp (25.0°C)	4 Select sub menu **Set Cond Ref Temp** and press **OK**. The current reference temperature value is shown. 25 °C is the default setting. The range is 0.00–99.9 °C.
Set Cond Ref Temp (25.0°C) 25.0	5 The current reference temperature value is displayed as default. Adjust the reference temperature value setting as necessary and press **OK**.

C.2.10 Setup adjust conductivity

Normally, it is not necessary to adjust the cell constant as the flow cell is pre-calibrated on delivery. Adjustment is only necessary when replacing the conductivity flow cell with a flow cell whose cell constant is unknown. We recommend that the conductivity flow cell is recalibrated after cleaning.

Note: *The conductivity temperature compensation must not be used when adjusting the cell constant. Set the* ***Set Cond Temp Comp*** *to 0 (see section C.2.8). The temperature sensor must be calibrated before adjusting the cell constant (see section C.2.16).*

1 Prepare a calibration solution of 1.00 M NaCl, 58.44 g/l. Let the solution stand until it is at room temperature. This is important for exact measurements.

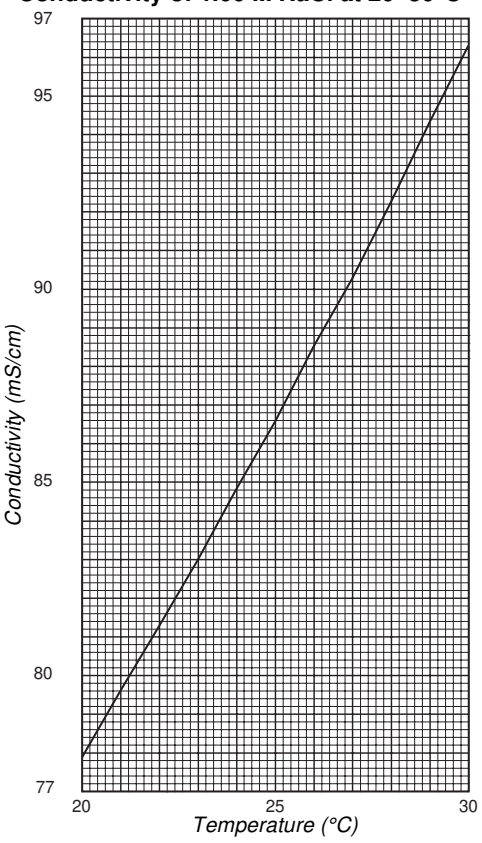
2 Fill the flow cell completely with the calibration solution by pumping at least 15 ml through the cell with a syringe.

3 Stop the flow and wait 15 minutes until the temperature is constant in the range 20–30 °C.

4 Read the conductivity value displayed and compare it with the theoretical value from the graph below at the temperature of the calibration solution. If the displayed value and the theoretical value correspond, no further action is required.

If the values differ, proceed with the actions below.

Conductivity of 1.00 M NaCl at 20–30°C



Set Parameters	5 From the main menu, select menu **Set Parameters** and press **OK**.
Setup and calibration	6 Select sub menu **Setup and calibration** and press **OK**.
Setup Cond	7 Select sub menu **Setup Cond** and press **OK**.
Set Adjust Cond (80.32mS/cm)	8 Select sub menu **Set Adjust Cond**. The current conductivity value is shown. Press **OK**.
Warning! This will change cell calibr.	9 A warning message is shown until confirmed by pressing **OK**.
Set Adjust Cond (80.32mS/cm) 83.55	10 The current value is displayed as default. Enter the theoretical conductivity value according to the graph and press **OK**. The new cell constant is automatically calculated. The range is 1.000–999.9 mS/cm.

C.2.11 Setup adjust cell constant

After replacing the flow cell, the cell constant has to be set. (The cell constant is shown on the cell packaging.)

Set Parameters	1 From the main menu, select menu **Set Parameters** and press **OK**.
Setup and calibration	2 Select sub menu **Setup and calibration** and press **OK**.
Setup Cond	3 Select sub menu **Setup Cond** and press **OK**.
Set Adj Cell Const ($83.56 cm^{-1}$)	4 Select sub menu **Set Adj Cell Const**. The current cell constant is shown. Press **OK**.
Warning! This will change cell calibr.	5 A warning message is shown until confirmed by pressing **OK**.
Set Adj Cell Const ($83.56 cm^{-1}$) 83.55	The current cell constant is displayed as default. Enter the new cell constant as read from the packaging and press **OK**. The range is 0.1–300.0 cm^{-1}.

C.2.12 Setup show conductivity

Set Parameters	1 From the main menu, select menu **Set Parameters** and press **OK**.
Setup and calibration	2 Select sub menu **Setup and calibration** and press **OK**.
Setup Cond	3 Select sub menu **Setup Cond** and press **OK**.
Set Show Cond (on)	4 Select sub menu **Set Show Cond**. The current status for showing conductivity is shown. If **on** is shown, current conductivity is displayed in the running display. If **off** is shown, no conductivity is displayed in the running display. Press **OK** to change the setting.
Set Show Cond (on) on off	5 Change the setting as desired and press **OK**.

C.2.13 Setup UV averaging filter constant

To filter the noise in the UV-signal, a moving average filter is used. The averaging time is the time interval used for calculating the moving average of the absorbance signal. A long averaging time will smooth out noise efficiently, but it will also distort the peaks. Peaks narrower than the minimum peak width value according to the table below may be distorted. Because of this, the averaging time should be as short as possible. On delivery, the averaging time is set to 1.3 s.

Set Parameters	1 From the main menu, select menu **Set Parameters** and press **OK**.
Setup and calibration	2 Select sub menu **Setup and calibration** and press **OK**.

Setup UV

3 Select sub menu **Setup UV** and press **OK**.

Set Averaging (1.3 s)

4 Select sub menu **Set Averaging**. The current set averaging time is shown. Press **OK** to change the setting.

Set Averaging (1.3 s) 0.64

5 Set the desired value and press **OK**. Values allowed are 0.02, 0.04, 0.08, 0.16, 0.32, 0.64, 1.3, 2.6, 5 and 10 s.

Averaging time (s)	*Corresponding time constant (s) (approximately)*	*Min. peak width at half height (s)*
10.0	5	50
5.1	2	32
2.6	1	16
1.3	0.5	8.0
0.64	0.2	3.2
0.32	0.1	1.6
0.16	0.05	0.8
0.08	0.03	0.5
0.04	0.01	0.2
0.02	0.01	0.1

C.2.14 Setup lamp run time

When the UV lamp is replaced, reset the **Lamp Run Time** counter.

Set Parameters

1 From the main menu, select menu **Set Parameters** and press **OK**.

Setup and calibration

2 Select sub menu **Setup and calibration** and press **OK**.

Setup UV

3 Select sub menu **Setup UV** and press **OK**.

Set Lamp Run Time Hg (2000 h)

4 Select sub menu **Set Lamp Run Time** press **OK**.

Set Lamp Run Time Hg (2000 h) 000

5 Set the **Lamp Run Time** counter to zero with the dial. Press **OK** to acknowledge.

C.2.15 Setup show UV

Normally, UV absorbance is shown in the running display. If not required, the UV absorbance display can be set to off.

Set Parameters

1 From the main menu, select menu **Set Parameters** and press **OK**.

Setup and calibration

2 Select sub menu **Setup and calibration** and press **OK**.

Setup UV

3 Select sub menu **Setup UV** and press **OK**.

Set Show UV (on)

4 Select sub menu **Set Show UV.** The current status for showing UV is shown. If **on** is shown, current UV is displayed in the running display. If **off** is shown, no conductivity is displayed in the running display.
Press **OK** to change the setting.

Set Show UV (On) on off

5 Change the setting as desired and press **OK**.

C.2.16 Setup adjust temperature

Calibration of the temperature sensor in the conductivity flow cell is only necessary if the monitor is used in high accuracy measurement or if the conductivity flow cell is replaced.

1 Place the flow cell together with a precision thermometer inside a box or empty beaker to ensure that they are not exposed to draught. Leave them for 15 minutes to let the temperature stabilize.

2 Read the temperature on the thermometer.

Set Parameters

3 From the main menu, select menu **Set Parameters** and press **OK**.

Setup and calibration

4 Select sub menu **Setup and calibration** and press **OK**.

Setup Temp

5 Select sub menu **Setup Temp** and press **OK**.

Set Adjust Temp (25.0°C)

6 Select sub menu **Set Adjust Temp**. The current temperature is shown. Press **OK**.

Warning! Temp calibr will be changed.

7 A warning message is shown until confirmed by pressing **OK**.

Set Adjust Temp (25.0°C) 25.0

8 The current adjustment value is displayed as default. Enter the temperature shown on the thermometer and press **OK**.

C.2.17 Setup show temperature

The display of the temperature in the conductivity flow cell, shown in the running display, can be enabled or disabled.

Set Parameters

1 From the main menu, select menu **Set Parameters** and press **OK**.

Setup and calibration

2 Select sub menu **Setup and calibration** and press **OK**.

Setup Temp

3 Select sub menu **Setup Temp** and press **OK**.

Set Show Temp **(on)**

4 Select sub menu **Set Show Temp**. The current status for showing temperature is displayed. If **on** is shown, current temperature is displayed in the running display. If **off** is shown, no temperature is displayed in the running display.
Press **OK** to change the setting.

Set Show Temp **(on)** **on** **off**

5 Change the setting as desired and press **OK**.

C.2.18 Setup mixer chamber volume

If the mixer chamber is exchanged for a chamber with another volume, the new chamber volume can be set. On delivery, the chamber volume is set to 2.0 ml.

Set Parameters

1 From the main menu, select menu **Set Parameters** and press **OK**.

Setup and **calibration**

2 Select sub menu **Setup and calibration** and press **OK**.

Set Mix Chamber Vol **(2 ml)**

3 Select sub menu **Set Mix Chamber Vol**. The current chamber volume is shown. Press **OK** to change the volume.

Set Mix Chamber Vol **(2 ml)** **2.0**

4 Change the setting as desired and press **OK**. Possible values are 0.6, 2.0, 5.0 and 12.0 ml.

C.2.19 Setup Delay UV to Frac

The volume (in µl) of the delivery tubing between the UV flow cell and the fraction collector can be set. This will ensure that event marks on the UV absorbance curve are synchronized with the tube changes.

Calculate the volume of the tubing from the UV flow cell to the very end of the tubing. The volume of 0.75 mm i.d. tubing is 44.2 µl/10 cm.

Set Parameters

1 From the main menu, select menu **Set Parameters** and press **OK**.

Setup and **calibration**

2 Select sub menu **Setup and calibration** and press **OK**.

Set Delay UV to Frac **(380 µl)**

3 Select sub menu **Set Delay UV to Frac**. The current delay volume is shown. Press **OK** to change the volume.

Set Delay UV to Frac **(380 µl)** **380**

4 Change the setting as desired and press **OK**.

C.2.20 Calibrating the flow rate

Set Parameters

1 From the main menu, select menu **Set Parameters** and press **OK**.

Start Pump Calibr. **800 pulses**

2 Select **Start Pump Calibration** in the **Setup and calibration** menu. Press **OK**.

Set Flow Rate **(10 ml/min) 2.0**

3 Enter a suitable calibration flow rate in the **Set Flow Rate** menu and press **OK**.

4 Measure the volume of the water collected in the bottle.

Enter Collected **Volume (ml) 2.05**

5 Enter the measured volume in ml in the **Enter Collected Volume** menu and press **OK**.

6 At the **Pump Calibrated OK** menu, press **OK**.

C.2.21 Calibrating the pressure offset

The pump should be calibrated when required.

1 Make sure that the pressure sensor is exposed to atmospheric pressure only, i.e. no back-pressure.

Change Press Offset **(1005 mV)**

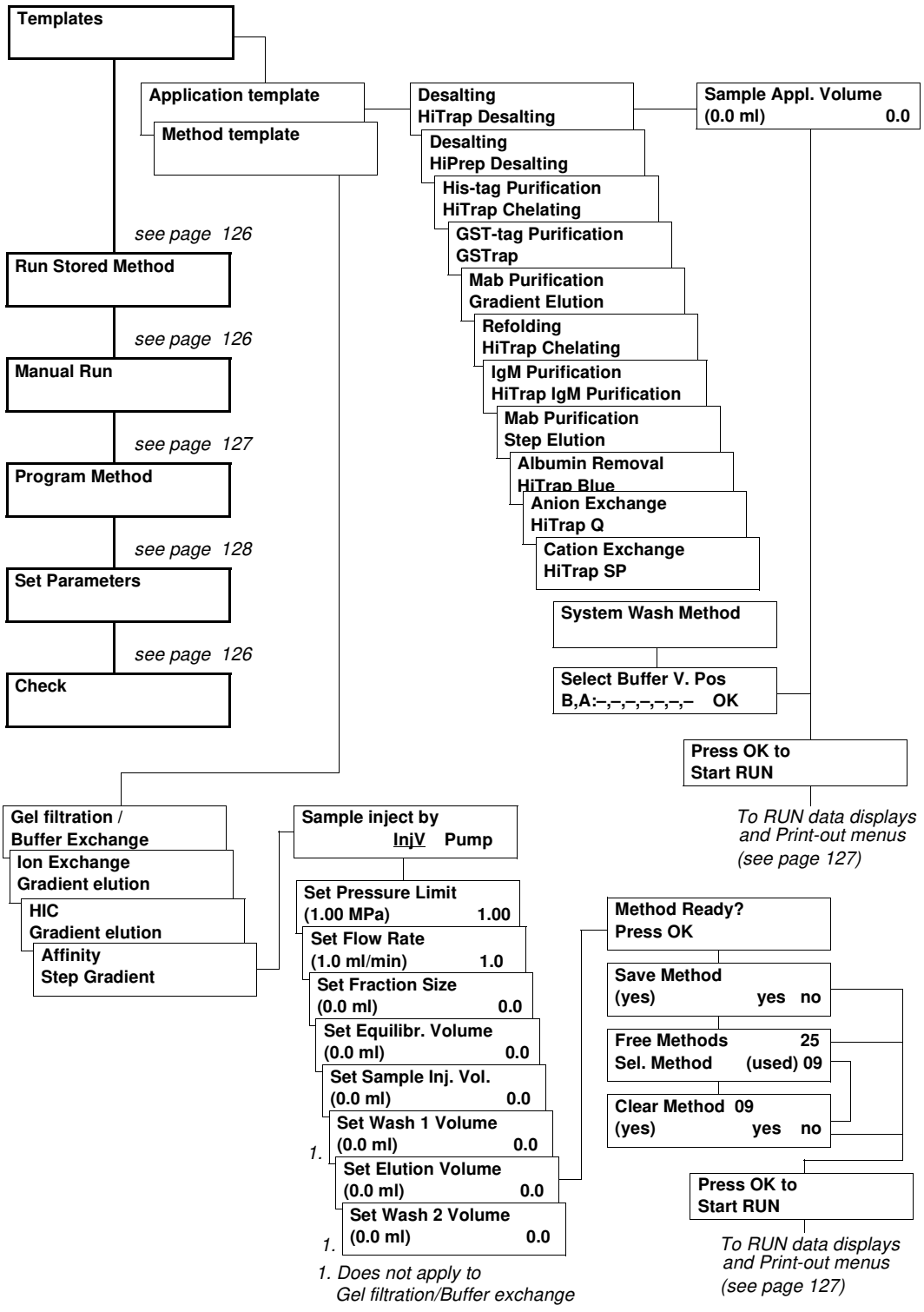
2 Select the **Change Press Offset** menu in the **Setup and calibration** menu. Press **OK**.

Set zero pressure **to calib. Press OK**

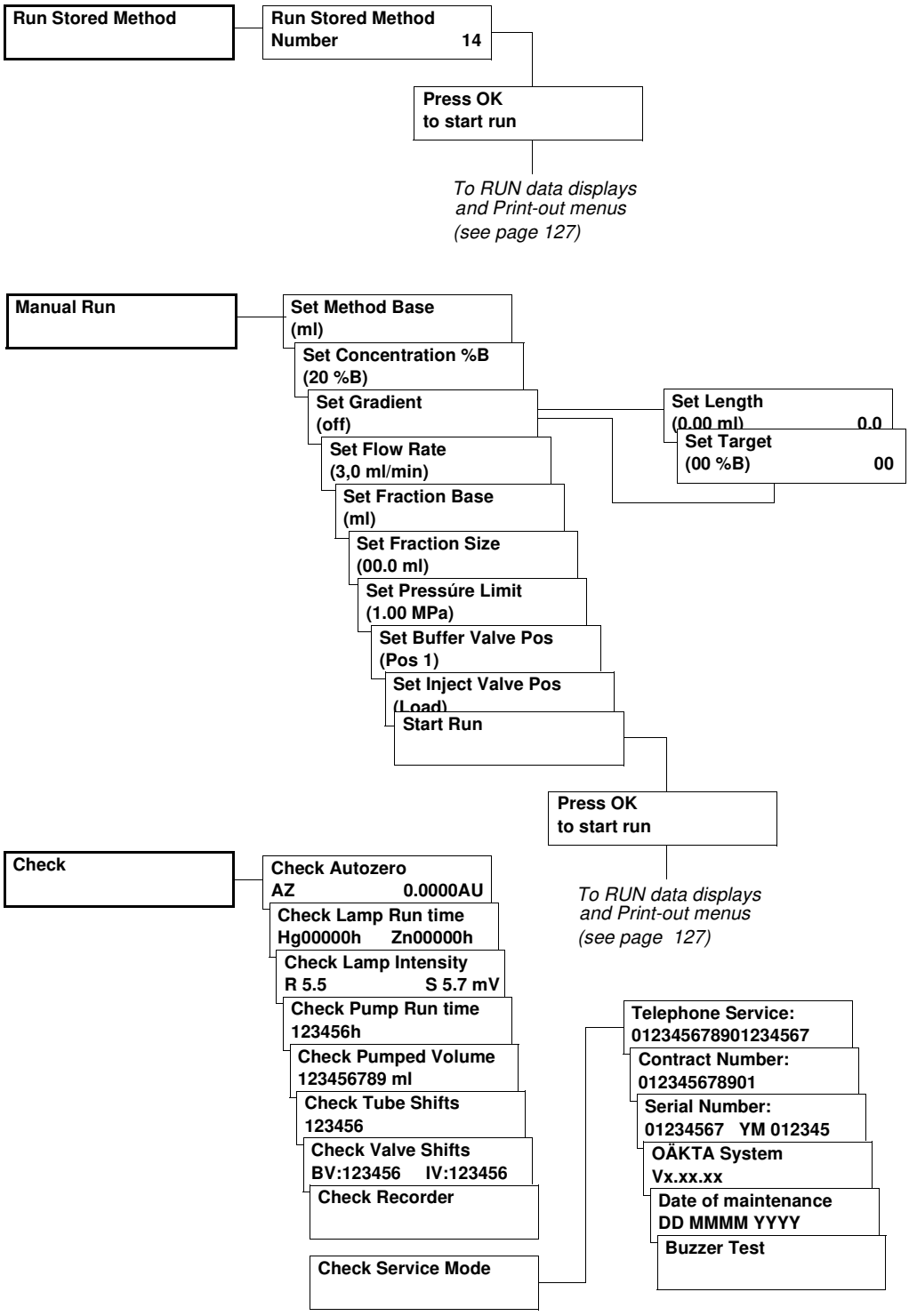
3 At the **Set zero pressure to calib.** menu, press **OK**.
The calibration only takes a few seconds.

4 Press **OK** at the **Calibrating Offset Done!** menu.

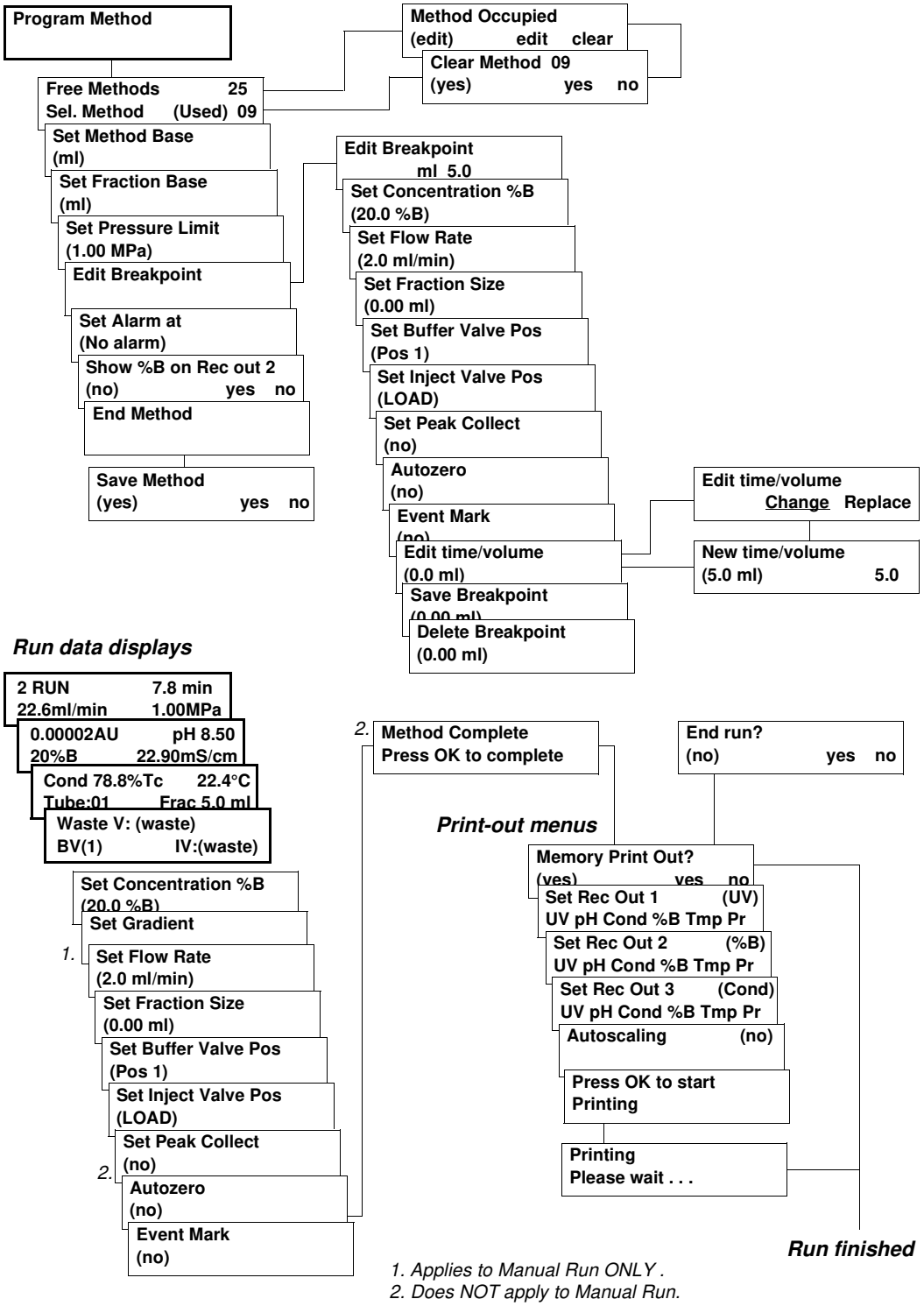
C.3 Menu overview



Menu overview (cont.) 2

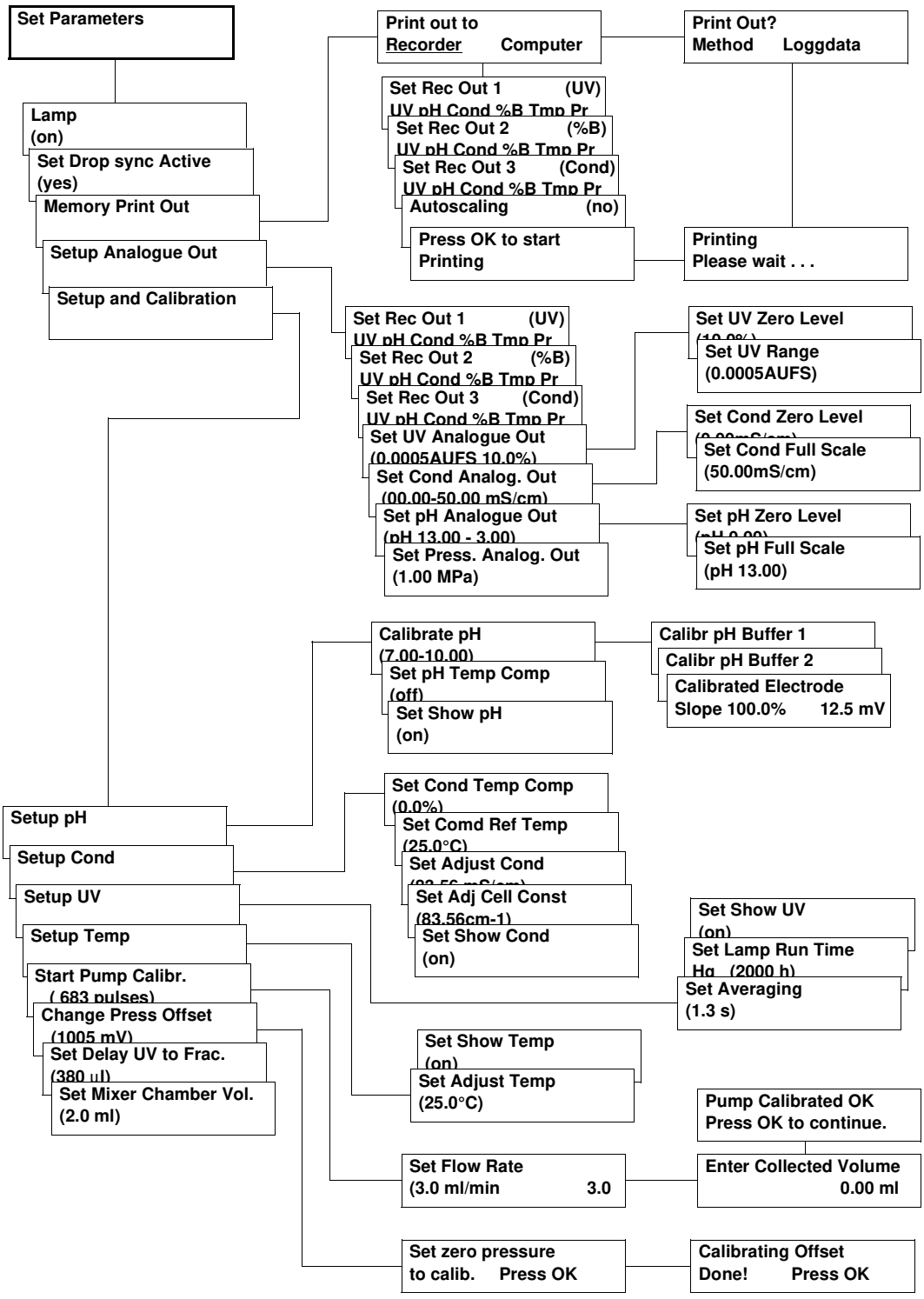


Menu overview (cont.) 3



1. Applies to Manual Run ONLY .
2. Does NOT apply to Manual Run.

Menu overview (cont.) 4



D Technical specifications

Relevant system and component specifications are listed below.

D.1 Operating data

System pump

Flow rate range	0.1–50 ml/min in steps of 0.1 ml/min
Pressure range	0–1.0 MPa (10 bar, 145 psi)
Pressure pulsation	< ±20% (dP/P) of mean value
pH stability range	1–14 (spec. valid between pH 2–12)
Viscosity	
< 5 ml/min	Max. 10cP
> 5 ml/min	Max. 5 cP
Flow rate reproducibility	rsd < 2% or 0.2 ml whichever is greater (at 0.1–50 ml/min)
Flow rate accuracy	< ±4% or ±0.1 ml/min whichever is greater
Gradient composition	
accuracy	±3% at 0.1–50 ml/min
reproducibility	±1.0% at 0.1–50 ml/min
Leakage	< 1.0 µl/min (complete system)
Pressure sensor	
range	0–1.0 MPa
scale error	±5%

UV measurement

Wavelengths	
Hg lamp, fixed	254 and 280 nm
by changing filter (optional)	313, 365, 405, 436 and 546 nm
Zn lamp (optional)	214 nm
Absorbance range	0.01–5.0 AU
Autozero range	-0.2–2.0 AU
Baseline adjust	Adjustable 0–100% of full scale
Linearity	< 3% up to 2 AU at 254 nm < 5% up to 1 AU at 280 nm
Static noise	
short term	$40x10^{-6}$ AU at 254 nm
long term	$40x10^{-6}$ AU at 254 nm
Static drift	$\pm100x10^{-6}$ AU/hour at 254 nm
Flow sensitivity	$2x10^{-4}$ AU min/ml

UV flow cell, 2 mm

Flow rate	0–100 ml/min
Max. pressure	4.0 MPa
Max. back-pressure	0.05 MPa at 100 ml/min
Liquid temperature range	+4 to +60 °C
Optical path length	2 mm
Cell volume	2 µl (30 µl detector volume)

UV flow cell, 5 mm (optional)

Flow rate	0–20 ml/min
Max. pressure	4.0 MPa
Max. back-pressure	0.02 MPa at 20 ml/min
Optical path length	5 mm
Cell volume	6 µl (10 µl detector volume)

Conductivity measurement

Conductivity range	1 µS/cm to 999.9 mS/cm
Reproducibility	
short term	Max. ±1% or ±5 µS/cm whichever is greater
long term	Max. ±3% or ±15 µS/cm whichever is greater
Noise	Max. ±0.5% of full scale calibrated range
Response time	< 3 s (0–95% of step)
Temperature sensor	
accuracy	±2.0 °C
drift	±0.5 °C per 10 h
Flow rate sensitivity	±1% within 0–100 ml/min

Conductivity flow cell

Flow rate	0–100 ml/min
Max. pressure	5 MPa (50 bar, 725 psi)
Max. back-pressure	0.01 MPa at 100 ml/min

Fraction collection

Tube capacity	175 in tube rack 12 mm (optional) 95 in tube rack 18 mm 40 in tube rack 30 mm (optional)

pH measurement

pH range	0 to 14 (spec. valid between 2 and 12)
Accuracy	
temperature compensated	±0.1 pH within +4 to +40 °C
not compensated	±0.2 pH within +15 to 25 °C, ±0.5 pH within +4 to +15 °C and +25 to +40 °C
Response time	< 10 s (0–95% of step)
Long term stability	Dev. max. 0.1 pH per 10 h at constant conditions (4–40 °C)
Flow rate sensitivity	Dev. max. 0.1 pH units

pH cell

Flow rate	0.1–100 ml/min
Max. pressure	0.5 MPa (5 bar, 72 psi)
Max. back-pressure	0.02 MPa at 100 ml/min

D.2 Physical data

Control	Via membrane keyboard and display (2x20 characters)
Degree of protection	
housing	IP 20
flow cells	IP 44
Power requirement	100–120/220–240 V ~, 50–60 Hz
Power consumption	Max. 90 VA
Fuse specification	T 1.6 AH/250 VAC, approved type (not replaceable by operator)
Dimensions, H x W x D	530 x 400 x 450 mm
Weight	13 kg
Environment	+4 to +40 °C, 10–95% relative humidity (non-condensing), 84–106 kPa (840–1060 mbar atmospheric pressure).
EMC standards	This product meets the requirements of the EMC Directive 89/336/EEC through the harmonized standard EN 61326-1 (emission and immunity). **Note**: The declaration of conformity is valid for the instrument if it is: • used in laboratory locations • used in the same state as it was delivered from Amersham Biosciences except for alterations described in the User Manual • connected to other CE labelled Amersham Biosciences modules or other products as recommended.
Safety standards	This product meets the requirement of the Low Voltage Directive (LVD) 73/23/EEC through the harmonized standard EN 61010-1.

D.3 ÄKTA*prime* component materials

The wetted materials of ÄKTA*prime* are listed below:

	FFKM	PEEK	PTFE	FEP	ETFE	ECTFE	PP	PE	Titanium alloy	Quartz	Glass	Gold	Kalrez, Simriz	Ceramic
System pump		X						X	X				X	X
Monitor	X	X	X						X	X	X			
Fraction collector			X			X								
Mixer	X	X	X											
Pressure sensor														
Buffer valve/ Injection valve		X												
Gradient switch valve/ Flow diversion valve			X			X								
Flow restrictor		X	X		X							X		
Superloop	X	X			X						X			
Tubing		X		X										
Inlet filters							X		X					
Unions/Connectors		X			X									

FFKM = perfluororubber
PEEK = polyetheretherketone
PTFE = polytetrafluoroethylene
FEP = perfluoroethylenepropylene copolymer
ETFE = ethylenetetrafluoroethylene
ECTFE = ethylenechlorotrifluoroethylene
PE = polyethene
PP = polypropylene

E Chemical resistance guide and chemical compatibility

The chemical resistance of ÄKTA*prime* to some of the most commonly used chemicals in liquid chromatography is indicated in the table below.

The ratings are based on the following assumptions:

1 The synergistic effects of the chemical mixtures have not been taken into account.

2 Room temperature and limited over-pressure is assumed.

Note: *Chemical influences are time and pressure dependent. Unless otherwise stated, all concentrations are 100%.*

Chemical	***Exposure***	***Comments***
Acetic acid, 0.1 M	OK	
Acetone, 1%	OK	
Aqueous buffers, pH 2–12	OK	
Decon 90, 10%	OK	For washing only
Ethanol, 20%	OK	
Ethanol, 96%	OK	For washing only
Ethylene glycol	OK	
Formic acid, 1%	OK	
Guanidin, 6 M	OK	
HCl, 0.1 M	OK	
Isopropanol, 30%	OK	
Lysozyme, 2 mg/ml	OK	
Methanol, 20%	OK	
NaOH, 0.1 M	OK	
NaOH, 1 M	OK	For washing only
SDS, 10%	OK	Short term use
TFA, 0.2%	OK	
Triton-X, 2%	OK	Short term use
Urea, 8 M	OK	

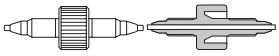
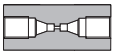
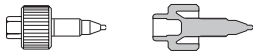
F Accessories and consumables

Item	*Quant./pack*	*A/C**	*Code no.*
Optical unit			
Hg lamp & housing complete	1	C	18-1128-22
Zn lamp & housing complete	1	C	18-1128-23
UV flow cell 5 mm	1	C	18-1128-24
UV flow cell 2 mm	1	C	18-1128-25
Filter 214 nm	1	C	18-0622-01
Filter 254 nm	1	C	18-0620-01
Filter 280 nm	1	C	18-0621-01
Filter 313 nm	1	C	18-0623-01
Filter 365 nm	1	C	18-0624-01
Filter 405 nm	1	C	18-0625-01
Filter 436 nm	1	C	18-0626-01
Filter 546 nm	1	C	18-0627-01
Filter wheel complete	1	A	18-0647-01
pH electrode			
pH electrode, round tip, incl. flow cell and holder	1	C	18-1134-84
pH electrode, round tip	1	C	18-1111-26
pH flow cell, round tip, incl. dummy electrode	1	A	18-1112-03
Dummy electrode, round tip	1	A	18-1111-03
Mixer			
Mixing chambers:			
0.6 ml	1	A	18-1118-90
2 ml	1	A	18-1118-91
5 ml	1	A	18-1118-92
12 ml	1	A	18-1118-93
Injection fill port 0.7 mm	1	C	18-1127-37
Sample loops:			
10 µl	1	C	18-1120-39
100 µl	1	C	18-1113-98
500 µl	1	C	18-1113-99
1 ml	1	C	18-1114-01
2 ml	1	C	18-1114-02
5 ml	1	C	18-1140-53

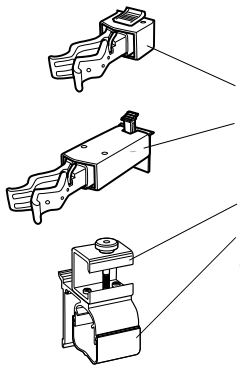
*) A = accessory, C = consumable

Item	Quant./pack	A/C*	Code no.
Fraction collector			
Tube racks, complete with bowl, tube support, holder and guide:			
12 mm	1	A	19-8684-03
18 mm	1	A	19-3050-03
30 mm	1	A	19-1124-67
Tube support	1	A	18-3054-02
Tube holder and guide:			
12 mm	1	A	19-7242-02
18 mm	1	A	19-8689-02
30 mm	1	A	18-1124-68
Eppendorf tube holder for 12 mm rack	100	A	18-8522-01
Flow diversion valve, FV-903 incl. mounting bracket	1	A	18-1114-50
Tubing holder	1	A	18-6464-01
Drive sleeve	5	C	19-6067-02
Superloop 10 ml, 50 ml			
Superloop 10 ml, complete	1	A	18-1113-81
Superloop 50 ml, complete	1	A	18-1113-82
Inner end piece	1	A	19-7846-01
Outer end piece	1	A	19-5167-01
O-ring, inner end piece	5	C	19-7595-01
O-ring, movable seal	2	C	18-1104-97
Movable seal	1	A	19-7845-01
Protective jacket (50 ml)	1	A	19-7849-01
Glass tube with thread and groove (10 ml)	1	A	19-7593-01
Glass tube with thread and groove (50 ml)	1	A	19-5165-01
Tubing kit for Superloop (10 ml)	1	A	18-1113-83
Tubing kit for Superloop (50 ml)	1	A	18-1113-84
Superloop 150 ml			
Superloop 150 ml, complete	1	A	18-1023-85
Movable seal	1	A	18-1029-58
Inner end piece	1	A	18-1029-59
O-ring, inner end piece	2	C	18-1029-60
O-ring, movable seal	1	C	18-1134-49

*) A = accessory, C = consumable

	Item	Quant./pack	A/C*	Code no.
	Cables			
	Mains cable, 120 V	1	A	19-2447-01
	Mains cable, 240 V	1	A	19-2448-01
	Mains distribution lead 0.3 m	1	A	18-1119-05
	Mains distribution lead 1 m	1	A	18-1132-08
	Signal cable, ÄKTA*prime*	1	A	18-1141-35
	Connectors and unions			
	Tubing connector, inlet nut for o.d. 3/16”, PEEK	10	A	18-1112-49
	Ferrule, for 3/16” o.d. tubing, PEEK	10	A	18-1112-48
	Union, 1/16” female/M6 male, PEEK	6	A	18-1112-57
	Union, luer female/1/16” male, PEEK	2	A	18-1112-51
	Union, M6 female/1/16” male, PEEK	8	A	18-1112-58
	Union, 1/16” male/1/16” male, for 1/16” o.d. tubing, PEEK	10	A	18-1120-92
	Union, 1/16” female/1/16” female, for 1/16” o.d. tubing, titanium	1	A	18-3855-01
	Fingertight connector 1/16”, for PEEK tubing o.d. 1/16”	10	A	18-1112-55
	Stop plug, 1/16”, PEEK	5	A	18-1112-52
	Stop plug, 5/16”, PEEK	5	A	18-1112-50
	Tubing			
	Teflon tubing, i.d. 2.9 mm, o.d. 3/16” (IN)	3 m	A	18-1112-47
	PEEK tubing, i.d. 0.50 mm, o.d. 1/16”	2 m	A	18-1113-68
	PEEK tubing, i.d. 0.75 mm, o.d. 1/16” (G)	2 m	A	18-1112-53
	PEEK tubing, i.d. 1.0 mm, o.d. 1/16” (W)	2 m	A	18-1115-83
	Sample tubing kit	1	A	18-1115-77

*) A = accessory, C = consumable



Item	*Quant./pack*	*A/C**	*Code no.*
Miscellaneous			
Accessory kit	1	C	56-3097-54
Inlet filter assembly	2	A	18-1113-15
Inlet filter set	10	C	18-1114-42
On-line filter	1	A	18-1112-44
On-line filter kit	10	C	18-1027-11
Flow restrictor, FR-902	1	A	18-1121-35
Flow restrictor, FR-904	1	A	18-1119-63
Cramp, for column holder, ÄKTAprime	1	A	18-1142-71
Column holder, for one column, short	1	A	18-1113-17
Column holder, for one column, long	1	A	18-1126-32
Extension arm, for column holders	1	A	18-3064-40
Flow cell holder for optical unit	1	A	18-3055-87
Clamp, conductivity flow cell	1	A	18-1111-14
Tubing cutter	1	A	18-1112-46
U-wrench, M6	1	A	19-7481-01
U-wrench, 1/4”	1	A	18-1112-45
U-wrench, 5.5 mm	1	A	18-1128-65
Allen key, 2.5 mm	1	A	19-4442-01
Chart recorder REC 112, 2 channel	1	A	18-1132-33

*) A = accessory, C = consumable

User Documentation			
ÄKTA*prime* User Manual	1		18-1135-24
Cue cards			
Mab purification, step elution	1		18-1138-01
His-tag Purification	1		18-1138-02
Desalting on HiTrap Desalting	1		18-1138-03
Desalting on HiPrep Desalting	1		18-1138-04
Cation Exchange	1		18-1138-05
GST-tag Purification	1		18-1138-06
Anion exchange	1		18-1139-39
Mab purification, gradient elution	1		18-1139-41
IgM Purification	1		18-1139-42
Refolding	1		18-1139-43
Albumin removal	1		18-1139-44
Method Templates value table	1		18-1139-45

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