GE Healthcare

ÄKTAprime plus

User Manual

ÄKTA

Important user information

All users must read this entire manual to fully understand the safe use of ÄKTAprime plus.

WARNING!

The WARNING! sign highlights instructions that must be followed to avoid personal injury. It is important not to proceed until all stated conditions are met and clearly understood.

CAUTION!

The Caution! sign highlights instructions that must be followed to avoid damage to the product or other equipment. It is important not to proceed until all stated conditions are met and clearly understood.

Note

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

Declaration of conformity

This product meets the requirements of applicable CE-directives. A copy of the corresponding Declaration of Conformity is available on request.

The **CE** symbol and corresponding declaration of conformity, is valid for the instrument when it is:

- used as a laboratory device. It is not intended for clinical or in vitro use, of for diagnostic purposes.
- used as a stand-alone unit, or
- connected to other CE-marked GE Healthcare instruments, or
- connected to other products recommended or described in this manual, and
- used in the same state as it was delivered from GE Healthcare except for alterations described in this manual.

Recycling

This symbol indicates that the waste of electrical and electronic equipment must not be disposed as unsorted municipal waste and must be collected separately. Please contact an authorized representative of the manufacturer for information concerning the decommissioning of equipment.

Contents

About this manual

This manual describes how to run ÄKTAprime plus chromatography system using preprogrammed application templates and method templates. It also describes how to create methods and running the system manually.

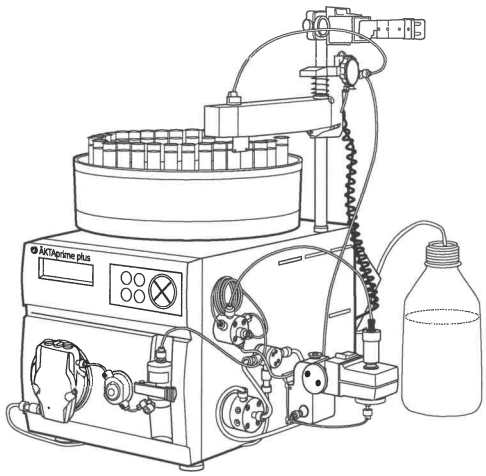
System description and instructions for installation, maintenance and troubleshooting are also included in this manual.

1 Introduction

This section is an introduction to ÄKTAprime™ plus, the documentation included and the content in this manual. It also contains an overview of the safety instructions.

1.1 General

ÄKTAprime plus is a compact liquid chromatography system designed for one-step purification of proteins at laboratory scale. When compared to traditional operations, ÄKTAprime plus offers significant advantages in terms of speed, capacity and fraction collection.



ÄKTAprime plus features:

- Easy unpacking and installation.
- Pre-programmed application templates for specific common purification steps.
- Method templates for all common chromatography techniques.
- Cue cards for simple and quick operation.
- Compatible with a range of prepacked columns, such as HiTrap™, HiPrep™ and RESOURCE™.
- High accuracy and reproducibility.
- Flow rates up to 50 ml/min and pressures up to 1 MPa.

The control system, monitor, pump and fraction collector, together with valves for buffer selection, sample injection, gradient formation and flow diversion, form a single, compact unit. The high precision on-line monitor includes the possibility to measure UV, conductivity and pH (optional).

The system is operated using the push buttons and LCD display at the front panel.

ÄKTAprime plus can be delivered with PrimeView™, a software that allows real time monitoring, evaluation and report generation on an external computer. The system can also be delivered with Recorder 112 for simpler data presentation.

A brief system description of ÄKTAprime plus can be found in chapter 4 System overview.

1.2 ÄKTAprime plus user documentation

The following user documentation is included in ÄKTAprime plus:

User documentation	Content
ÄKTAprime plus User Manual	System description. How to use the system, including safety instructions, concepts, operation, maintenance and troubleshooting.
ÄKTAprime plus Cue Cards	Short step-by-step instructions for selected applications using the preprogrammed application templates. System preparation and value table for the method templates.

For related literature, such as handbooks, methods and principles, see Ordering information.

1.3 ÄKTAprime plus User Manual

This manual has the following content:

Chapter	Content
Important user information	Regulatory and safety information.
1. Introduction	Brief introduction to ÄKTAprime plus, information about the user documentation and safety instructions.
2. Installation	Preparing the initial installation and performing the installation.
3. Making your first run	Short step-by-step instructions for preparing the system and performing a run using an application template.
4. System overview	Description of ÄKTAprime plus.
5. Making further runs	Detailed instructions for performing a run, for example for sample application, fraction collection, cold room operation, and storage.
6. Method programming	Programming a method using method templates or by editing line-by-line.
7. Template description	Detailed description of the application templates and method templates.
8. Installing and modifying components	Instructions for installing and assembling parts in the system. Instructions for recorder REC 112.
9. Maintenance	Maintenance schedules and instructions for preventive maintenance, replacing spare parts and calibration.
10. Troubleshooting	Overview of error symptoms, possible causes, and corrective actions. Error messages.
11. Reference information	Detailed hardware description, technical and chemical specifications, ordering information.

1.4 Safety

IMPORTANT! ÄKTAprime plus is intended for laboratory use only, not for clinical or *in vitro* use, or for diagnostic purposes.

- 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, take all suitable protective measures, such as wearing protective glasses and gloves resistant to the chemicals used. Follow local regulations and instructions for safe operation and maintenance of the system.

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 GE Healthcare may be used for maintaining or servicing the system.

WARNING! Use ONLY tubings supplied by GE Healthcare 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.

1.5 *Typographical conventions*

Keyboard options, menu selections and text on labels and panels are identified in the text by **bold** typeface. Menu commands, field names and dialog box prompts in PrimeView are also identified in the text by **bold** typeface.

A colon separates menu levels, thus **File:Open** refers to the **Open** command in the **File** menu.

2 Installation

This chapter describes unpacking and installation of ÄKTAprime plus. It also describes how to connect a computer to the system for using PrimeView for data collection.

2.1 General

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

ÄKTAprime plus is assembled, calibrated and fully tested before shipping. For safe transportation some components have been secured and need to be released from strappings.

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

After the installation procedure has been performed, ÄKTAprime plus is ready for purification work.

2.1.1 Installation procedure overview

2.2 Pre-requisites

- ÄKTAprime plus requires a working area of about 120 x 80 cm (width x depth). The mains power switch on the rear panel must always be easy to access.
- The system should be installed on a stable laboratory bench. To ensure correct ventilation, the system requires a free space of 0.1 m behind and in front of it. Do not place soft material under the system. It might block the ventilation inlet.
- The system can be operated at normal ambient temperatures in the range +4 to +40 °C. It should be located in a place of low temperature variations, away from heat sources, draughts and direct sunlight.
- ÄKTAprime plus requires 100–120/220–240 V~, 50–60 Hz electrical supply with safety grounding.
- Flasks for buffers and waste are needed.

2.3 Unpacking the system

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 Remove the cardboard hood, the red strap that secures the system to the pallet, and other packing material.

2 Check the contents against the enclosed packing list. Also check enclosed packages.

3 Store all boxes and plastic bags in a convenient nearby place.

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

CAUTION! Do not lift the system by the pillar.

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

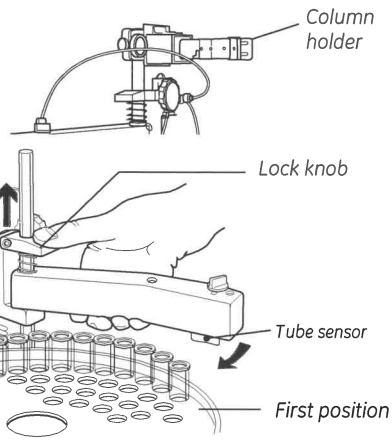
5 Open the plastic cover from the top and fold down to uncover the system.

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

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

2.4 Installing the system

1 Raise the column holder to the top position.

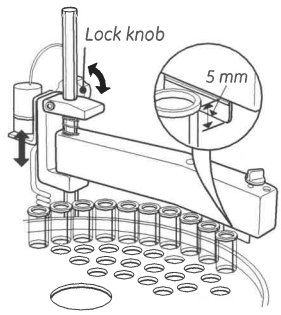


2 Put at least 20 collection tubes in the bowl, starting at the first position.

3 Loosen the lock knob holding the delivery arm and raise the arm.

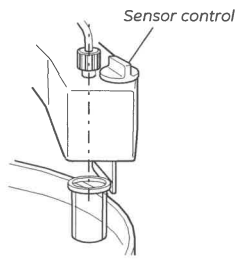
4 Adjust the delivery arm so that the tube sensor touches the collection tubes of the outer track.

5 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

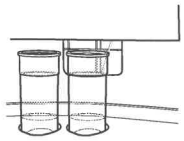


6 Lock the arm bracket at this height with the lock knob.

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



8 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.



9 Remove the inlet tubings and the brown waste capillaries from the plastic bag.

10 Put the inlet tubing **A1** and **B** in deionized water.

11 Put the waste capillaries **W1–W3** in waste.

2.5 *Connecting the mains cable*

WARNING! ÄKTAprime plus must be connected to a grounded mains socket to prevent system parts from becoming live.

WARNING! Only use mains cables delivered or approved by GE Healthcare.

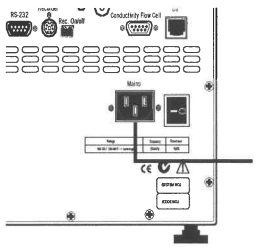
1 Turn ÄKTAprime plus to access the rear.

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 properly connected to the rear panel.

5 Turn ÄKTAprime plus to access the front.



To mains outlet

WARNING! Do not block the rear panel of the system. The mains power switch must always be easy to access.

2.6 Connecting a computer for PrimeView to the system

ÄKTAprime plus can be delivered with PrimeView™, a software that allows data collection, result evaluation and report generation on an external computer.

PrimeView package contains a software CD, user manual and serial cable.

Note: *Before connecting the computer, install PrimeView software on the computer according to PrimeView User Manual.*

WARNING! The computer should be installed and used according to the instructions provided by the manufacturer of the computer.

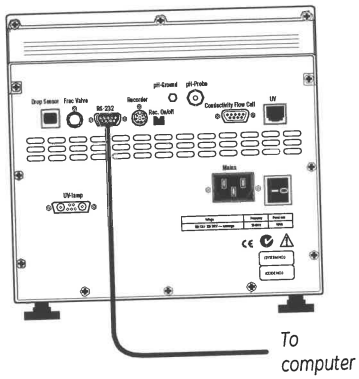
To connect a computer to the system:

1 Make sure the mains power to ÄKTAprime plus and the computer is turned off.

2 Connect the serial signal cable to socket **RS-232** on the system.

3 Connect the other end of the cable to the serial interface on the computer.

Note: *Data is sent through pin 3 and received through pin 2. Ground is on pin 5.*

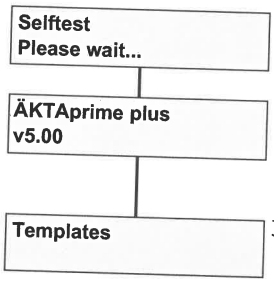


4 Switch on the mains power to ÄKTAprime plus and the computer.

5 Start PrimeView according to PrimeView User Manual.

2.7 System self-test

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



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

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.

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.

Note: *The system can be used for most applications after 15 min of lamp warm-up but the full specifications are not obtained until after 1 hour.*

Note: *At delivery, the flow path is filled with 20% ethanol as protection. The ethanol should be removed before a purification run. See section 3.8.1 Removing storage solution from the flow path*

3 Making your first run

This chapter contains step-by-step instructions for performing a typical purification run after the installation using an application template.

Note: *It can also be used as a guide for running the system on a daily basis.*

3.1 General

The run described in this section is an affinity purification of Histidine-tagged proteins using a HisTrap™ HP column. The sample is injected from the sample loop. PrimeView is used for monitoring, result evaluation and report generation.

Histidine-tag is mostly used for facilitating purification of expressed recombinant proteins. The Histidine-tag is small and does not usually interfere with the function, activity or structure of the protein. HisTrap HP column is designed for this purpose and has, for example high binding capacity and compatibility with several different additives.

For more information on performing a run, for example sample application, fraction collection, running method templates, and cold room operation, see chapter 5 Making further runs.

WARNING! When using hazardous chemicals, take all suitable protective measures, such as wearing protective glasses and gloves resistant to the chemicals used. Follow local regulations and instructions for safe operation and maintenance of the system.

3.2 Purification work flow

A typical purification run consists of the following main steps:

- Buffer preparation
- Sample preparation
- Purification setup
- Selecting template and starting the run
- Viewing the run
- Viewing the result
- Creating and printing a report

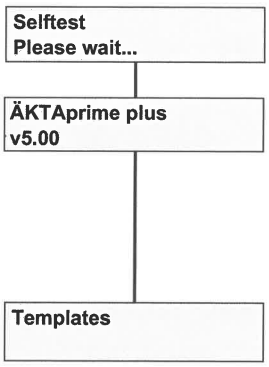
3.3 Pre-requisites

The system and computer must be installed and functioning as described in chapter 2 Installation.

IMPORTANT! Before using ÄKTAprime plus, read all the safety information in section 1.4 Safety.

3.4 Starting ÄKTAprime plus

If the system is not already turned on:



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

2 First the system name and software version number is displayed. Several messages are then shown during the self-test. If an error is detected during the self-test, an error message is shown.

3 All parameters are automatically set to factory default values during the self-test.

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

Note: *The system can be used for most applications after 15 min of lamp warm-up but the full specifications are not obtained until after 1 hour.*

3.5 Starting PrimeView

If the computer and PrimeView are not already started.

1 Turn on the computer.

2 Click **PrimeView** icon on the desktop of the Microsoft® Windows® operating system to open PrimeView module.

For customizing the view panes, see *PrimeView User Manual.*

3.6 Buffer preparation

- Use high-purity water and chemicals.
- Filter the buffers through a 0.45 µm filter before use.

Prepare at least 500 ml of the following buffers

Type of buffer	Solution
Binding buffer (A1)	20 mM sodium phosphate, 0.5 M NaCl and 30 mM imidazole, pH 7.4
Elution buffer (B)	20 mM sodium phosphate, 0.5 M NaCl and 0.5 M imidazole, pH 7.4

3.7 Sample preparation

1 Adjust the sample composition to the binding buffer by:

- diluting the sample in binding buffer, *or*
- buffer exchange using HiTrap Desalting or HiPrep 26/10 Desalting column.

2 Filter the sample through a 0.45 µm filter.

3.8 Purification setup

3.8.1 Removing storage solution from the flow path

At delivery and during storage, the flow path is filled with 20% ethanol. This should be removed before continuing the setup.

Note: *Do not use buffer with high salt concentration to flush out the ethanol. It might cause too high backpressure.*

To flush out the ethanol using deionized water:

1 Put the inlet tubing **A1–A8** that is used and **B** in deionized water.

Note: *At delivery, only A1 and B are installed.*

2 Put all waste capillaries, **W1–W3**, in waste.

Templates

3 Select **Templates** in the main menu using the △ and ▽ buttons and press **OK**.

Application template

4 Select **Application Template** and press **OK**.

System Wash Method

5 Select **System Wash Method** and press **OK**.

Select Buffer V. Pos
B,A: 2, 3, 4, 5, _, _, _ OK

6 Choose to wash the **A2–A8** inlet tubing that is used by pressing **OK** at those cursor positions. **A1** and **B** will always be washed.

Note: *At delivery, only A1 and B are installed.*

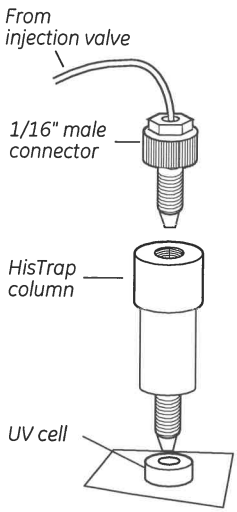
7 Scroll to **OK** and press the **OK** button.

Press OK to start run

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

9 When the method is finished, replace the first collection tube. It will contain a small amount of water after the system wash.

Removing large amounts of air from the inlet tubing
If there are large amounts of air in the inlet tubing, use the Purge kit to remove it as described in section 5.2.2 Purging pump and inlet tubing.



3.8.2 Preparing the tubing and column

1 Put inlet tubing **A1** in the binding buffer.

2 Put inlet tubing **B** in the elution buffer.

3 Put the three waste capillaries (brown color) from port **4** and **5** on the **INJECTION VALVE** and port **NO** on the fraction collector valve in waste.

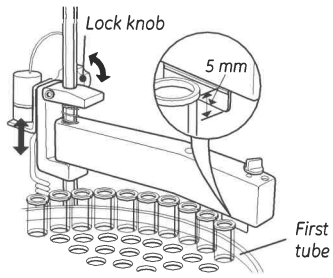
4 Connect the HisTrap HP 1 ml column between port **1** on the **INJECTION VALVE** and the upper port of the UV flow cell. Use a suitable length of PEEK tubing and 1/16" male connectors.

Note: *Other unions and connectors might be required for other columns.*

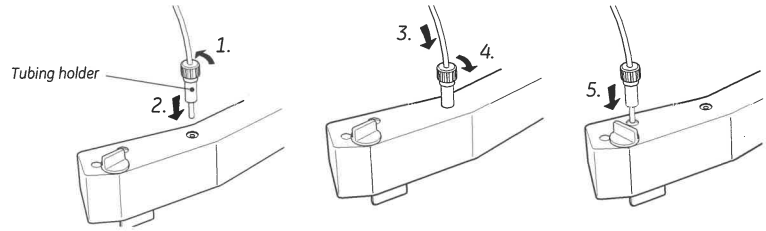
3.8.3 Preparing the fraction collector

1 Fill the fraction collector rack with 18 mm tubes (minimum 40 pcs.).

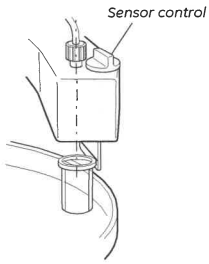
2 Adjust the height of the delivery arm using the lock so that the bottom of the tube sensor is about 5 mm below the top of the tubes. The tubes should always be below the horizontal mark on the tube sensor.



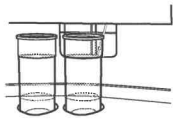
3 If necessary, adjust the length of the tubing exposed according to the sequence shown below.



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



5 Rotate the rack by hand until the rear half of the tube sensor rests against tube 1.



6 Press **feed tube** on the front panel. The bowl moves to the correct position to collect the first fraction in tube 1.

Set Parameters

Set Drop Sync Active (yes)

7 Make sure that drop synchronization is turned on.

Note: *Drop synchronization can NOT be used at flowrates above 3 ml/min.*

3.8.4 Preparing the monitors

1 Check the UV lamp filter position and the lamp position. See section 8.2 Optical unit.

2 Calibrate the pH electrode (optional). See section 9.20.5 Calibrating the pH electrode (optional).

3.8.5 Filling the buffer inlet tubing

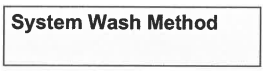
When running an application templates, the buffer inlet tubing will automatically be filled with buffer. The procedure below can then be ignored.

For other applications, fill the inlet tubing manually with buffer as described in the following procedure.

1 If there are large amounts of air in the tubing, fill the tubing using the Purge kit. See section 5.2.2 Purging pump and inlet tubing.

2 Select **Templates** in the main menu using the △ and ▽ buttons and press **OK**.

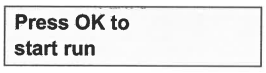
3 Select **Application Template** and press **OK**.



4 Select **System Wash Method** and press **OK**.

5 Select the used tubing. By default, **A1** and **B** will always be washed.

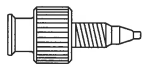
6 Move the cursor to **OK** and press **OK**.



7 Press **OK** to start the method.

8 When the method is finished, empty the first collection tube. It will contain a small amount of liquid after the system wash.

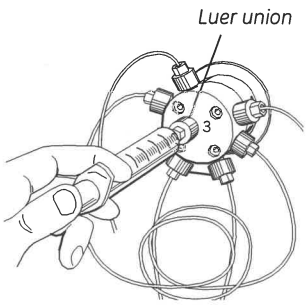
3.8.6 Filling the sample loop



Luer union

Using an injection fill port

1 Connect a sample loop between port **2** and **6** on the **INJECTION VALVE**. Make sure that the sample loop is large enough for your sample.



2 Connect a luer female/1/16" male union to port **3**.

3 Fill a syringe with five loop volumes of deionized water or binding buffer.

4 Fit the syringe in the luer union and carefully inject the buffer.

5 Remove the syringe and fill it with at least two loop volumes of the sample.

6 Carefully inject the sample into the sample loop.
Do NOT remove the syringe after the injection because the loop might otherwise be emptied due to self-drainage.

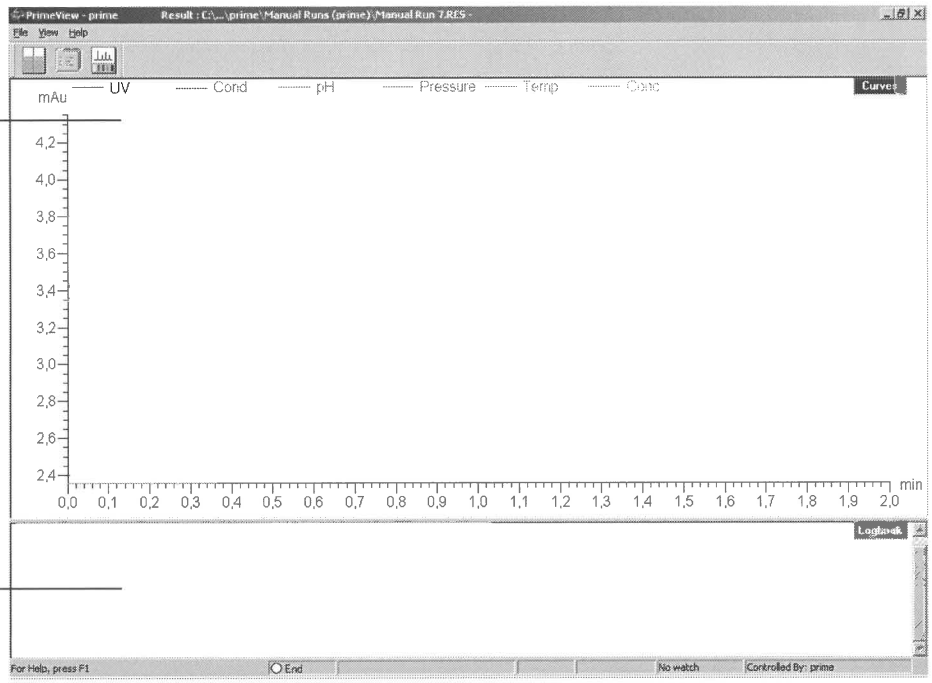
3.9 Selecting template and starting the run

Templates	1 Select **Templates** in the main menu and press **OK**.
Application Template	2 Select **Application Template** and press **OK**.
His Tag Purification HisTrap	3 Select **His Tag Purification HisTrap** and press **OK**.
Sample appl. volume 0.0	4 Set the sample volume and press **OK**.
Press OK to start run	5 Press **OK** to start the purification run.

3.10 Viewing the run

3.10.1 Viewing the run in PrimeView

When the pump starts running, the progress of the run can be viewed in the two panes in PrimeView.



- The **Curves** pane displays monitor signal values graphically.
- The **Logbook** pane displays all actions (e.g. method start and end, base instructions and method instructions) and unexpected conditions (e.g. warnings and alarms). The log is saved in the result file.

Selecting curves to be displayed

1 In **PrimeView** module, select **View:Properties**.

2 In the **Properties** dialog, click the **Curves** tab.

3 In the **Display curves** list, select the curves you want to display.

4 Click **OK**.

For more information on customizing the view panes, see *PrimeView User Manual*.

3.10.2 Finishing the run

Method Complete Press OK to continue

1 Press **OK** at the **Method Complete** prompt. This will cause all valves to return to their default positions.

3.10.3 Aborting a run

To abort a run before it is completed:

1 Press the **end** button.

End method? (yes) yes no

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

3.11 Viewing the result

PrimeView Evaluation module provides facilities for the presentation and evaluation of separation results.

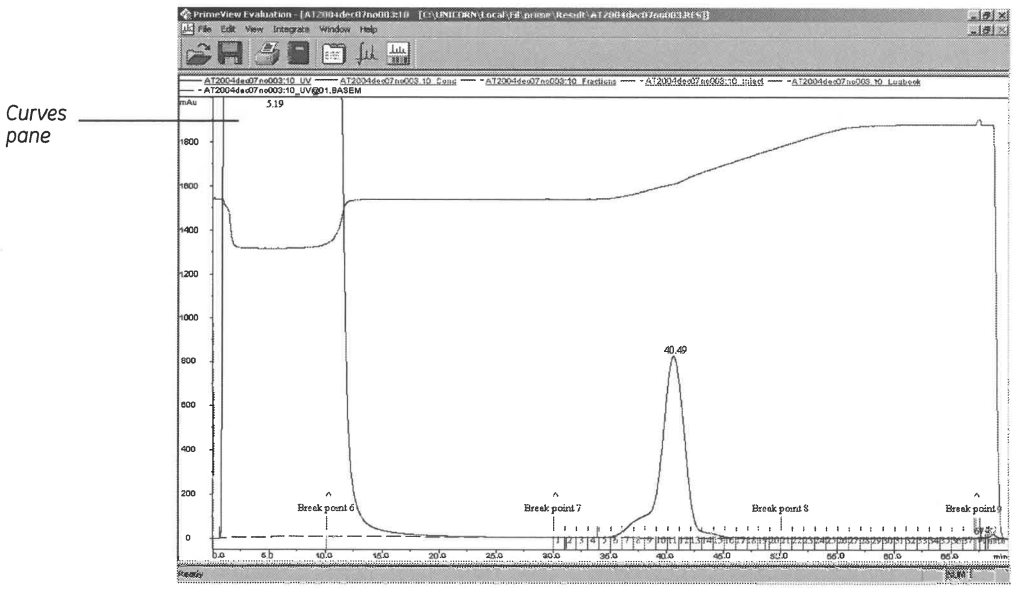
1 To start PrimeView Evaluation module, click **PrimeView Evaluation** icon on the Windows desktop.

3.11.1 Opening a result file

1 Select **File:Open** to open the Open Result dialog.

2 Select the result file (example: **AT2005feb12no001.res**) and click **OK**.

All contents of the opened result file are transferred to the Evaluation module and the chromatogram is automatically opened.



3.11.2 Changing the chromatogram layout

The chromatogram includes a number of curves that have been created during the method run, such as UV, conductivity, pH and fraction marks.

To change the layout of the chromatogram:

1 Right-click in the chromatogram window and select **Properties**.

The Chromatogram Layout dialog is opened.

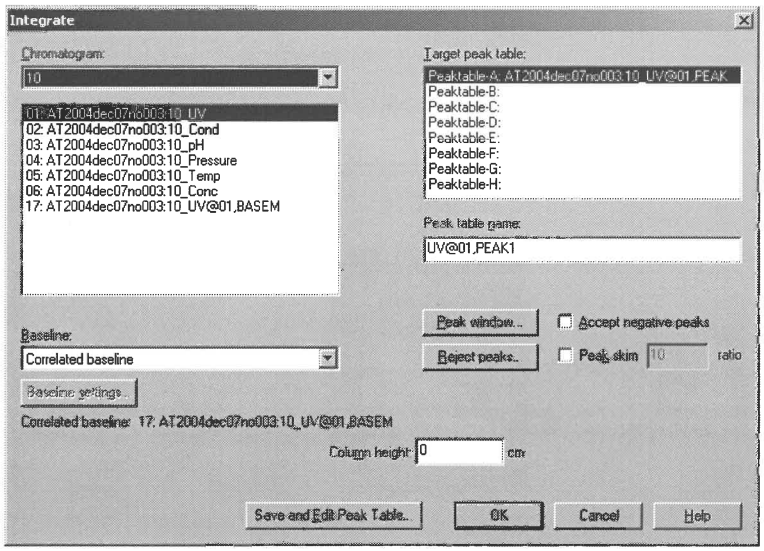
2 Carry out the changes on the different tabs to get the desired layout for header, curves and peak table.

3 Click **OK**.

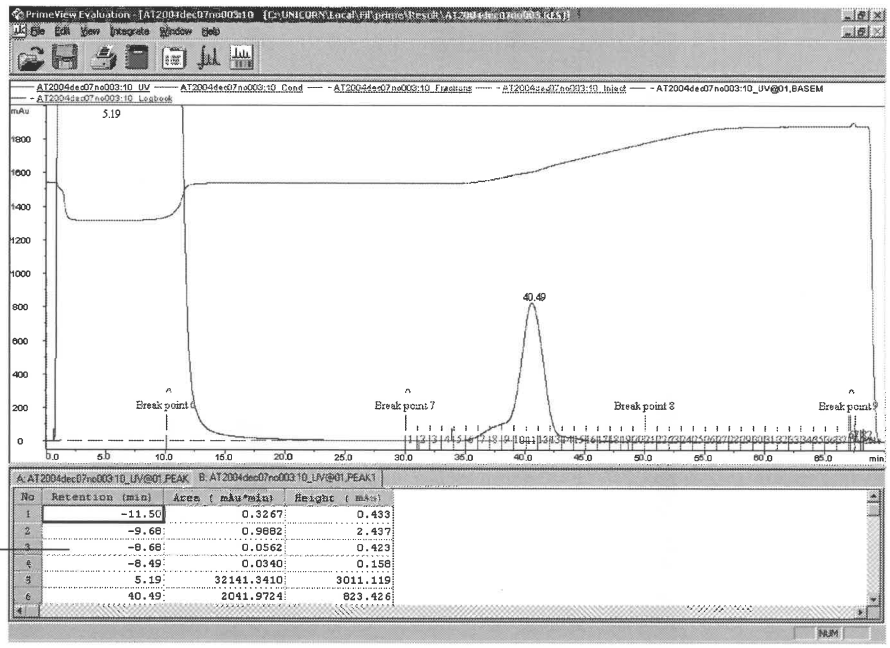
3.11.3 Integrating peaks

To make a simple integration of the UV curve peaks:

1 Select **Integrate:Peak integrate** to open the Integrate dialog.



2 Click **OK**. A peak table is added at the bottom of the module.

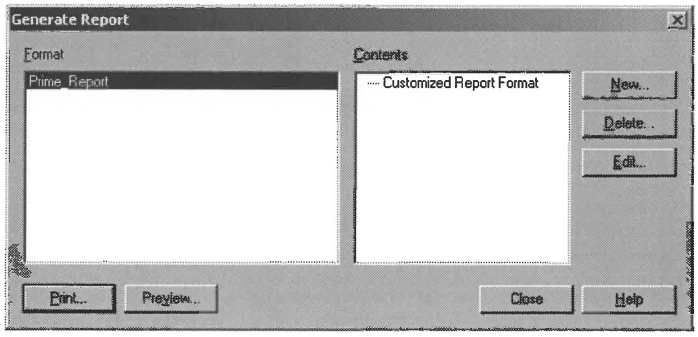


3.12 *Creating and printing a simple report*

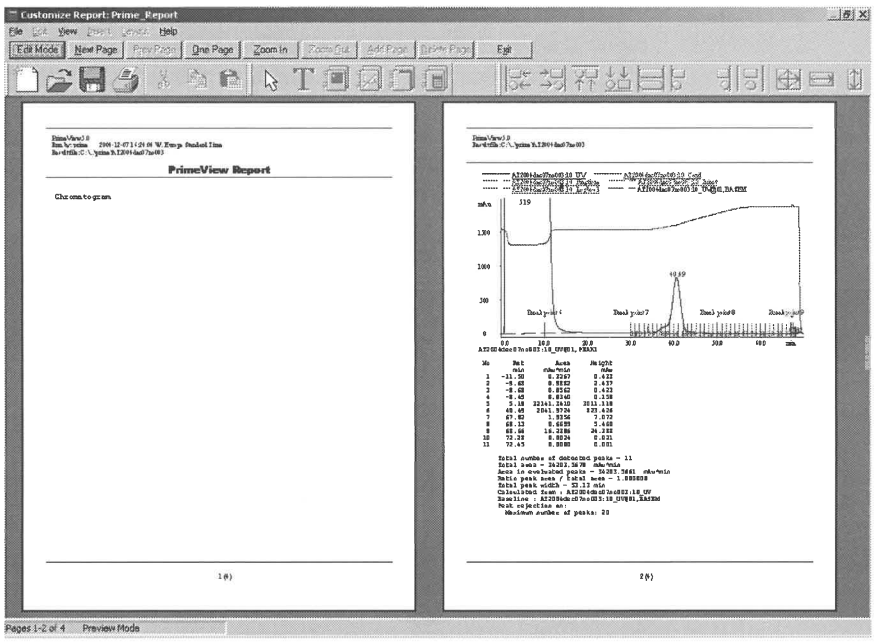
This description describes how create and print a simple report. However, it is possible choose from a variety of objects to include in a report, including chromatograms, methods, documentation, free text and more in the customized report interface.

To create and print a report:

1 Select **File:Report** to open the Generate Report dialog.

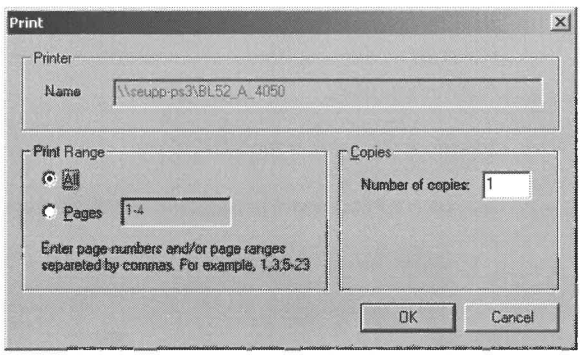


2 Click **Preview** to open the Customize Report and see the entire report layout.



3 Click **Exit** to return to the Generate Report dialog.

4 Click **Print** to open the Print dialog.



5 Click **OK** to print the report.

3.13 Cleaning after a run

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 might occur.

Buffers not containing any salt can be left in the system for a short time after a run, even overnight (not in the pH electrode, see instructions below).

CAUTION! If a buffer containing salt has been used, the flow path must be flushed with deionized water.

To flush the flow path:

1 Fill a syringe with five times the sample loop volume of deionized water.

2 Rinse the sample loop by injecting the water through the fill port on the injection valve.

3 Put all used inlet tubings in water.

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

5 Select the used inlet ports. Inlets **A1** and **B** will always be washed.

6 Press **OK** to start the method. The system flow path is now automatically flushed.

For information on cleaning and long-term storage, see section 5.11 Cleaning after a run and storage.

3.14 Making further runs

This chapter contains instructions for performing a specific purification run. More information on using ÄKTAprime plus further is found in chapter 5 Making further runs.

Chapter 5 Making further runs contains information on the following topics:

Other topics:

4 System overview

This chapter contains a brief overview of ÄKTAprime plus, including the system components, a system flow scheme with functional description and a description of the operator interface.

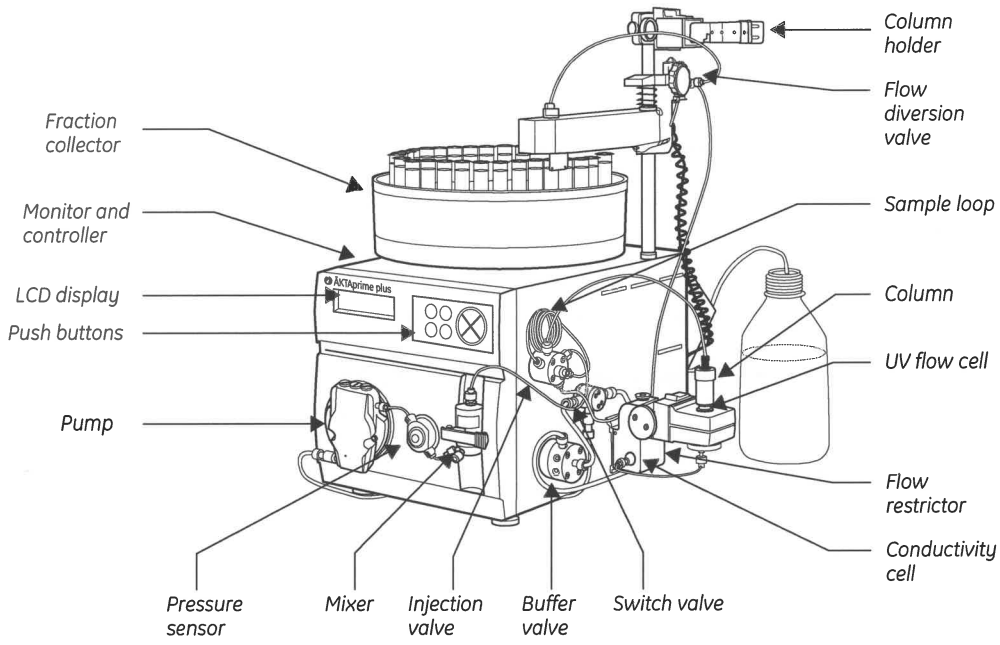
4.1 General

ÄKTAprime plus is a single, compact unit designed for simple protein purification at laboratory. The unit includes the control system, monitor, pump, fraction collector, and valves for buffer selection, sample injection, gradient formation and flow diversion.

The system is operated from the push buttons and LCD display at the front panel.

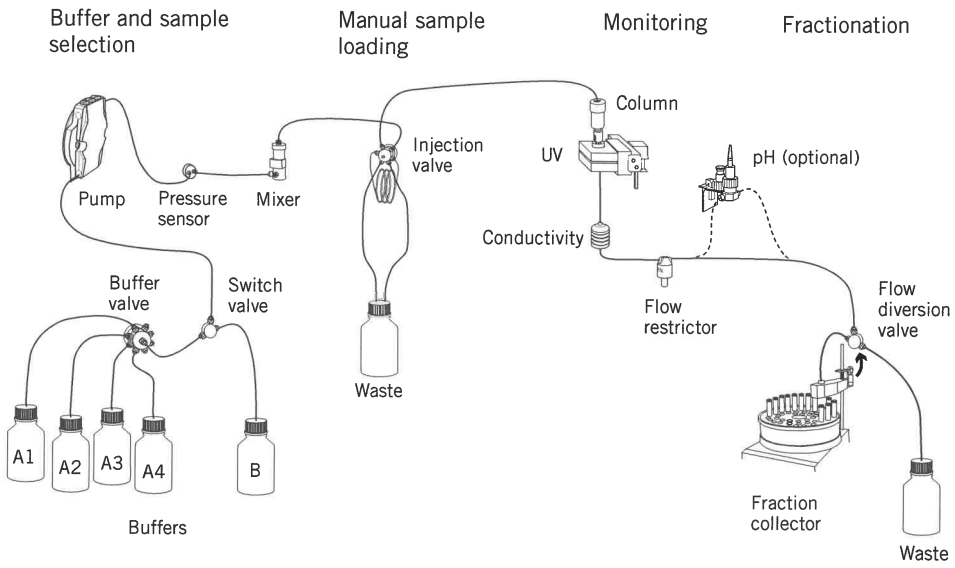
4.2 System components

The location of the system components are shown below.



4.3 System flow path

The illustration below shows the flow path of ÄKTAprime plus.



Functions available in ÄKTAprime plus:

- Automatic buffer and sample selection.
- Manual sample application.
  - Fixed volume loops for applying samples from 100 µl to 5 ml.
  - Superloop™ 10 ml, Superloop 50 ml and Superloop 150 ml.
- Sample loading through the pump for large sample volumes.
- Online monitoring of UV and conductivity (optional for pH).
- Peak detection and collection of up to 95 small fractions.
- Data presentation using PrimeView or Recorder 112 (optional).

4.4 *Functional description*

4.4.1 System control

ÄKTAprime plus controls all components within the system, including programming of the fraction collector. Application templates, method templates for the most common chromatographic techniques, or line-by-line programming can be used to create and run a purification. Up to 40 user-defined programs can be stored.

4.4.2 Liquid delivery

The single-channel pump delivers liquid with high precision over a wide flow rate range which provides fast and reproducible purifications. A 3-port switch valve and a mixer are used for gradient formation and a pressure sensor prevents damage to the column in case of too high pressure increase.

4.4.3 Flow path control

Two motorized rotary valves automatically control the flow path. The 8-port valve is used for buffer or sample selection and the 7-port valve for sample injection.

4.4.4 Monitoring

The high precision online monitor makes it possible to measure UV absorbance, conductivity and pH (optional). A temperature sensor inside the conductivity cell provides automatic temperature compensation for pH and conductivity measurements.

UV absorbance

UV detection wavelengths 254 and 280 nm are supplied with the system. Other wavelengths for special applications are available, including 214 nm if higher sensitivity is required.

Conductivity

The conductivity monitor gives reliable measurements over the range of values typically seen during purification of biomolecules.

pH (optional)

The true pH conditions during purification can be monitored and recorded by placing a flow cell containing the pH electrode into the flow path after the conductivity flow cell.

4.4.5 Fraction collection

Fractionation is performed by fixed volume collection or automatic peak fractionation. Peak fractionation can be based on peak detection using slope sensing. Fraction marks and fraction numbers allow easy identification of fractions and peaks.

A 3-port flow diversion valve allows automatic diversion to waste so that only required fractions are collected. A selection of rack sizes is available.

4.4.6 Data presentation

ÄKTAprime plus can be delivered with either PrimeView software for monitoring, evaluation and report generation or with Recorder 112 for simpler data presentation of runs.

PrimeView
PrimeView offers real time monitoring of the chromatography run for documentation and evaluation. Documentation is simplified since result files contain a complete record of a run including method, curve data and a run log. PrimeView allows rapid preparation of customized reports.

Recorder 112
Recorder 112 can be used to display data during the run, for example the UV trace and conductivity gradient, and other run data that has been stored during the run, such as flow, pressure, pH and theoretical gradient.

4.5 Columns and tubing

A wide range of pre-packed columns for the most commonly used techniques, such as ion exchange, gel filtration, hydrophobic interaction and affinity chromatography, can be used with ÄKTAprime plus. A list of the recommended pre-packed columns is given in section 11.5.1 Recommended columns.

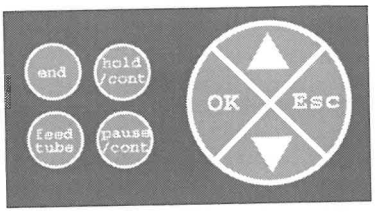
On delivery, the system is equipped with i.d. 1.6 mm inlet tubing, i.d. 0.75 mm tubing from the pump to the outlet, and i.d. 1.0 mm waste tubings.

When running columns with low maximum pressure limits at high flow rates, PEEK tubing with a larger inner diameter can be used instead to prevent increased backpressure, which might cause damage to the column.

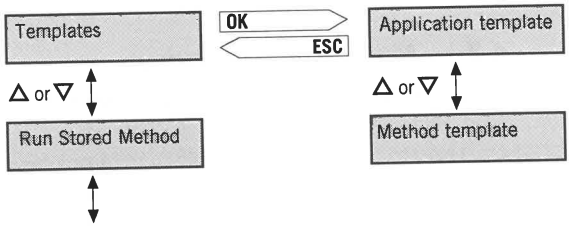
Note: *If tubings are changed, the delay volume to the fraction collctor must be changed.*

4.6 *Operator interface*

4.6.1 Menu navigation



- △ or ▽: Find a specific menu option.
- **OK**: Enter a menu.
- **Esc**: Return one menu level.



4.6.2 Control keys

end

- Interrupt method operation before the run is completed.
- Stop manual operation.

hold /cont

- Hold method time or volume and the gradient at the current concentration. Pump and fraction collector continue uninterrupted.
- Continue the normal method operation.

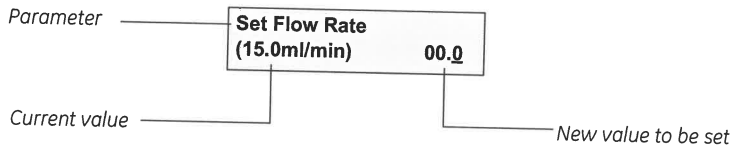
pause /cont

- Pause all operation without ending the method. All functions, including pump and fraction collector, are stopped.
- Continue the normal method operation.

feed tube

- Advance the fraction collector one position.

4.6.3 Changing a parameter value



To change a parameter value:

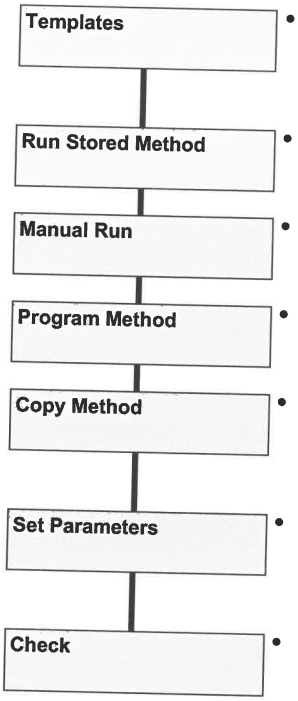
1 Press **OK** to enter the set value mode.

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**.

4.7 *Main menu overview*

The main menu contains the following options:



- Used to run pre-made application templates and method templates. This menu appears after the self-test when turning on the system.
  See sections 5.6 Starting a run and 5.8 Running a method template.
- Used to run methods that are programmed by the user.
  See section 5.9 Running a stored method.
- Used to run the system manually without using methods.
  See section 5.10 Running the system manually.
- Used to program user-specific methods.
  See chapter 6 Method programming.
- Used to copy methods between ÄKTAprime plus and an external computer connected to the serial interface of the system.
  See section 6.5 Copying a method.
- Used to calibrate and set parameters, for example for UV, conductivity, temperature, pressure and flow rate.
  See sections 9.20 Calibrations and 11.2.1 Set Parameters menus.
- Used to check system parameters, such as serial number, pump run time and lamp intensity.
  See section 11.2.2 Check menus.

5 Making further runs

This chapter describes operations and procedures related to the daily work with ÄKTAprime plus. It also gives further information to chapter 3 Making your first run.

The chapter also contains a list of other common topics and where to find more information about them.

5.1 General

This chapter contains information on the following topics:

Other topics:

5.2 *Preparing the system further*

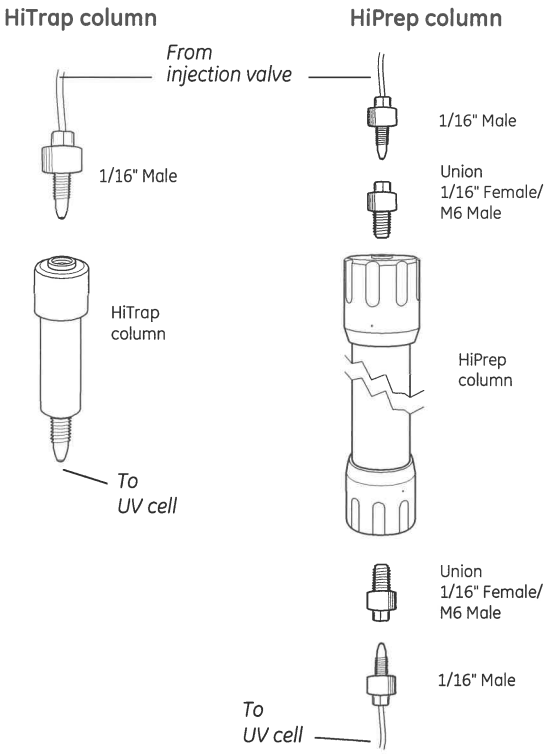
To prepare ÄKTAprime plus for a run, follow the instructions in chapter 3 Making your first run. It covers the most commonly used applications. This section contains additional instructions, for example how to connect different types of columns, purging the system flow path and different ways of injecting the sample.

5.2.1 Connecting different types of columns

1 If the column is large, use a column holder to secure it.

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

The illustration shows two columns, one with 1/16" fingertight fittings (HiTrap) and one with M6 fittings (HiPrep).



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

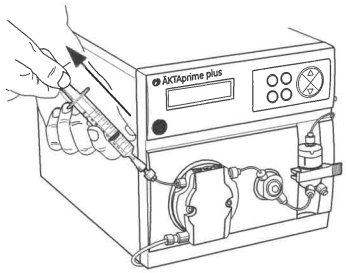
5.2.2 Purging pump and inlet tubing

If there are large amounts of air in the tubing or if you suspect air in the pump, use the Purge kit to purge the flow path . Air bubbles that still are trapped in the pump (causing increased pulsation) can be removed by flushing 100% ethanol through the pump. These two procedures are described in the following two sections.

Purging the flow path using the Purge kit

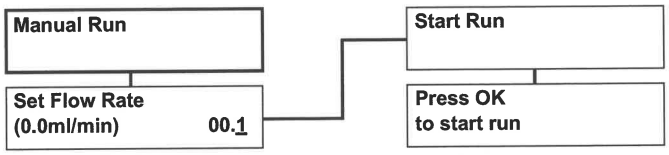
1 Remove the stop plug from the pump.

2 Connect the Purge kit to the pump



3 Put the used inlet tubing in the appropriate buffers.

4 Run the pump at 0.1 ml/min.



Filling inlet tubing A1–A8:

1 Go to **Set Buffer Valve** using the arrow buttons.

2 Set the chosen A inlet and click **OK**. The valve switches to the selected port.

3 Draw buffer with the purge syringe until liquid enters the syringe.

4 Repeat step 1–3 until all chosen A inlet tubing is filled.

Filling inlet tubing B:

1 Go to **Set Concentration %B** and set the concentration to **100%**

2 Click **OK**. The switch valve turns to the inlet B port.

3 Draw buffer with the purge syringe until liquid enters the syringe.

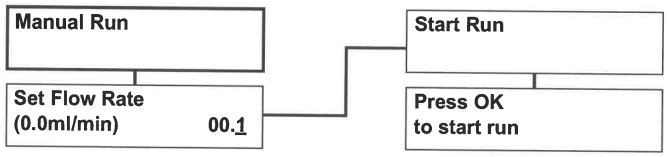
4 Replace the purge tubing with the stop plug.

5 Stop the pump by pressing **end** and then **OK**.

Flushing the pump with 100% ethanol

1 Put inlet tubing A1 in deionized water.

2 Run the pump at 40 ml/min for 1 min and press **pause/cont**.



3 Move inlet tubing A1 to 100% ethanol

4 Press **pause/cont**, run the pump for 10–20 s and press **pause/cont**.

5 Set the flow rate to 5 ml/min using the arrow buttons.

6 Press **pause/cont**, run the pump for at least 30 s and press **pause/cont**.

7 Move inlet tubing A1 to deionized water.

8 Press **pause/cont** and run the pump for 1 min.

9 Finish by pressing **end** and then **OK**.

5.3 Calibrations schedule

The table below lists the type and frequency of calibrations that can be done on ÄKTAprime plus. Refer to section 9.20 Calibrations for descriptions of how to perform these calibrations.

Component	How often
Pump	Only necessary after replacing wear parts.
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

5.4 *Applying the sample*

In ÄKTAprime plus, the sample can be applied in three ways depending on the volume to inject. The table below lists the options together with the recommended sample volume ranges.

Volume to inject	Sample application technique
25 µl–5 ml	Sample loop, manual filling
1–150 ml	Superloop, manual filling
> 10 ml	Pump together with buffer valve

The different sample techniques are described in the following sections.

5.4.1 Applying 25 µl–5 ml of sample using a sample loop

Partial or complete filling

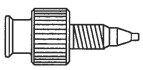
The sample loop can be filled partially or completely depending on whether high recovery or reproducible sample volumes are most important.

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.

Complete filling is used when reproducible sample volumes are required. An excess of sample is used to ensure that the sample loop is filled completely, . In preparative applications, the sample volume should be at least two times the volume of the sample loop. For analytical reproducibility, use five times the volume of the sample loop as sample volume. About 2–3 loop volumes of sample are required to achieve 95% of maximum loop volume. Five loop volumes gives better precision.

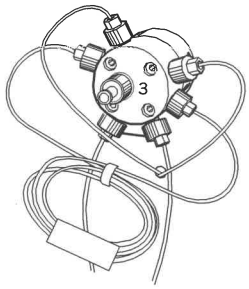
Preparation

Prepare the injection valve as follows:



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

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

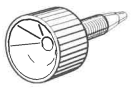


3 Mount the sample loop between ports 2 and 6 of the injection valve.

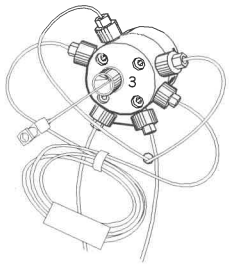
Five sizes of sample loops are available:

Sample loop	Code no.
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, 1 MPa	18-1140-53

An *injection fill port* can be used instead of the luer union connector. Then 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.

Note: *Use a needle with a round tip.*

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.

Filling the sample loop

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

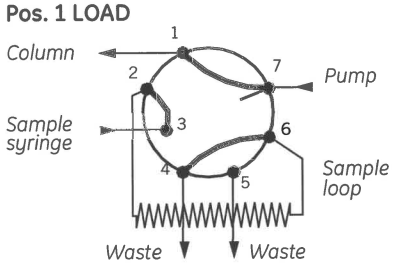
Partial filling

Partial loop 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.

Pos. 1 LOAD



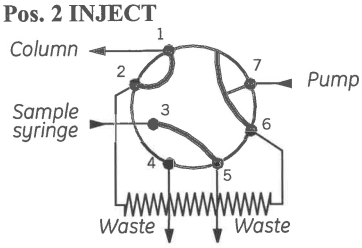
2 Load the syringe with a large volume of deionized water or binding buffer (at least 5 times the loop volume).

3 Fill the sample loop carefully.

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 enter the sample loop.*

Pos. 2 INJECT



5 Load the syringe with the required volume of sample (maximum 50% of the sample loop volume).

6 Insert the syringe into the luer union on the injection valve.

7 Set the injection valve to position LOAD.

8 Carefully inject the sample into the sample loop.
Do NOT remove the syringe after the injection! Otherwise, the loop might be emptied due to self-drainage.

9 The sample will be injected onto the column when the valve is switched to INJECT in the method.

Complete filling

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

Complete filling is achieved as follows:

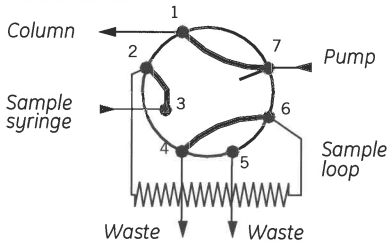
Note: *The flow must be off. For example, when running the system manually, press Pause.*

1 Set the injection valve to position LOAD.

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

3 Carefully inject the sample into the sample loop.
Do NOT remove the syringe after the injection! Otherwise, the loop might be emptied due to self-drainage.

Pos. 1 LOAD



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

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

5.4.2 Applying 1–150 ml of sample using a Superloop

Superloop allows introducing larger volumes of sample (1–150 ml) onto the column.

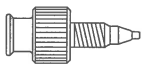
Superloop is an accessory available in three sizes:

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.

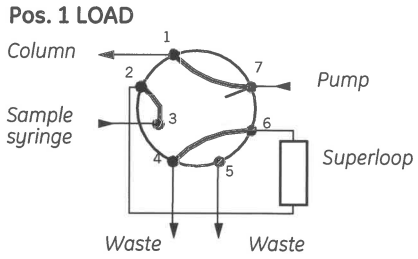
Preparation

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 Make sure that tubing for the waste is connected to port 4 of the injection valve.



3 Make sure 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.

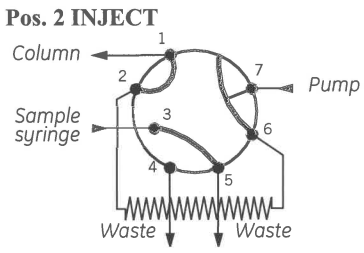
5 Connect the bottom tubing to injection valve port 2.

6 Connect the top tubing to injection valve port 6.

7 Make sure that all connections are fingertight.

Filling Superloop

Fill the Superloop as follows:



1 Set the injection valve to position INJECT.

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

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

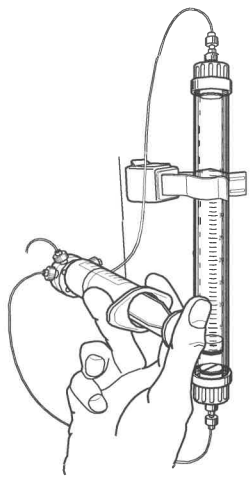
4 Load a large volume syringe with sample.

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.



5.4.3 Applying 10 ml or more of sample using the buffer valve

Larger sample volumes can be applied directly from a sample vessel through the buffer valve.

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

Preparation

1 The sample must be particle-free and filtered through a 0.45 µm filter. Otherwise, the inlet filter might get clogged quickly.

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

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

4 Fill the inlet tubing with sample. See section 5.2.2 Purging pump and inlet tubing.

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 must be set to position 8 and the injection valve to position LOAD when applying the sample.

2 In method templates, select sample application with pump.

3 Set the required parameters and the sample volume.

4 Start the run.

Note: *When using the buffer valve 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 the **System Wash** method. The standard recommendation is to pump 1 M NaOH for 30 minutes and then wash out immediately with buffer.

5.5 Collecting fractions

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

- Fixed volume fractionation
- Peak fractionation

Note: *Make sure that the fraction collector is properly installed and prepared before starting the run. See section 8.4 Fraction collector.*

5.5.1 Fixed volume fractionation

Fixed volume fractionation means that fixed fractions (fixed volume, time or number of drops) are collected within a set interval of time or volume. The fraction properties are preset in the application templates and the method templates. In method templates and other stored methods, they are set in the menus **Set Fraction Base** and **Set Fraction Size**. 0 means no fractionation.

Set Fraction Base
(ml) min ml drp

Set Fraction Size
(1.00 ml)

5.5.2 Peak fractionation

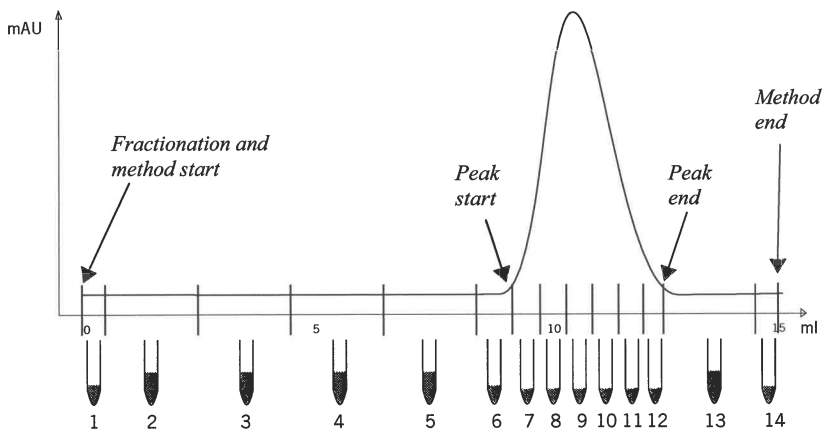
Peak fractionation allows you to collect peaks during the elution, besides fixed volume fractions. In this case, the slope of the curve decides when the actual fractionation should start and end.

Set Peak Collect
(no)

The properties for controlling the start and end points are set in the menu **Set Peak Collect**. **no** means no peak fractionation. Slope setting is described in section Setting peak collection on page 76.

Set Slope
(0.00 mAU/min) 0.00

The illustration shows a UV curve where fixed volumes are collected in tube 1–6 until the peak is detected. The peak is collected in tube 7–12.



5.6 Starting a run

5.6.1 Final checks

Before starting a 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 according to page 43. 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. For range setting, refer to section 8.6.5 Setting analog outputs.

6 Calibrate the pH electrode if required (optional). Refer to section 9.20.5 Calibrating the pH electrode (optional).

7 Check that the fraction collector has at least 40 tubes fitted.

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

9 If using a chart recorder or a computer for monitoring the run, make sure that it is set correctly.

5.6.2 Selecting an application template

ÄKTAprime plus 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 describes how to select and 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.

  See section 5.8 Running 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.

  See section 5.9 Running 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.

  See section 5.10 Running the system manually.

This section describes how to use an application template for performing a run.

This information can also be found on the cue cards supplied.

To use an application template:

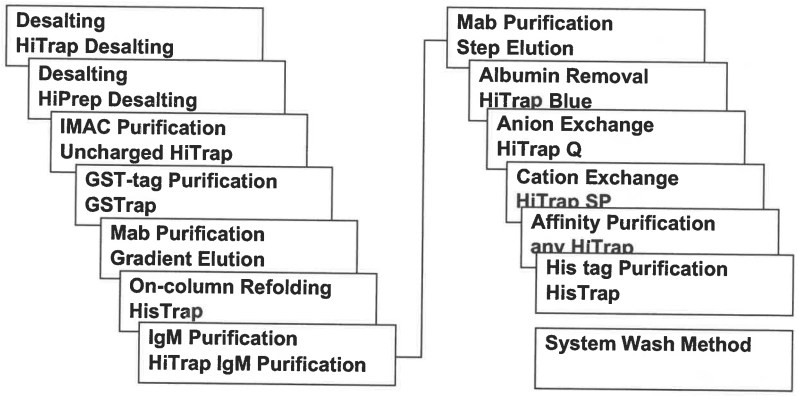
Templates

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**.

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 7 Template description.

5.7 During a run

5.7.1 Viewing the run

Besides viewing the run in PrimeView, the process parameters can be viewed directly on the front panel display.

Running display

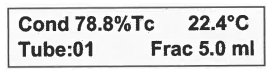
Four display alternatives with run data are available. Select the desired running display by pressing △ or ▽ .

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.

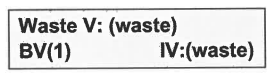
The available running modes are:

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.

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



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



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 on the recorder. See section 8.6 Recorder REC 112.

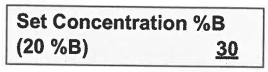
5.7.2 Changing parameters

Some of the process parameters can be changed during the run. They can be changed at any time during the run, except the gradient. The gradient can only be changed if no gradient is running or if the system is paused (press **pause/cont**) or held (press **hold/cont**).

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

Changing the concentration of buffer B

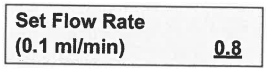
1 Select menu **Set Concentration %B**. The current setting is displayed. Press **OK**.



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

Changing the flow rate

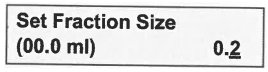
1 Select menu **Set Flow Rate**. The current setting is displayed. Press **OK**.



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 5.10 Running the system manually.

5.7.3 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.

5.7.4 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. If using a chart recorder, press **yes** for making a print-out (see section 8.6.6 Printing curves directly after a run). Otherwise, select **no**.

The run is now completed.

If using PrimeView, you can now evaluate the run and create a report.

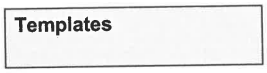
5.8 *Running a method template*

ÄKTAprime plus 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.

5.8.1 Selecting method template

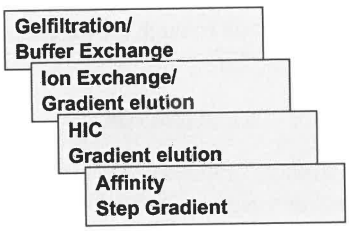
1 Perform the general preparation of the system according to the description in chapter 5.2 Preparing the system further.



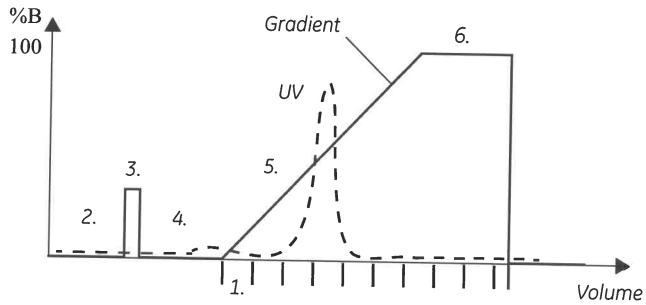
2 Select main menu **Templates** and press **OK**.

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

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



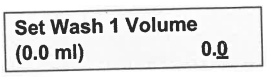
5.8.2 Setting the parameters



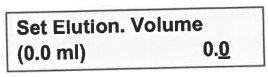
The figure above shows the theoretical gradient (concentration of B), the UV curve and fractionation marks. The numbers correspond to steps in the method to be defined before starting the run:

1. *Fraction size*
2. *Column equilibration volume*
3. *Sample application volume*
4. *Wash volume to remove unbound sample, etc. (Wash 1)*
5. *Elution volume*
6. *Wash volume to remove residues, etc. (Wash 2)*

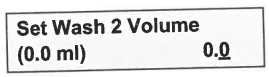
Sample inject by **InjV Pump**	1	Select sample injection through the injection valve or through the system pump.
Set Pressure Limit **(1.00 MPa) 1.00**	2	Set the pressure limit and press **OK**.
		Note: *The pressure limit should be set to the maximum backpressure limit of the column used* ***+ 0.2 MPa*** *(the back-pressure contribution from flow restrictor). The maximum backpressure limit is found in the column instruction. More information on using the flow restrictor is found on page 161.*
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 (*1.* in the figure) and press **OK**.
Set Equilibr. Volume **(0.0 ml) 0.0**	5	Set the equilibration volume (2.) and press **OK**.
Set Sample Inj. Volume **(0.0 ml) 0.0**	6	Set the sample volume (*3.*) to be injected and press **OK**.
		Note: *The sample volume entered should include sample wash out volume if needed.*



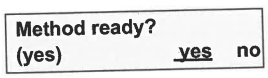
7 Set the wash 1 volume (4.) and press **OK**.
This setting does NOT apply to the Gel filtration method template.



8 Set the elution volume (5.) and press **OK**.

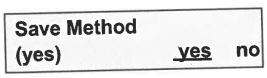


9 Set the wash 2 volume (6.) and press **OK**.
This setting does NOT apply to the Gel filtration method template.

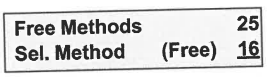


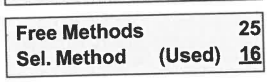
10 Select **yes** at the **Method ready?** prompt and press **OK**.

5.8.3 Storing the method



1 Select **yes** to store the method, then press **OK**.
Otherwise, select **no** and press **OK**.





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.

5.8.4 Starting the run

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

2 See section 5.7 During a run for a description of viewing and printing run data, and changing parameters during the run.

5.8.5 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 or from the computer. This is described in section 8.6.6 Printing curves directly after a run.

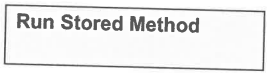
5.9 *Running a stored method*

The ÄKTAprime plus system can store up to 40 programmed methods (a computer connected to the system can store up to 999 methods). The methods are made either by using a method template or by programming line-by-line. Programming a method is described in chapter 6 Method programming.

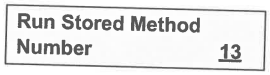
Go through the procedure below to run a stored method.

5.9.1 Selecting a stored method

1 Perform the general preparation of the system according to the description in chapter 5.2 Preparing the system further.



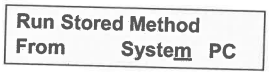
2 Select main menu **Run Stored Method** and press **OK**.



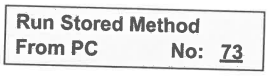
3 Select the method number and press **OK**.

If a computer running PrimeView is connected to the system, do as follows:

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

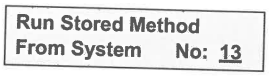


2 Select a method from either the system (**System**) or the computer (**PC**). Press **OK**.

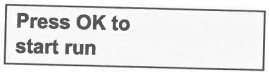


3 Select the method number and press **OK**.

or



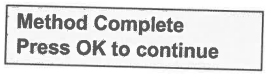
5.9.2 Starting the run



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

The progress of the method can be viewed on the computer monitor. See section 5.7 During a run for a description of viewing and changing parameters during the run.

5.9.3 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**.

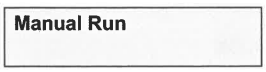
2 When the run is finished, the curves obtained can be printed from the computer.

5.10 Running the system manually

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

5.10.1 Preparing a manual run

1 Perform the general system preparation (refer to section 5.2 Preparing the system further).



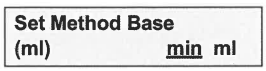
2 In the main menu, select menu **Manual Run** and press **OK**.

5.10.2 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**.

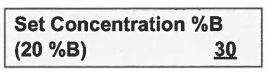


2 Select time (**min**) or volume (**ml**) and press **OK**.

Setting the concentration

Set the start concentration of buffer B as follows:

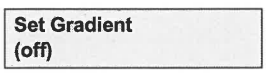
1 Select menu **Set Concentration %B**. The current setting is displayed. Press **OK**.



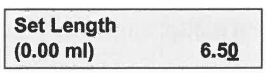
2 Set the desired concentration and press **OK**.

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**.



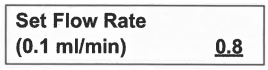
2 Set the length (volume or time) for the target concentration of buffer B to be reached. Press **OK**.

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

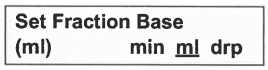
1 Select menu **Set Flow Rate**. The current setting is displayed. Press **OK**.



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

Setting the fraction base

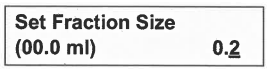
1 Select menu **Set Fraction Base**. The current setting is displayed. Press **OK**.



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

Setting the fraction size

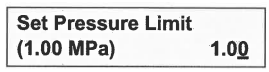
1 Select menu **Set Fraction Size**. The current setting is displayed. Press **OK**.



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**.

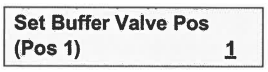


2 Set the pressure limit and press **OK**.

Note: *The pressure limit should be set to the maximum backpressure limit of the column used* ***+ 0.2 MPa*** *(the back-pressure contribution from flow restrictor). The maximum backpressure limit is found in the column instruction. More information on using the flow restrictor is found on page 161.*

Setting the buffer valve position

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



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**.

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.

5.10.3 Starting the run

Press OK to start run

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

5.10.4 During the run

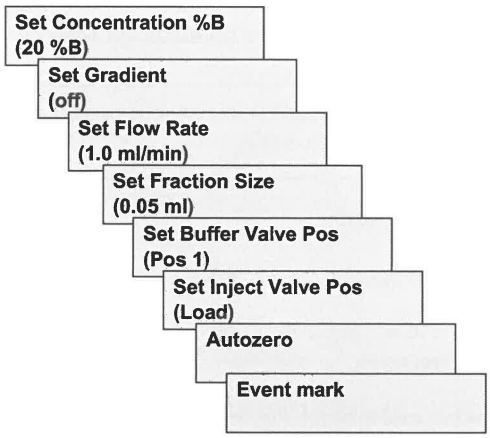
Viewing parameters

The progress of the method can be viewed on the computer monitor. See section 5.7 During a run for a description of viewing and changing parameters during the run.

Changing parameters

Most parameter values can be changed at any time during a manual run. The gradient can only be changed if no gradient is running or if the system is paused (press **pause**) or held (press **hold**).

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 5.10.1 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**.

5.10.5 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 can be printed from the computer.

5.11 Cleaning after a run and storage

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

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.

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.

5.11.1 Between runs

Buffers not containing any salt can be left in the system for a short time after a run, even overnight (not in the pH electrode, see instructions below).

CAUTION! If a buffer containing salt has been used, the flow path must be flushed with deionized water.

To flush the flow path:

1 Fill a syringe with five times the sample loop volume of deionized water.

2 Rinse the sample loop by injecting the water through the fill port on the injection valve.

3 Put all used inlet tubings in water

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

5 Select the used inlet ports. Inlets **A1** and **B** will always be washed.

6 Press **OK** to start the method. The system flow path is automatically flushed.

5.11.2 Storage overnight

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

CAUTION! If a buffer containing salt has been used, the flow path must be flushed with deionized water.

5.11.3 Weekend and long term storage

If you are not using the system for a few days or longer:

1 Wash all tubing and flow paths used with deionized water, for example by running the **System Wash Method** with all tubing inlets in water.

2 Replace the column with a bypass capillary.

3 Replace the pH electrode (optional) with a dummy pH electrode.

4 Wash the system with 20% ethanol and store it in 20% ethanol.

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.

5.12 Cold room operation

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

5.12.1 Preparation

1 Place the separation unit in the cold room.

2 Turn on the system. If the system already is turned on, turn on the UV lamp.

3 Allow the UV lamp to warm up for at least 15 minutes.

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

5 Tighten any leaking connector.

5.12.2 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.

Note: *The UV lamp should be turned off overnight.*

5.12.3 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.

6 Method programming

This section describes how to create customized methods using method templates or by programming line-by-line, how to edit stored methods and to copy methods.

6.1 General

Fully customized methods for purification can be created either by using a method template or by programming line-by-line.

A method consists of a series of *breakpoints* which define changes of one or more parameter values. The methods are programmed on a time or a volume base. ÄKTAprime plus can store up to 600 breakpoints in totally 40 user-defined methods. A computer connected to the system can store up to 999 methods.

To plan the method programming:

1 Illustrate the run by the progress of the gradient (the concentration of buffer B) during the run.

2 Define all breakpoints and the actions at the breakpoints that are required to achieve this progress.

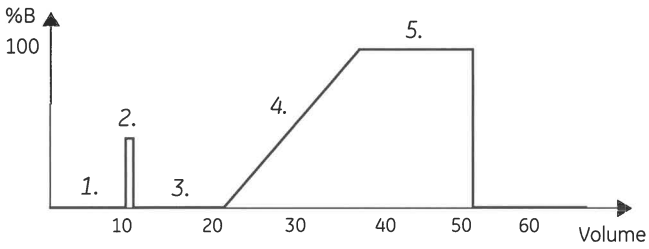
6.2 Programming using method templates

In the method templates, the breakpoints are predefined. Only the length between the breakpoints needs to be set, for example the length of the elution, in volume or time.

The illustration below shows an typical method for gradient elution with a linear gradient from 0 to 100%. The sample is loaded manually through the injection valve. The fraction collection 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.

Note: *The example only shows the general principles for programming using method templates. Some applications might need additional parameters. This is described in detail in chapter 7 Template description.*



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

6.2.1 Selecting method template

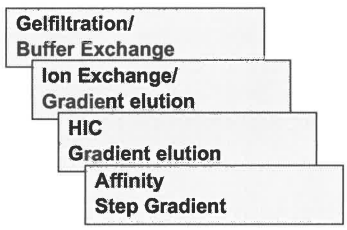
Templates

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**.



The method templates are described in chapter 7 Template description.

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

6.2.2 Setting the parameters

Sample inject by **InjV Pump**	1 Select sample injection through the injection valve or through the system pump. Refer to section 5.4 Applying the sample for more information about sample application.

Note: *When using the system pump for sample application, the sample tubing should be connected to port 8 on the Buffer valve.*

Set Pressure Limit **(1.00 MPa) 1.00**	2 Set the pressure limit and press **OK**.

Note: *The pressure limit should be set to the maximum backpressure limit of the column used* ***+ 0.2 MPa*** *(the back-pressure contribution from flow restrictor). The maximum backpressure limit is found in the column instruction. More information on using the flow restrictor is found on page 161.*

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**.

6.2.3 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

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 occupied.
Select a free method number and press **OK**. Alternatively, press **OK** to clear the number in the **Clear Method** menu.

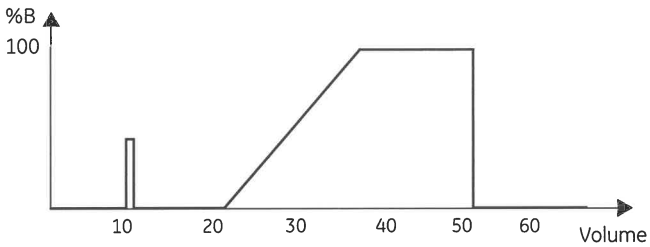
Free Methods		25
Sel. Method	(Used)	16

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

6.3 *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 in method. Values actively entered for each breakpoint are shown in bold. "S" represents the sample volume.



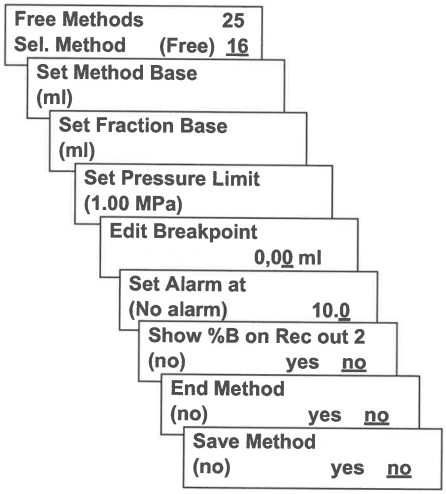
Breakpoint [ml]	Parameter	Conc %B	Flow	Fract.	Buffer V	Inject V
0	Equilibration	0	**1**	0	pos 1	LOAD
10	Sample applic.	0	1	0	pos 1	**INJECT**
10+S	Elution delay/wash	0	1	0	pos 1	**LOAD**
20+S	Elution, start fraction, start gradient	0	1	**1**	pos 1	LOAD
35+S	Column wash	**100**	1	1	pos 1	LOAD
49.9+S	End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

To program a new method line-by-line:

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.



6.3.1 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.

Method Occupied
(edit) edit clear

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

Clear Method 09 25
(yes) yes no

3 Select **yes** in the **Clear Method** menu and press **OK**.

6.3.2 General parameter set-up

Setting the method base

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

```
Set Method Base
(ml)          min ml
```

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

Setting the fraction base

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

```
Set Fraction Base
(ml)       min ml drp
```

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

Setting the pressure limit

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

```
Set Pressure Limit
(1.00 MPa)        1.00
```

2 Set the pressure limit and press **OK**.

Note: *The pressure limit should be set to the maximum backpressure limit of the column used* ***+ 0.2 MPa*** *(the back-pressure contribution from flow restrictor). The maximum backpressure limit is found in the column instruction. More information on using the flow restrictor is found on page 161.*

6.3.3 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

```
Edit Breakpoint
              0.00 ml
```

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**.

```
Edit Breakpoint
                NEW
```

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:

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

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

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

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

Setting the buffer valve position

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

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

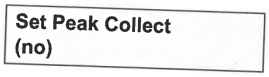
2 Set the position and press **OK**.

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.

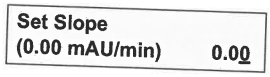
Setting peak collection

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 slope of the UV curve when a peak should be detected, 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**.

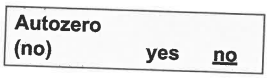


2 In the **Set Slope** menu, set the slope and press **OK**.

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

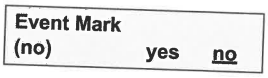
1 Select menu **Autozero** and press **OK**.



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

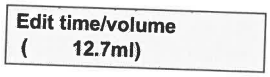
Setting an event mark

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

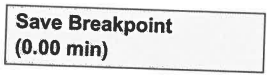


1 Select menu **Edit time/volume** and press **OK**.

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**.

6.3.4 Setting an alarm

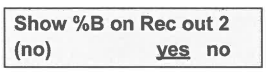
If an alarm should sound during or after the run:

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.

6.3.5 Printing the method

If using a chart recorder, print the programmed method (concentration of B curve) on recorder channel 2 as follows:



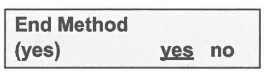
1 Go to the **Show %B on Rec out 2** menu.

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

If using a computer, it also possible to print the method from the computer. Refer to the *PrimeView User Manual* for more information.

6.3.6 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.

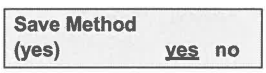
2 Select **yes** and press **OK**.

3 Select a final breakpoint with the same parameters.

6.3.7 Saving the method

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

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

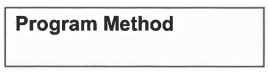


2 Select **yes** and press **OK**.

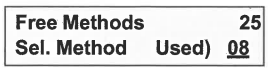
6.4 *Editing a stored method*

To edit an existing method, follow the instruction below. Refer to section 6.3 Programming line-by-line for more detailed information about setting the parameters.

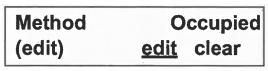
6.4.1 Selecting method



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

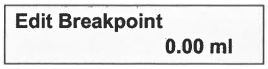


2 Select the number of the method and press **OK**.



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 6.3 Programming line-by-line).

6.4.2 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.

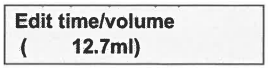
5 Save the new parameter values by pressing **OK**.

6.4.3 Editing time/volume of a breakpoint

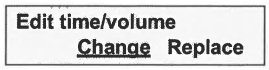
1 Go to the **Edit Breakpoint** menu.

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

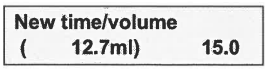
3 Press **OK** at the desired breakpoint to enter the parameter menus.



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

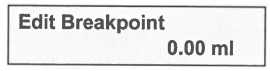


5 Select **Change** or **Replace** and press **OK** to edit the time/volume of the breakpoint.
Change will also change the time/volume of all the following breakpoints accordingly.
Replace will not change the time/volume of the other breakpoints.



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

6.4.4 Inserting a new 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**.

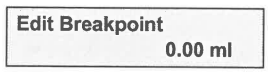
3 Press **OK** at breakpoint **NEW** to create a new breakpoint. This breakpoint will have the value **0.00**.

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**.

6.4.5 Deleting a breakpoint

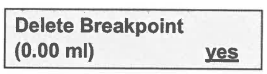


1 Go to the **Edit Breakpoint** menu.

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

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

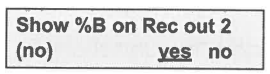
4 Select the **Delete Breakpoint** menu and press **OK**.



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

6.4.6 Printing the method

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

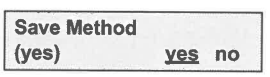


1 Go to the **Show %B on Rec out 2** menu.

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

6.4.7 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**.

6.5 *Copying a method*

An existing method can be copied to another method number in the system. The system totally has 40 method numbers.

By connecting a computer running PrimeView to the system, an extra 999 methods can be stored for future use. However, a method always has to be stored on the system first. The Copy Method function can then be used to copy it to the computer.

To copy a method to another method number:

Note: *Displays including the **PC** option only appear when a computer running PrimeView is connected to the system.*

Copy Method

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

Copy Method From ?
System PC

2 At the **Copy Method From ?** sub menu, select from which unit you want to copy the method (**System** = ÄKTAprime plus system, **PC** = computer). Press **OK**.

Copy Method From
System No: 25

3 Enter the number of the method and press **OK**.

Copy Method To ?
System PC

4 At the **Copy Method To ?** sub menu, select to which unit you want to copy the method. Press **OK**.

Copy Method To
System (free) No: 29

Copy Method To
PC (used) No: 285

5 Enter the new number of the method and press **OK**.

Free means that the selected number is free for storing a new method.
Used means that the number is already occupied.

Method occupied
Clear it? yes no

6 If the **Method occupied** display appears, select to clear the number or not (**yes** or **no**). Press **OK**.

7 Confirm the selected procedure by pressing **OK**.

Copy Method Press OK
PC 274 -> System 29

Copy Method Press OK
System 25 -> PC 285

Copy Method Press OK
PC 274 -> PC 285

Copy Method Press OK
System 25 -> System 29

Copy Method
Please wait!

The method is copied to the new method number.

7 Template description

7.1 General

ÄKTAprime plus contains a collection of templates for common chromatographic applications.

The templates are grouped into two categories:

- Application templates.
  Only the sample volume needs to be entered before starting a run.
- Method templates.
  Require more information to be entered, e.g. flow rate, elution volume and equilibration volume. Methods that are made from the method templates can also be stored for future use.

7.2 Application templates

7.2.1 General

The following application templates are included:

- Desalting
- Affinity purification
- Purification of Histidine-tagged proteins
- Purification of GST-tagged proteins
- Purification of monoclonal antibodies
- IgM purification
- Removal of albumin
- Protein on-column refolding
- Anion exchange
- Cation exchange
- System Wash Method

The process parameters in the application templates can not be changed. Only the sample volume needs to be entererd.

- Sample application is always made by using a syringe and a sample loop.
- The buffer solutions to use are described in the application cue card.

For more information on how to run an application template, refer to section *5.6 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.

7.2.2 Selecting 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 Select the desired template with the up and down buttons.

7.2.3 HiTrap desalting

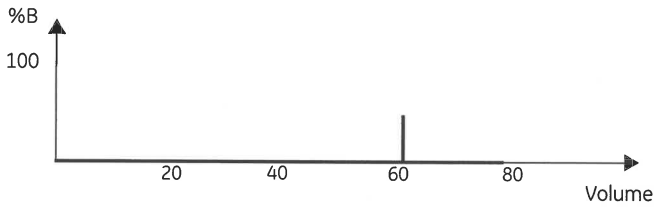
The HiTrap desalting application template is used for desalting a sample solution.

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



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

7.2.4 HiPrep desalting

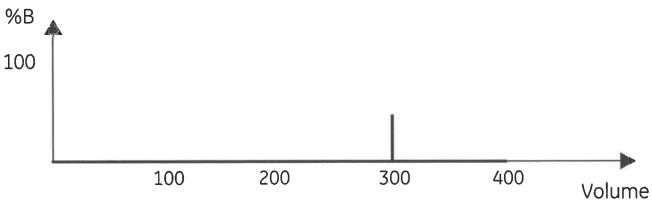
The HiPrep desalting application template is used for desalting a sample solution.

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



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

7.2.5 IMAC purification

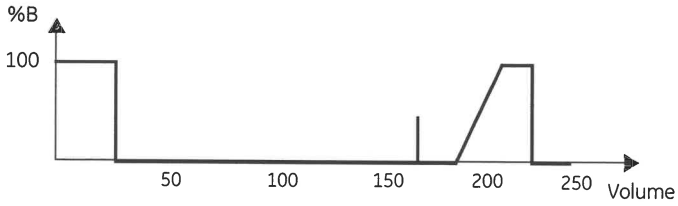
The IMAC purification application template is used for purification of Histidine-tagged proteins.

IMAC Purification Uncharged HiTrap

To access the template, select **IMAC Purification Uncharged HiTrap** and press **OK**.

Column: HiTrap Chelating

Total run time: approx. 87 min + sample application time



Action	Volume	Conc %B	Flow	Fract.	Buffer V	Inject V
Priming B	0	100	40	0	pos 2	WASTE
End priming B	25	100	40	0	pos 2	WASTE
Priming A2	25.1	0	40	0	pos 2	WASTE
Water wash	60	0	1	0	pos 2	LOAD
Priming A3	65	0	40	0	pos 3	WASTE
Metal ion application	100	0	1	0	pos 3	LOAD
Priming A2	101	0	40	0	pos 2	WASTE
Water wash	116	0	1	0	pos 2	LOAD
Priming A1	121	0	40	0	pos 1	WASTE
Equilibration	156	0	1	0	pos 1	LOAD
Autozero	166					
Sample application	166	0	1	0	pos 1	INJECT
Buffer wash	166+S	0	1	0	pos 1	LOAD
Elution	186+S	0	1	1	pos 1	LOAD
Elution wash out	206+S	100	1	1	pos 1	LOAD
End wash	223+S	100	1	0	pos 1	LOAD
Priming A2	223.1+S	0	40	0	pos 2	WASTE
Re-equilibration	238+S	0	1	0	pos 2	LOAD
End method	243+S					

7.2.6 GST-tag purification

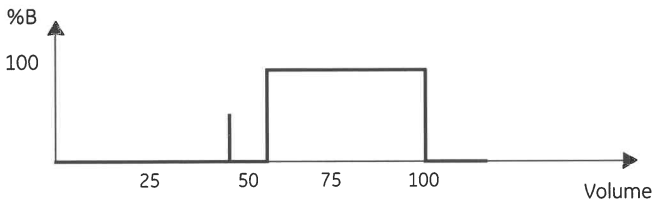
The GST-tag purification application template is used for purification of GST-tagged proteins.

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



Action	Volume	Conc %B	Flow	Fract.	Buffer V	Inject V
Priming A1	0	0	40	0	pos 1	WASTE
Equilibration	35	0	1	0	pos 1	LOAD
Autozero	45					
Sample application	45	0	0.3	0	pos 1	INJECT
Wash	45+S	0	1	0	pos 1	LOAD
End wash	55+S	0	1	0	pos 1	LOAD
Priming B	55.1+S	100	40	0	pos 1	WASTE
Elution	90+S	100	1	1	pos 1	LOAD
End elution	100+S	100	1	0	pos 1	LOAD
Priming A1	100.1+S	0	40	0	pos 1	WASTE
Re-equilibration	115+S	0	1	0	pos 1	LOAD
End method	120+S					

7.2.7 Mab purification (step elution)

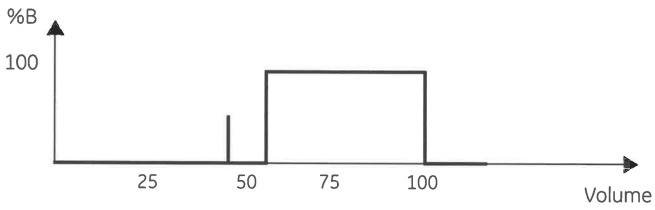
The Mab purification (step elution) application template is used for purification of monoclonal antibodies by step elution.

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



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

7.2.8 Albumin removal

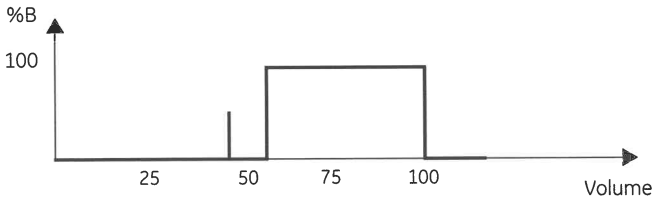
The Albumin removal application template is used for removing albumin.

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



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

7.2.9 Mab purification (gradient elution)

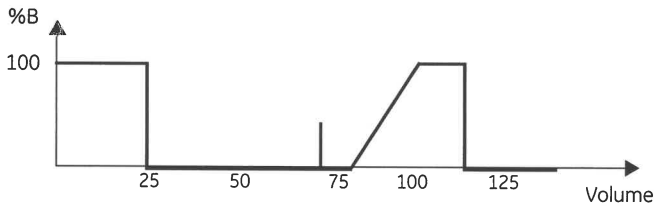
The Mab purification (gradient elution) application template is used for purification of monoclonal antibodies using a pH gradient

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



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

7.2.10 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

For buffer gradient and parameter table, see Mab purification (gradient elution).

7.2.11 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

For buffer gradient and parameter table, see Mab purification (gradient elution).

7.2.12 IgM purification

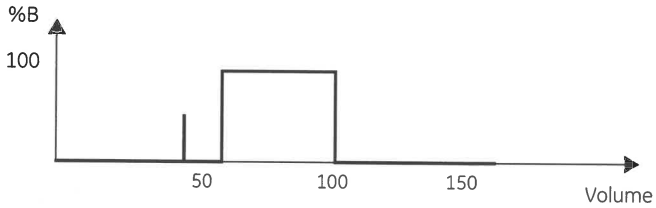
The IgM purification application template is used for purification of monoclonal antibody IgM.

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



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

7.2.13 On-column refolding

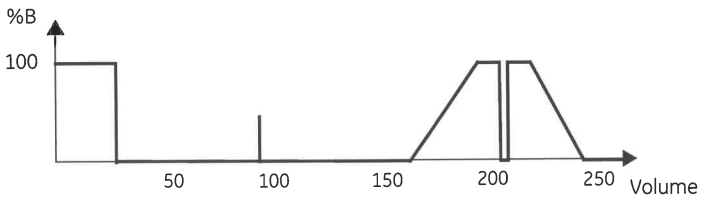
The On-column Refolding application template is used for protein renaturation on a column.

On-column Refolding HisTrap

To access the template, select **On-column Refolding HisTrap** and press **OK**.

Column: HisTrap 1 ml

Total run time: approx. 160 min + sample application time



Action	Volume	Conc %B	Flow	Fract.	Buffer V	Inject V
Priming B	0	100	40	0	pos 3	WASTE
End priming B	25	100	40	0	pos 3	WASTE
Priming A3	25.1	0	40	0	pos 3	WASTE
Priming A1	50	0	40	0	pos 1	WASTE
Equilibration	85	0	1	0	pos 1	LOAD
Autozero	95					
Sample application	95	0	1	0	pos 1	INJECT
Buffer wash 1	95+S	0	1	0	pos 1	LOAD
Priming A2	115+S	0	40	0	pos 2	WASTE
Buffer wash 2	150+S	0	1	0	pos 2	LOAD
Refolding	160+S	0	0.5	0	pos 2	LOAD
End refolding	190+S	100	0.5	0	pos 2	LOAD
Priming A3	200+S	100	40	0	pos 3	WASTE
Buffer wash 3	200.1+S	0	40	0	pos 3	WASTE
Priming B	205+S	0	40	0	pos 3	WASTE
Buffer wash B	205.1+S	100	40	0	pos 3	WASTE
Elution	220+S	100	1	1	pos 3	LOAD
Re-equilibration	240+S	0	1	1	pos 3	LOAD
End method	257+S					

7.2.14 Affinity purification

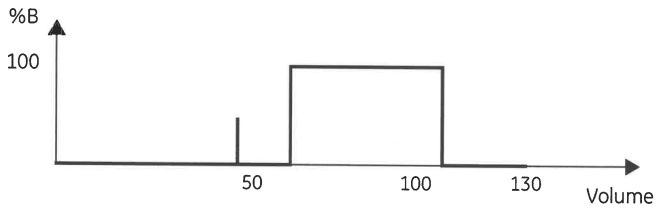
The Affinity purification application template is used for purification of, for example benzamidine.

Affinity Purification any HiTrap

To access the template, select **Affinity Purification any HiTrap** and press **OK**.

Column: any HiTrap column

Total run time: approx. 47 min + sample application time



Action	Volume	Conc %B	Flow	Fract.	Buffer V	Inject V
Priming A1	0	0	40	0	pos 1	WASTE
Equilibration	35	0	1	0	pos 1	LOAD
Autozero	45					
Sample application	45	0	1	1	pos 1	INJECT
Wash (incl. frac.)	45+S	0	1	1	pos 1	LOAD
Wash (excl. frac.)	52+S	0	1	0	pos 1	LOAD
End wash	65+S	0	1	0	pos 1	LOAD
Priming B	65+0.1+S	100	40	0	pos 1	WASTE
Elution	100+S	100	1	1	pos 1	LOAD
End elution	110+S	100	1	0	pos 1	LOAD
Priming A1	110+0.1+S	0	40	0	pos 1	WASTE
Re-equilibration	125+S	0	1	0	pos 1	LOAD
End method	130+S					

7.2.15 Histidine-tag purification

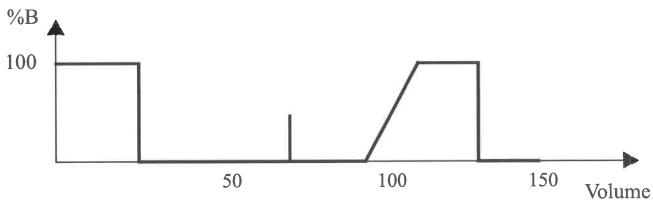
The Histidine-tag purification application template is used for purification of Histidine-tagged proteins.

His Tag Purification HisTrap

To access the template, select **His Tag Purification HisTrap** and press **OK**.

Column: HisTrap HP 1 ml

Total run time: approx. 74 min + sample application time



Action	Volume	Conc %B	Flow	Fract.	Buffer V	Inject V
Priming B	0	100	40	0	pos 1	WASTE
End priming B	25	100	40	0	pos 1	WASTE
Priming A1	25+0.1	0	40	0	pos 1	WASTE
Equilibration	60	0	1	0	pos 1	LOAD
Autozero	70					
Sample application	70	0	1	0	pos 1	INJECT
Wash 3	70+S	0	1	0	pos 1	LOAD
Elution	90+S	0	1	1	pos 1	LOAD
Wash 4	110+S	100	1	1	pos 1	LOAD
End wash 4	127+S	100	1	0	pos 1	LOAD
Priming A2	127+0.1+S	0	40	0	pos 1	WASTE
Re-equilibration	142+S	0	1	0	pos 1	LOAD
End method	147+S					

7.2.16 System wash

The System wash template is used for rinsing and priming the tubings and the components in the system 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: *A1 and B are pre-selected and will always be washed.*

Total run time: depends on the number of buffer inlets selected. If all are selected, the approximate run time is 9 min.

The table below shows how the tubings are washed.

Action	Volume	Conc %B	Flow	Fract.	Buffer V	Inject V
Wash B	0	100	50	0	pos 8	WASTE
End wash B	24,9	100	50	0	pos 8	WASTE
Wash A8	25	0	50	0	pos 8	WASTE
Wash A7	50	0	50	0	pos 7	WASTE
Wash A6	75	0	50	0	pos 6	WASTE
Wash A5	100	0	50	0	pos 5	WASTE
Wash A4	125	0	50	0	pos 4	WASTE
Wash A3	150	0	50	0	pos 3	WASTE
Wash A2	175	0	50	0	pos 2	WASTE
Wash A1	200	0	50	0	pos 1	WASTE
Air wash out	225	0	50	0	pos 1	WASTE
Wash Inject v. load	250	0	0.5	0	pos 1	LOAD
Wash Frac tubing	251	0	0.5	2	pos 1	LOAD
End Method	252					

7.3 Method templates

7.3.1 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**.

The following selections must be made before starting the run:

- Pressure limit, flow rate, fraction size and the volumes during the main steps of the run. The selected values depend on the choice of column (see the *Method Templates value table* cue card for recommended values).
- Sample injection using the sample pump or via the sample port.
  Any volume changes due to the selection are handled automatically within the templates.

  Note: *When using the pump and buffer valve sample application, the sample is always applied through port 8 on the buffer valve.*

In the following sections, the method templates are illustrated by the buffer gradient during the run. The table below the gradient shows how the parameters to be entered correspond to the steps during the run.

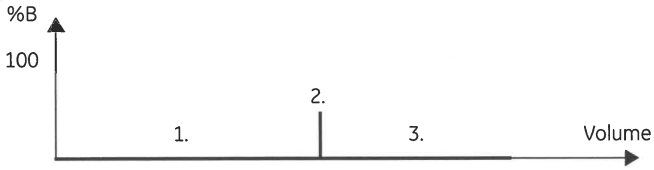
"F" in the tables represents the recommended flow rate of the column that is used (see the *Method Template value table* cue card).
"V" represents the fractionation volume.

For more information on how to run a method template, refer to section 5.8 Running a method template.

7.3.2 Gel filtration/buffer exchange

Gelfiltration/ Buffer Exchange

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



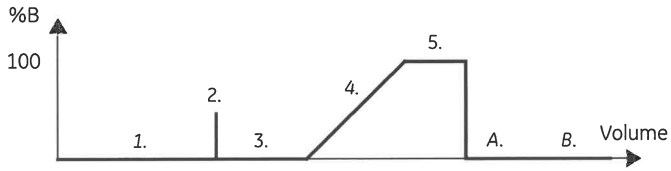
Action	Step	Conc %B	Flow	Fract.	Buffer V	Inject V
Equilibration	**1**	0	F	0	pos 1	LOAD
Sample application	**2**	0	F	V	pos 1[1]	INJECT[1]
Elution	**3**	0	F	V	pos 1	LOAD

[1] If using the pump for sample application, Buffer V = pos 8 and Inject V = LOAD.

7.3.3 Ion exchange

Ion Exchange Gradient elution

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



Action	Step	Conc %B	Flow	Fract.	Buffer V	Inject V
Equilibration	**1**	0	F	0	pos 1	LOAD
Sample application	**2**	0	F	0	pos 1[1]	INJECT
Wash 1[2]	**3**	0	F	0	pos 1	LOAD
Elution	**4**	0	F	V	pos 1	LOAD
Wash 2 (+ 12 ml)	**5**	100	F	V	pos 1	LOAD
Priming, 20 ml of buffer A	A[3]	0	40	0	pos 1	WASTE
Re-equilibration (= equilibr. volume)	B[3]	0	F	0	pos 1	LOAD

[1] If using the pump for sample application, Buffer V = pos 8 and Inject V = LOAD.

[2] If using the pump for sample application, 15 ml is automatically added to Wash 1.

[3] These steps are hidden in the template and cannot be changed.

7.3.4 HIC (hydrophobic interaction chromatography)

HIC
Gradient elution

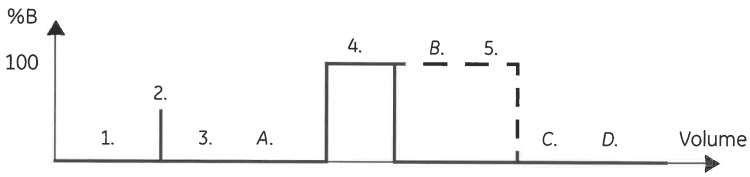
To access the template, select **HIC Gradient elution** and press **OK**.

For buffer gradient and parameter settings table, see the Ion exchange method template at the previous page.

7.3.5 Affinity

Affinity
Step Gradient

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



Action	Step	Conc %B	Flow	Fract.	Buffer V	Inject V
Equilibration	**1**	0	F	0	pos 1	LOAD
Sample application	**2**	0	F	0	pos 1[1]	INJECT[1]
Wash 1[2]	**3**	0	F	0	pos 1	LOAD
Priming, 15 ml of elution buffer B	A[3]	100	40	0	pos 1	WASTE
Elution	**4**	100	F	V	pos 1	LOAD
Priming, 15 ml of Wash 2 buffer (if Wash 2 is used)	B[3]	0	40	0	pos 2	WASTE
Wash 2 (optional)	5	0	F	V	pos 2	LOAD
Priming, 20 ml of buffer A	C[3]	0	40	0	pos 1	WASTE
Re-equilibration (= equilibr. volume)	D[3]	0	F	0	pos 1	LOAD

[1] If using the pump for sample application, Buffer V = pos 8 and Inject V = LOAD.

[2] If using the pump for sample application, 15 ml is automatically added to Wash 1.

[3] These steps are hidden in the template and cannot be changed.

8 Installing and modifying components

This chapter describes how to install and modify components in ÄKTAprime plus. It also describes how to install and use optional components.

8.1 General

Some applications might require that some of the components in ÄKTAprime plus have to be modified or changed. For example, the UV sensitivity can be changed by using another UV flow cell, the wavelength of the optical filter can be changed, or the rack in the fraction collector changed according to the tube size used.

The optional pH electrode allows for real-time monitoring of the pH and, if a recorder is used, a print-out.

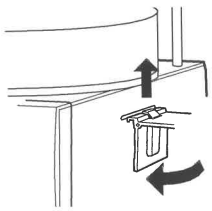
The chapter covers the following components:

- The optical unit, including flow cells and filters.
- The conductivity cell.
- The fraction collector.
- The pH flow cell and electrode (optional).
- The recorder REC 112 (optional).

8.2 *Optical unit*

8.2.1 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.



8.2.2 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.

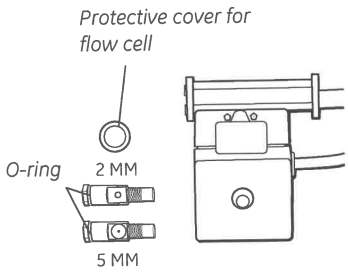
Change the flow cell as follows:

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

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

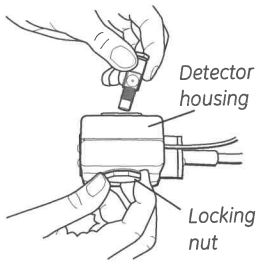
3 Remove the protective cover from the old flow cell and transfer it to the new flow cell.

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 8.2.5 Changing the lamp assembly (optional) section below.*

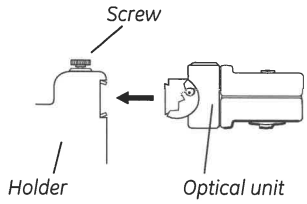
8.2.3 Connecting the optical unit to the system

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.



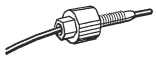
4 Connect the signal cable to the socket **UV** on the rear panel of the module.

8.2.4 Connection to the column

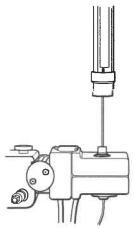
1 Fix the optical unit directly under the column if possible.

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.



Note: *The inlet port of the 5 mm UV cell is above the optical unit. The inlet port of the 2 mm UV cell is below the optical unit.*



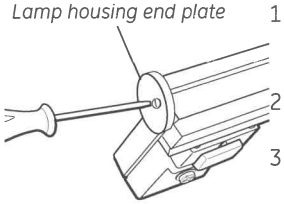
3 Connect the optical unit outlet tubing onto the opposite hole in the flow cell. Use fingertight connectors.

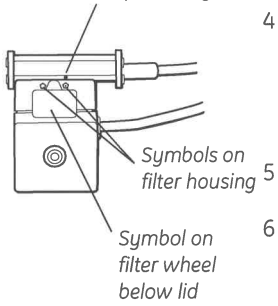
If no outlet tubing exists, cut a piece of PEEK tubing (i.d. 0.75 mm, o.d. 1/16"). The length should be 170 mm.

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

8.2.5 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.

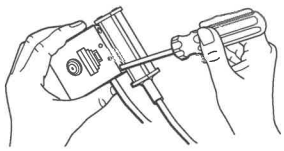
5 Refit the lamp housing end plate.

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.

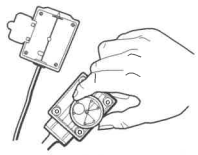
8.2.6 Changing the filter (optional)

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 8.2.5 Changing the lamp assembly (optional).

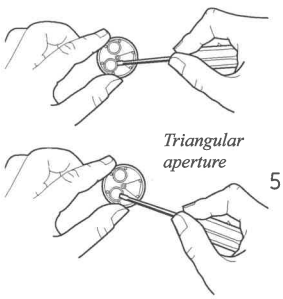
2 Remove the four screws in the filter housing. Separate the filter housing from the detector housing.



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.

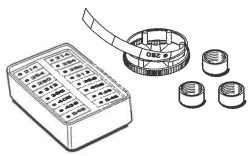
Note: *Filters are sensitive optical components. Never touch the optical surfaces or expose them to temperatures above 60 °C. Clean them with dry lens cleaning tissue and store them, when not in use, in the box in which they were supplied. Heavy contamination may be removed by using a lens tissue dipped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

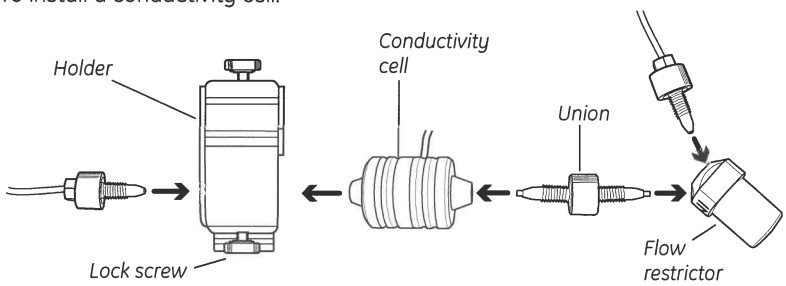
10 Check that all filters are clean. Insert the filter wheel back into the filter housing.

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.

8.3 Conductivity cell

To install a conductivity cell:

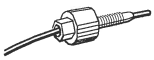


1 Connect the conductivity cell to the flow restrictor using the union.

2 Insert the cell into the holder and secure it with the lock screw.

Note: *When the conductivity flow cell is used 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.*

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



4 Connect the tubing with fingertight connectors.

8.4 Fraction collector

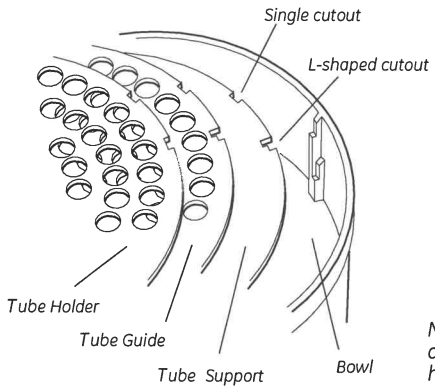
8.4.1 Assembling the tube rack

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

ÄKTAprime plus 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.

The 12 mm rack is available as accessory. 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.



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

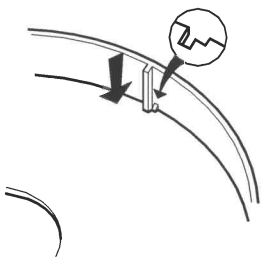
When assembling a rack, different cutouts are used for the various items depending on the length of the tubes. The cutouts are summarized in the tables below.

Tube racks, 12 and 18 mm

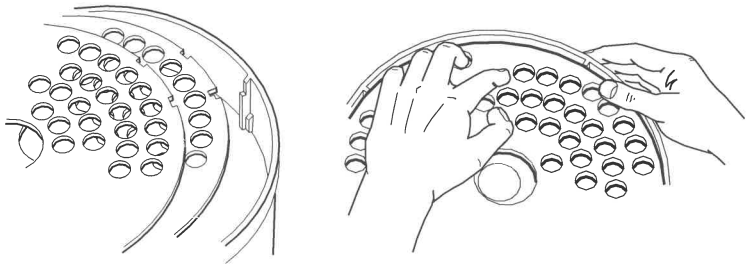
Item	50-85 mm tubes	85-100 mm tubes
Tube support	L-shaped cutout	Not required
Tube guide	Single cutout	L-shaped cutout
Tube holder	Single cutout	Single cutout

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.

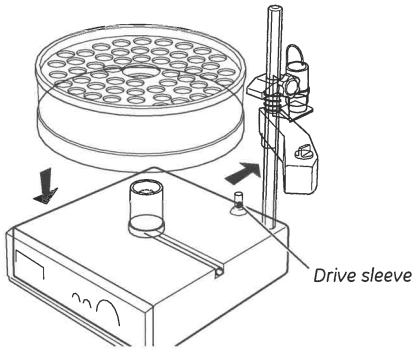


Do not force the holder into place as this may damage the lip. The surface of the holder should be level.

8.4.2 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.

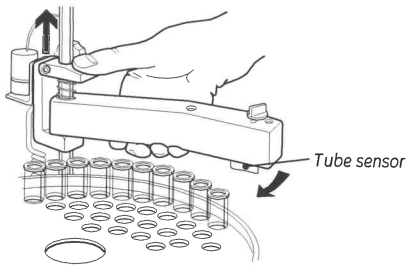


8.4.3 Inserting the collection tubes

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.

8.4.4 Adjusting the delivery arm

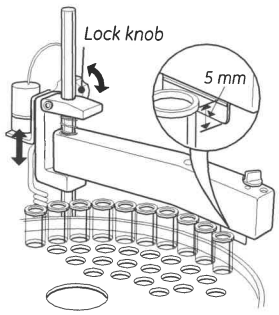
1 Lower the arm and allow it to move so that the tube sensor touches the collection tubes of the outer track.

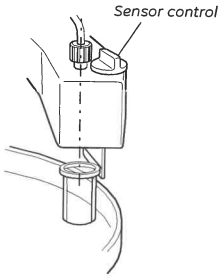


2 Adjust the arm bracket so that the bottom of the tube sensor is about 5 mm below the top of the tubes. The tubes should always be below the horizontal mark on the tube sensor

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.

8.4.5 Connecting tubing

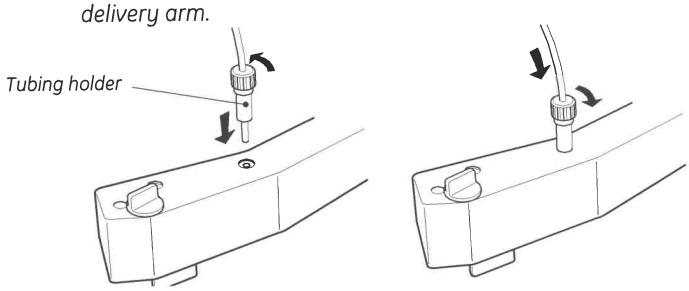
Select the tubing with the required inner diameter 0.75 mm. To change the tubing, follow steps 1–5.

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

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.

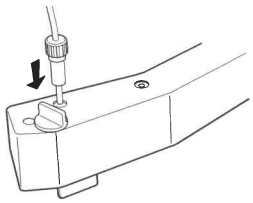
Note: *Check that the tubing end is cut leaving a straight edge.*

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



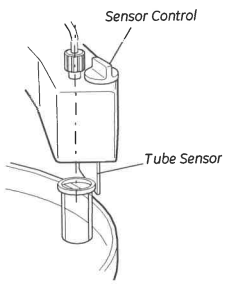
2 Place the tubing holder over the length guide (small hole) in the delivery arm, push the tubing down to the bottom of the guide and tighten the nut. This ensures that the correct length of tubing is exposed.

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

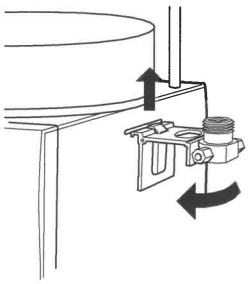


4 Set the red sensor control to position the tubing over the centre of the collection tube.

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



8.5 pH flow cell and electrode (optional)

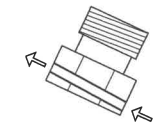


8.5.1 Mounting the flow cell holder

In the ÄKTAprime plus 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.

8.5.2 Inserting the pH electrode

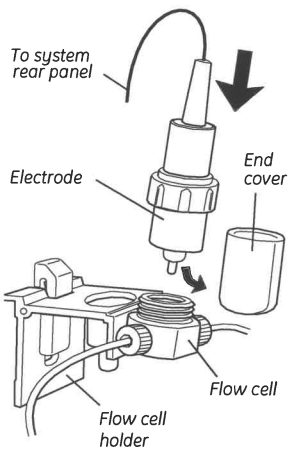
Note: *Handle the pH electrode with care.*

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.



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.

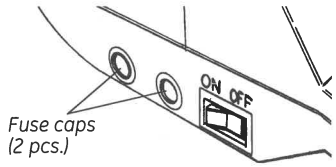
8.6 *Recorder REC 112*

ÄKTAprime plus can be delivered with a recorder, REC 112, for simpler data presentation. This section describes have to install and use the recorder.

8.6.1 Electrical connections

1 Make sure that the mains power switch is in the **OFF** position.

2 Insert the correct fuses into the fuse caps.



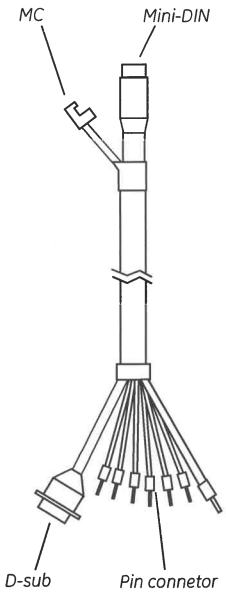
Fuse caps (2 pcs.)

- For 100–120 V operation, use the 250 mA fuses supplied.
- For 220–240 V operation, use the 125 mA fuses supplied.

3 Fit the fuse caps into the fuse holder sockets on the left side of the recorder.

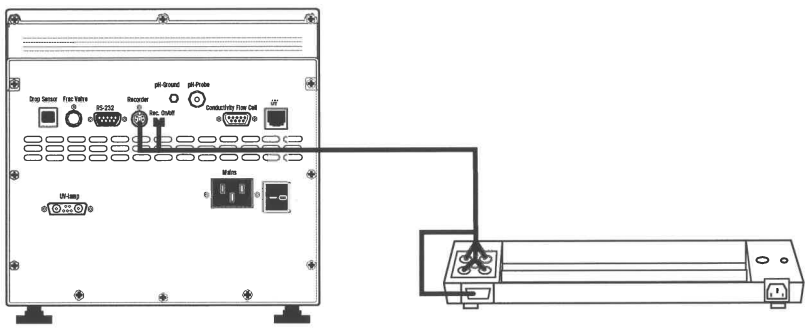
4 Make sure that the mains voltage selector at the rear of the recorder is set to the mains voltage of the laboratory.

CAUTION! The mains voltage selector must be set to the mains voltage of the laboratory. If not, electronics might be damaged.

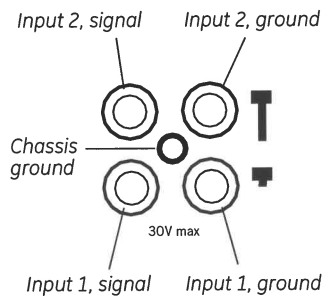


5 Connect the supplied signal cable to ÄKTAprime plus as follows:

- 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.



6 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.*

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

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

[1] The signals have the following default parameter settings:
Signal 1 – UV absorbance
Signal 2 – %B
Signal 3 – Conductivity
These settings can be changed (see section 8.6.5 Setting analog outputs).

8 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.

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

WARNING! The system must be connected to a grounded mains socket to prevent system parts from becoming live.

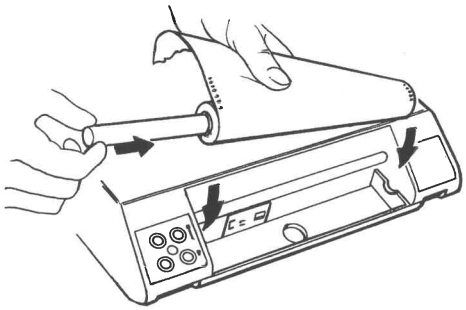
10 Turn on the mains power to the recorder.

8.6.2 Loading the chart paper roll

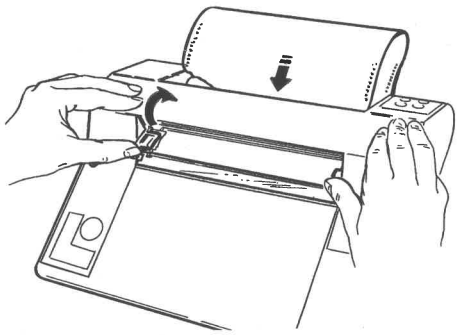
To load the chart paper roll:

1 Unpack the paper roll supplied with the recorder and put into place (1.). Make sure the paper roll is positioned so that the printed grid faces the user during normal operation.

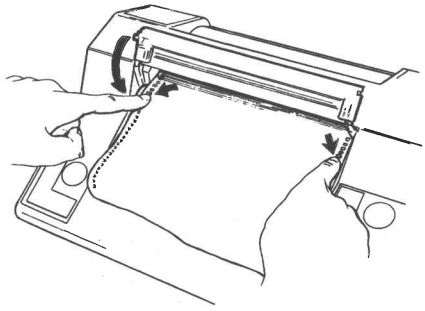
2 Lift the plastic transparent ruler (2.) by pushing it gently upwards so that it rests over the top of the recorder. Leave it in this position.



1.



2.



3.

3 Pull the paper forward. It should be parallel to the housing.

4 Use your fingertips to fit the holes in the edge of the paper over the sprockets. Do this on the right-hand side first and then on the lefthand side (3.). This is important because the holes in the right side guarantee exact paper positioning whereas the holes on the left side only support transport.

5 When the paper is correctly positioned over both sprocket wheels, hold the paper in position and lower the ruler.

6 Use both hands to gently apply pressure to both the left and right edge of the ruler. A double click indicates that the ruler is properly positioned.

8.6.3 Installing the pens

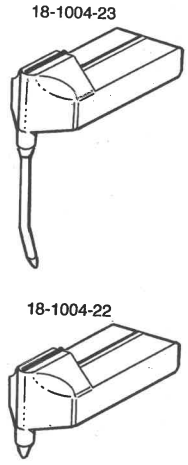
Recorder REC 112 has a writing system that is very simple and convenient. It uses reliable fibre-tip pens of the disposable cartridge type (1.), which leave a very fine trace and do not bleed.

To install the pens:

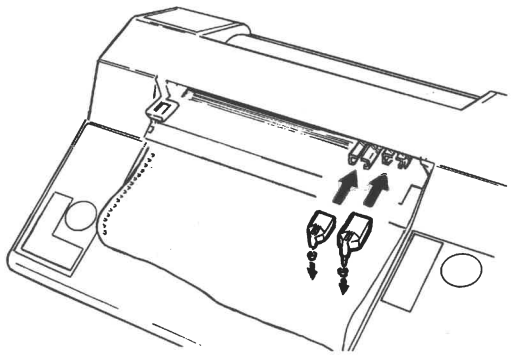
1 Remove the cap.

2 Gently push the pen into the plastic pen holder (2.), avoiding sidewards or upwards pressure.

Note: *To prevent the pens from drying when the recorder is not in use, cover the tips of the pens using the caps provided.*

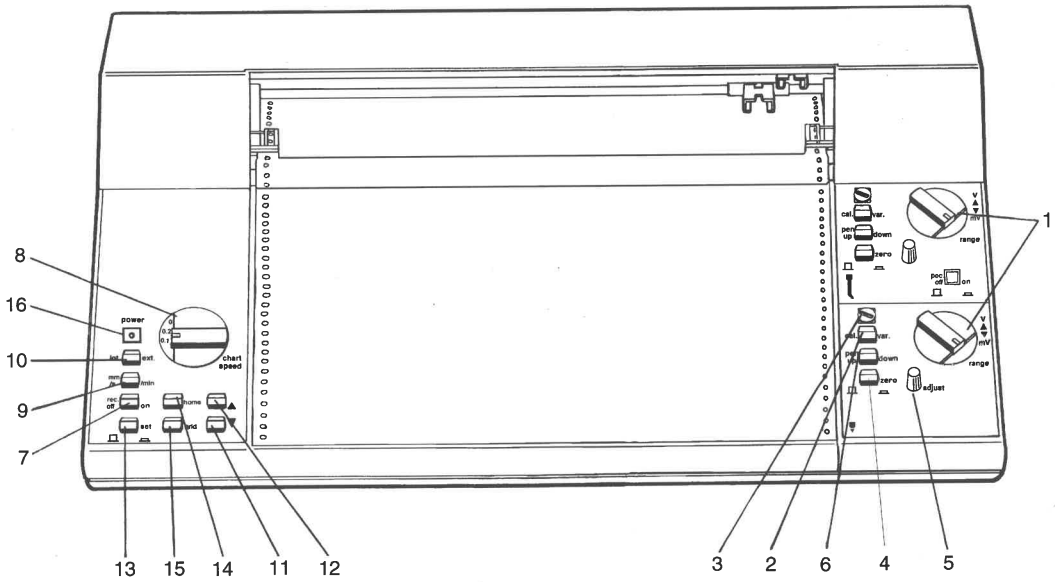


1.



2.

8.6.4 Preparing the recorder for a run



1 Turn on the mains power to the recorder.

2 Set the **zero** keys (4) to the **down** position.

3 Select a suitable low chart speed (0.5–2 mm/min) with the **chart speed** selector (8) and the **mm/s–mm/min** key (9).

4 Set both **pens** (6) to position **down**. Use the **adjust** knob (5) to make a coarse zero adjustment to the right-hand zero on the chart.

5 Set **rec.** (7) to position **on** and make a final zero adjustment.

6 Set **rec.** to position **off.** Use the forward **feed** key (11, 12) to align the short-nib pen with a grid line and press the **set** key (13), i.e. set start position.

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

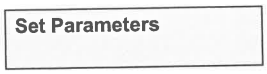
8 Select a suitable chart speed (2–20 mm/min) according to the length of the purification.

9 Select 1 V with the **range V/mV** selector (1).

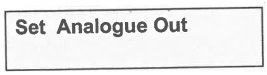
10 The recorder is made ready to use by setting the **rec.** key to **on**. The chart paper starts rolling when the run starts.

8.6.5 Setting analog outputs

The **Set Analogue Out** menu is used to select which measurement parameter that 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.



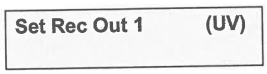
1 Select the **Set Parameters** menu in the main menu and press **OK**.



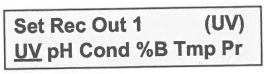
2 Select the **Setup Analogue Out** menu and press **OK** to enter the settings menu.

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.*

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.

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) 0.00

2 Press **OK** to access the settings menu. The current setting is displayed. Press **OK**.

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) 50.00

4 Press the down button to access the next settings menu. The current setting is displayed. Press **OK**.

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.

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) 00.00

2 Press **OK** to access the settings menu. Press **OK**.

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) 14.00

4 Press the down button to access the next settings menu. Press **OK**.

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.

Setting the Press analogue output

Set Press. Analogue Out
(1.00 MPa) 1.00

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
(2.00 MPa) 2.00

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.

8.6.6 Printing curves directly after a run

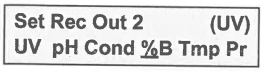
Selected run data is usually printed directly during the run. However, if a re-print of the result is desired, this can be done as a post-run print-out. This procedure is described below.

The system has three analog 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.

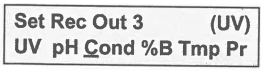
When the run is completed, or if it has been aborted, the prompt **Memory Print Out?** is displayed.

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

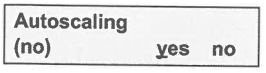
2 At the **Set Rec Out 1** menu, select the parameter to be printed on channel 1. Press **OK.**



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



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



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

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

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

8.6.7 Printing curves before the next run

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 in the precious section, or before the next run using the **Set Parameters** menu as described below.

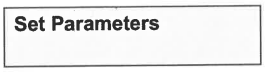
1 Set the **rec.** key on the recorder to **off**.

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

3 Increase the **chart speed** value about ten times. This is due to that the curves are printed ten times faster at the post-run print-out than during the actual run.

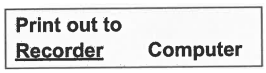
4 Set the **rec.** key to **on** to enable the print-out.

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



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

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



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

9 Select the desired parameters to be printed as in steps 2–5 on page 117.

The print-out will now overlay the previous curves.

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

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

9 Maintenance

This chapter contains a periodic maintenance schedule and instructions for maintenance, replacing components, and calibrations.

9.1 Periodic maintenance

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

WARNING! Remove liquid or dirt from the system surface using a cloth and, if necessary, a mild cleaning agent.

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

WARNING! If there is a risk that 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, take all suitable protective measures, such as wearing protective glasses and gloves resistant to the chemicals used. Follow local regulations and instructions for safe operation and maintenance of the system.

WARNING! When using hazardous chemicals, make sure that the entire system has been flushed thoroughly with bacteriostatic solution, e.g. NaOH, and distilled water before service and maintenance.

WARNING! NaOH is injurious to health. Avoid spillage.

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

WARNING! Use ONLY tubings supplied by GE Healthcare 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.

9.1.1 Preventive maintenance schedule

Interval	Action
Daily	
System	• Inspect the complete system for eluent leakage. • Never leave the system filled with buffers containing salt overnight. It will ruin the pumps. • If you are not using the separation unit for a few days: 1. Wash the flow path with distilled water. 2. Remove the column and the pH electrode (optional). 3. Wash the flow path with 20% ethanol and store it in 20% ethanol. Make sure that all tubing and all flow paths used are rinsed.
Pump	• Check for leakage. If there are signs of liquid leaking from the pump: – Check the tubing connections. – Check the O-rings in the connection part. Replace the O-rings if necessary. • If there are signs of erratic or pressure pulsation, flush the pump as follows: 1. Wash the system with distilled water to remove all buffers. 2. Flush the system with 100% ethanol.
pH electrode	• 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 9.5.

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 backpressure on the 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 display. 6. Calculate the backpressure 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 9.9 or 9.10. • Change the pH electrode. Refer to section 8.5.
Fraction collector	• Check the drive sleeve on the tube rack. Replace if worn. • Check the number of tube shifts according to section 9.7.
Superloop	• Check that the top, bottom and moveable seal O-rings are in good condition. Replace if necessary. • 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 9.17.
Yearly	
Injection valve and buffer valve	• Check for external or internal leakage. Replace channel plate and distribution plate when required. Refer to section 9.13.
Every 2 years	
Mixer	• Replace the complete mixing chamber. Refer to section 9.17.
Superloop	• Replace O-rings. Refer to section 4 in the instruction supplied with Superloop.
When required	
Monitor	• Clean the conductivity flow cell according to section 9.11. • Clean the pH electrode flow cell according to section 9.18.

Interval	Action
Pump	• Replace the check valve O-rings according to section 9.16. • Clean the check valves. If necessary, replace the check valve O-rings or the entire check valves according to section 9.16.

9.2 Cleaning the system

- Wipe the surface regularly with a damp cloth. Do not allow spilt liquid to dry on the instrument.
- Remove dirt from the surface using a cloth and a mild cleaning agent.
- Let the system dry completely before using it.

9.3 *Cleaning the system flow path*

WARNING! When using hazardous chemicals, make sure that the entire system has been flushed thoroughly with bacteriostatic solution, e.g. NaOH, and distilled water before service and maintenance.

Section 9.3.3 Monthly cleaning describes how to flush the system with a bacteriostatic solution.

Usually the column should be by-passed with a piece of i.d. 0.75 mm PEEK capillary before cleaning the system flow path. If not, make sure that the column withstands the expected flow and pressures.

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

9.3.1 Betweens runs

Buffers not containing any salt can be left in the system for a short time after a run, even overnight (not in the pH electrode, see instructions below).

CAUTION! If a buffer containing salt has been used, the flow path must be flushed with deionized water.

To flush the flow path:

1 Fill a syringe with five times the sample loop volume of deionized water.

2 Rinse the sample loop by injecting the water through the fill port on the injection valve.

3 Put all used inlet tubings in water

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

5 Select the used inlet ports. Inlets **A1** and **B** will always be washed.

6 Press **OK** to start the method. The system flow path is automatically flushed.

9.3.2 Weekend and long-term storage

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. Store the pH electrode fitted in the end cover filled with a 1:1 mixture of pH 4 buffer and 2 M KNO_3. Do NOT store in water only!

If you are not using the system for a few days or longer:

1 Wash all tubing and flow paths used with deionized water, for example by running the **System Wash Method** with all tubing inlets in water.

2 Replace the column with a bypass capillary.

3 Replace the pH electrode (optional) with a dummy pH electrode.

4 Wash the system with 20% ethanol and store it in 20% ethanol.

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.

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. After removing the pH electrode from the flow cell, insert a dummy electrode in the flow cell.

9.3.3 Monthly cleaning

WARNING! NaOH is injurious to health. Avoid spillage.

Clean the system every month, before service and maintenance, 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 Put all tubing inlets in 1 M NaOH.

3 Run **System Wash** 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.

9.3.4 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.

9.4 *Moving the system*

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

WARNING! When using hazardous chemicals, make sure that the entire system has been flushed thoroughly with bacteriostatic solution, e.g. NaOH, and distilled water before service and maintenance.

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.

9.5 *Checking the UV monitor*

9.5.1 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 assembly according to section 8.2.5 Changing the lamp assembly (optional), or contact GE Healthcare for lamp replacement.

9.5.2 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 assembly according to section 8.2.5 Changing the lamp assembly (optional), or contact GE Healthcare for lamp replacement.

9.5.3 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.

9.6 Checking the pump

9.6.1 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 wearing parts (glass tube, piston, sealings and O-rings) is typically 2000 hours at normal use.

When necessary, contact GE Healthcare for replacement.

9.6.2 Checking pumped volume

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

Check Pumped volume
194529452ml

2 Select menu **Check Pumped Volume**.

When required, contact GE Healthcare for replacement.

9.7 Checking the fraction collector

9.7.1 Checking tube shifts

Check Tube Shifts
17564

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

2 Select menu **Check Tube Shifts**.

When required, contact GE Healthcare for replacement.

9.8 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 plates according to section 9.13 Replacing plates in the rotary valves, or contact GE Healthcare for sealing replacement.

9.9 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.

9.10 Cleaning the UV flow cell off-line

A clean flow cell is essential for 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 syringe and flush the flow cell with distilled water (10 ml).

9.11 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.*

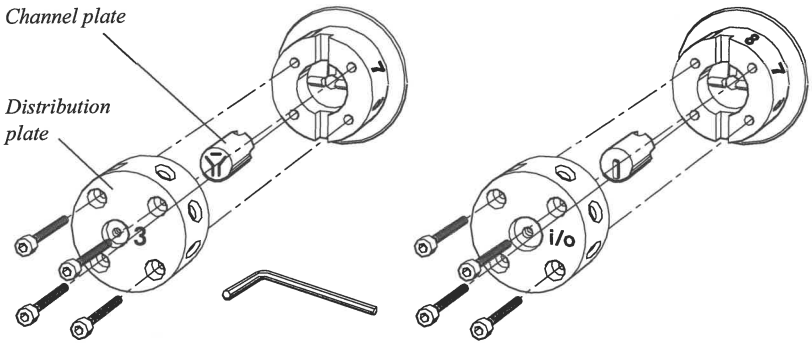
9.12 Replacing the conductivity cell

The conductivity flow cell can be replaced 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 9.20.3 Calibrating conductivity. If the cell constant is not known, it can be determined.

9.13 Replacing plates in the rotary valves

A replacement kit for each valve is available. Refer to 11.5 Ordering information.



Injection valve *Buffer valve*

1 Make sure 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 hex wrench. 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 a hex wrench, tighten the four screws in turn, a little at a time, until the distribution plate is fixed to the valve body.

9.14 *Removing and assembling the pump*

The connection part should be removed to allow access to the check valves and the O-rings.

9.14.1 Removing the connection part

Before disassembling the pump, move the input liquid bottle below the level of the pump to prevent siphoning.

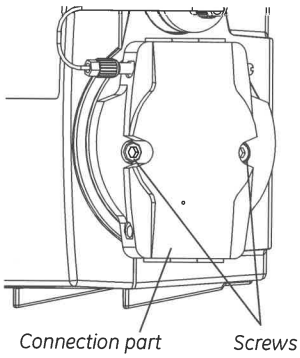
1 Turn off the system with the mains power switch.

2 Disconnect the inlet and the outlet tubing from the connection part.

3 Release the pump from the system.

4 Unscrew the two attachment screws using a 4 mm hex wrench.

5 Remove the connection part.



9.14.2 Installing the connection part

1 Wipe the back of the connection part and the other parts behind the connection part with a clean cloth.

2 Check that none of the seven O-rings on the rear side of the connection part has come loose.

3 Fit the connection part in position. Fasten the two attachment screws using the 4 mm hex wrench.

4 Reconnect the inlet and the outlet tubing.

5 Purge the pump carefully and check that the fault is corrected. See section 5.2.2 Purging pump and inlet tubing.

9.15 Replacing O-rings in the pump

9.15.1 Required spare parts

- O-ring kit (see Ordering information for code no.)

9.15.2 Replacing an O-ring

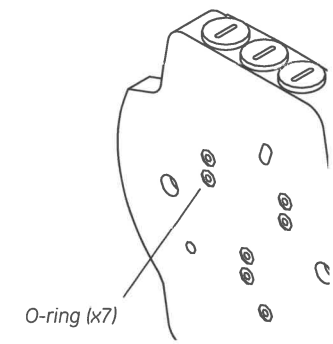
To replace an O-ring:

1 Remove the connection part according to section 9.14.1 Removing the connection part.

2 Remove the faulty O-ring carefully to avoid making scratches on the connection part.

3 Fit the new O-ring in position.

4 Reinstall the connection part according to section 9.14.2 Installing the connection part.



9.16 Cleaning and replacing check valves in the pump

Faulty operation of the check valves is usually indicated by irregular flow, very low flow, or unstable pressure traces. Probable causes are air, dirt, or a damage in a check valve preventing it from closing to seal and hold the pressure. Liquid appearing at a banjo fitting might indicate that a check valve O-ring is damaged.

9.16.1 Required spare parts

- Check valve (there are four different check valves. See Ordering information for code nos.)
- O-ring kit (see Ordering information for code no.)

9.16.2 Cleaning the check valves in-place

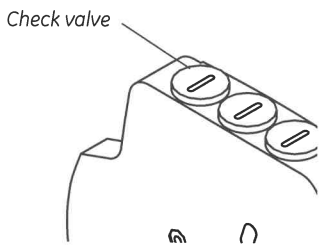
Try to clean the check valves in-place by pumping 100% ethanol for approximately 10 min. If this does not correct the problem, follow the instructions below to remove and then clean the valves. If necessary, a check valve or O-rings might need to be replaced.

9.16.3 Removing the check valves

If the condition of the check valve is not improved by in-place cleaning, remove it as follows:

1 Remove the connection part according to section 9.14.1 Removing the connection part.

2 The check valves in the connection part are locked in position with banjo fittings. Remove the check valves using the screwdriver.



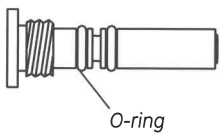
9.16.4 Cleaning the check valves off-line

1 Immerse the check valves in 100% ethanol and place in an ultrasonic bath for 5–10 minutes.

2 Repeat the ultrasonic bath with distilled water.

9.16.5 Replacing the check valve O-rings

Liquid appearing at a banjo fitting might indicate that a check valve O-ring is damaged. It might also cause reduced flow or pressure fluctuation.



Carefully replace both O-rings on the check valve with new ones if you suspect that an O-ring is damaged.

9.16.6 Installing the check valves

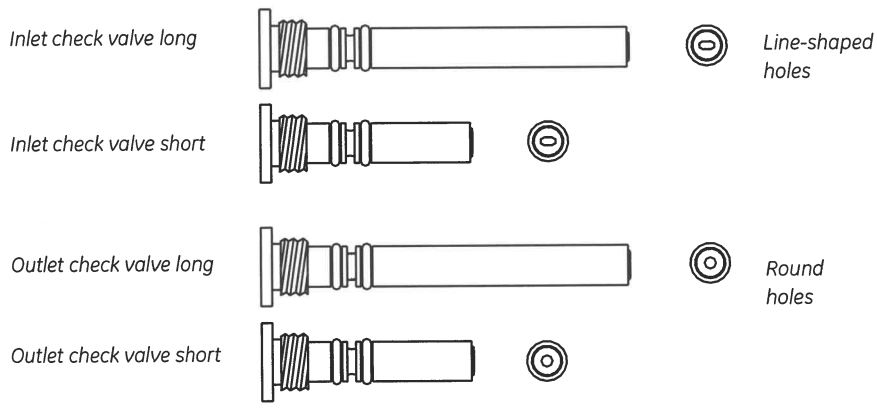
If cleaning of the check valve as described in section 9.16.4 Cleaning the check valves off-line does not correct the fault, replace the check valve with a new one.

Note: *Make sure that the check valves are installed in their correct positions. By mistake, an inlet check valve can be installed incorrectly in an outlet check valve position, and vice versa.*

The check valves are of four different types:

- Inlet check valve short (1 pc.)
- Inlet check valve long (2 pcs.)
- Outlet check valve short (2 pcs.)
- Outlet check valve long (1 pc.)

The inlet check valves have line-shaped holes.
The outlet check valves have round holes.



To install a check valve:

1 Carefully insert the check valve fully.

Note: *Make sure that the inlet check valves are installed next to the inlet (lower) port on the connection part, and the outlet check valves next to the outlet (upper) port.*

2 Fasten the check valve using the screwdriver.

3 Reinstall the connection part according to section 9.14.2 Installing the connection part.

9.17 Replacing mixer chamber

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

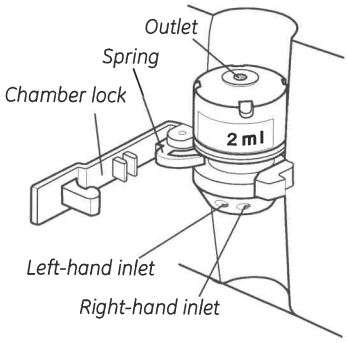
1 Make sure the pump is stopped.

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.

9.18 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.

9.19 Replacing the pH electrode (optional)

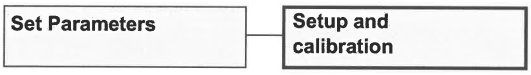
See section 8.5 pH flow cell and electrode (optional).

9.20 Calibrations

Follow the calibration schedule below to maintain accurate measurements.

Component	How often
Pump	Only necessary after replacing spare parts.
Pressure offset	When required.
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.
pH electrode (optional)	Every day

The calibrations are made in the **Setup and calibrations** menu.



9.20.1 Calibrating the pump

Calibrating the flow rate

Calibrate the pump after replacing spare parts.

1 Make sure no bubbles are trapped in the flow path.

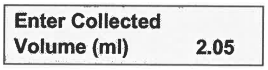
2 Immerse the inlet tubing A1 in a vessel filled with degassed buffer.

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).

4 Select **Start Pump Calibration** in **Setup and calibration** menu. Press **OK**.

5 Enter the intended flow rate as calibration flow rate and press **OK**.

6 Measure the volume of the water collected in the vessel.



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**.

9.20.2 Calibrating the pressure sensor

Calibrating the pressure offset
The zero pressure should be calibrated when required.

1 Make sure that the pressure sensor is exposed to atmospheric pressure only, i.e. no backpressure.

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.

9.20.3 Calibrating conductivity

Set up adjust cell constant
After replacing the flow cell, the cell constant has to be set. The cell constant is written 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}.

Set up adjust conductivity

Normally, it is not necessary to adjust the cell constant because 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 page 139). The temperature sensor must be calibrated before adjusting the cell constant (see page 140).*

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.

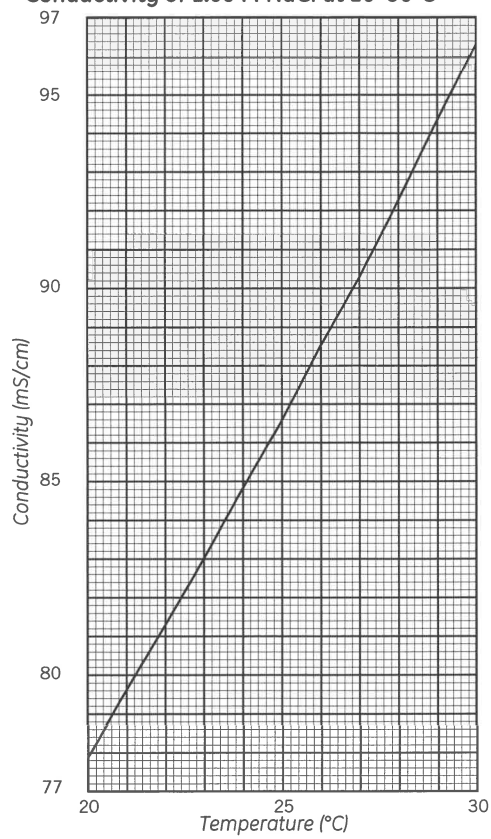
3 Stop the flow and wait 15 minutes until the temperature is constant in the range 20–30 °C.

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.

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.

Conductivity of 1.00 M NaCl at 20–30°C



Set up 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**.

Set up 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**.

9.20.4 Calibrating the temperature sensor

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	The current adjustment value is displayed as default. Enter the temperature shown on the thermometer and press **OK**.

9.20.5 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.

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!

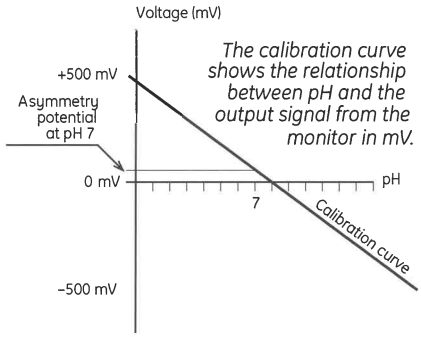
10 The text opposite disappears when the reading is stable and the text below is then shown.

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.

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.

Set up 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.

Set Parameters	1 From the main menu, select sub menu **Set Parameters** and press **OK**.
Setup and calibration	2 Select sub menu **Setup and calibration**. Press **OK**.
Setup pH	3 Select sub menu **Setup pH** and press **OK**.
Set pH Temp Comp (off)	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.
Set pH Temp Comp (off) on off	5 Select the desired setting and press **OK**.

9.21 Recycling

This symbol indicates that the waste of electrical and electronic equipment must not be disposed as unsorted municipal waste and must be collected separately. Please contact an authorized representative of the manufacturer for information concerning the decommissioning of equipment.

10 Troubleshooting

10.1 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 GE Healthcare.

ÄKTAprime plus v 5.00

When contacting GE Healthcare 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.

This symbol indicates that the waste of electrical and electronic equipment must not be disposed as unsorted municipal waste and must be collected separately. Please contact an authorized representative of the manufacturer for information concerning the decommissioning of equipment.

10.2 System

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

10.3 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. 3 A check valve in the pump might be clogged or damaged. Remove the check valve according to section 9.14. Clean the check valve in an ultrasonic bath. If the leakage persists, replace the assembly.
Irregular flow, noisy baseline signal, irregular pressure trace	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. 4 Remove any air bubbles according to section 10.13. 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. 4 A check valve in the pump might be clogged or damaged. Remove the check valve according to section 9.14. Clean the check valve in an ultrasonic bath. If the leakage persists, replace the assembly.

10.4 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 9.9 and 9.10. 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 10.13.
Ghost peaks	1 Check that there is no air in the eluent. If necessary, degas the eluent 2 Clean the system in accordance with section 9.3. 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.

10.5 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 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 pump. 5 Clean the flow cell according to the procedures in sections 9.11.
Conductivity measurement with the same buffer appears to change over time	1 Clean the flow cell according to the procedures in sections 9.11. 2 The ambient temperature may have changed. Use a temperature compensation factor.
Waves on the gradient	1 Check that the 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.
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.

10.6 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 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 9.18). 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 9.18.
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.
Small response to pH changes	1 Clean the pH electrode according to section 9.18 and recalibrate. 2 If the problem persists, replace the pH electrode.

Fault	Action
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 9.18. 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 9.18.
pH values vary with varied back-pressure	1 Replace the pH electrode.

10.7 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.

10.8 Pump

Fault	Action
Irregular flow	1 Check the inlet and outlet tubings. 2 Remove any air bubbles according to section 5.2.2. 3 If still irregular flow, calibrate the pump according to section 9.20.1. 4 A check valve in the pump might be clogged or damaged. Remove the check valve according to section 9.14. Clean the check valve in an ultrasonic bath. If still irregular flow, replace the assembly.
Leakage	1 An O-ring in a check valve or in the connection part might be damaged. Examine the O-rings. If necessary, replace them according to the section 9.15.2 or 9.16.5.

10.9 Fraction collector

Fault	Action
No tube change	1 Press the **feed tube** key. If the motor does not start and an error appears, call GE Healthcare. 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 GE Healthcare.
Tubes skipped	1 The spring tension may be insufficient. Perform the actions described in section 10.12.
Drop synchronization is not functioning	1 Check that the drop synchronization function is turned on. See section 11.2.1. 2 The drop sensor photocell located above the tube sensor is dirty. Clean the photocell with a damp cloth. 3 Make sure that the fraction collection tubing is properly connected to the delivery arm. See section 8.4.5

10.10 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.
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 9.13.
High back-pressure	1 Perform cleaning-in-place by flushing the system with detergent. 1 Change channel plate and distribution plate according to section 9.13.

10.11 Error messages

If the suggested actions do not correct the fault, call GE Healthcare.

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 11.2.1.
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.
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.

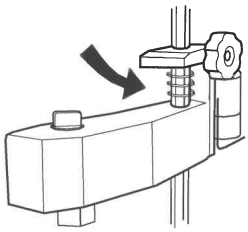
Messages	Action
69 ERROR Stop grad. set HOLD or PAUSE	1 Set the system in HOLD or PAUSE, or stop the gradient (%B).
70 Lamp disconnected If not, call service	1 Connect the lamp or call service.
71 WARNING low light intensity	1 Check the cables to the optical unit. 2 Check that lamp and filter position 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.
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.

Messages	Action
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 11.2.1.
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.
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 11.2.1.
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.

Messages	Action
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.

10.12 Adjusting the spring tension of the delivery arm

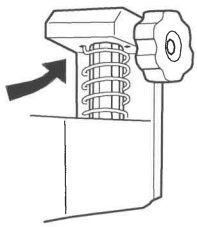
Incorrect spring tension can cause the fraction collector to skip tubes. The effect is greater as the arm moves towards the centre.



Spring tension is temperature sensitive. Low temperature, for example in a cold room, reduces the spring tension so it may be necessary to adjust the tension.

1 Remove the arm bracket from the stand.

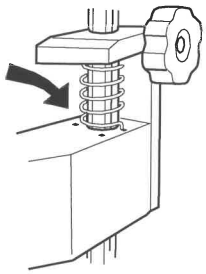
2 Dismantle the delivery arm from the bracket.



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 turn the spring counter-clockwise.
- To decrease the tension turn the spring clockwise.

10.13 Removing trapped air bubbles

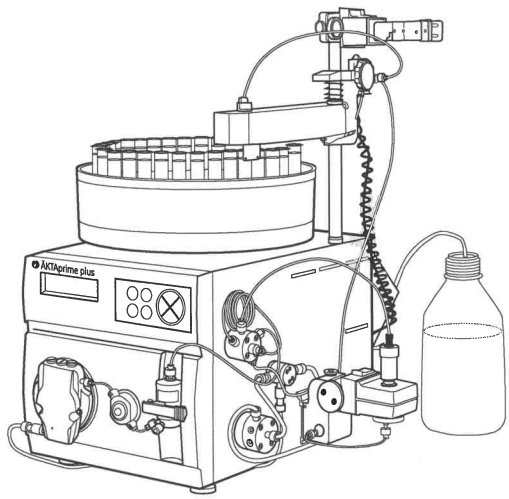
If there are large amounts of air in the tubing or if you suspect air in the pump, purge the flow path as described in section 5.2.2.

10.14 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.

11 Reference information

11.1 System description



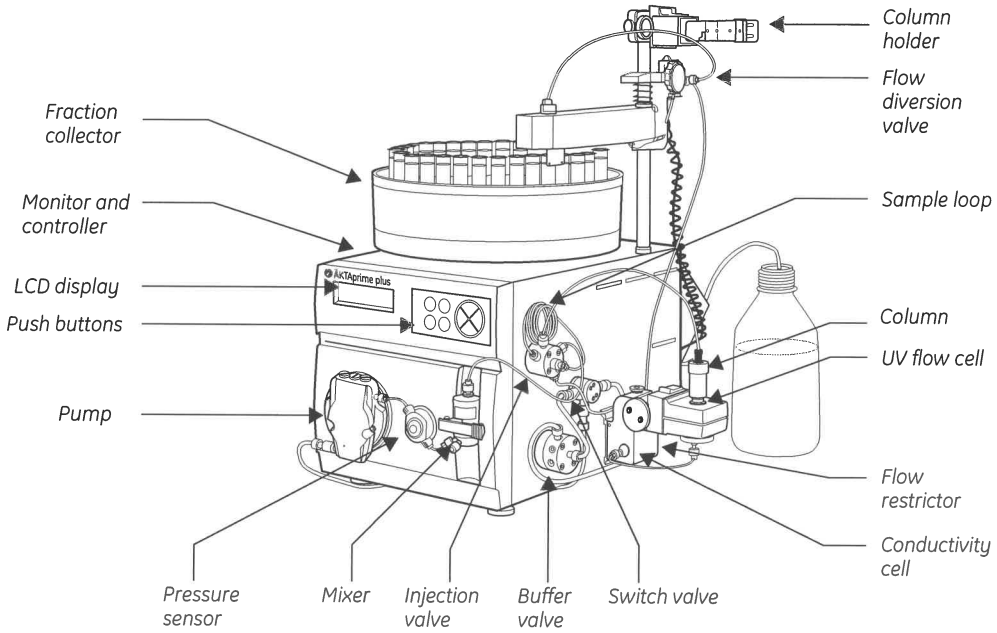
ÄKTAprime plus is a compact separation unit comprising components for fluid handling and for measuring UV-absorption, conductivity and pH (optional).

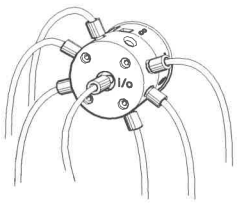
This section gives a brief description of the system and its components. It also describes optional components that may be connected to the system.

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.

11.1.1 Components description

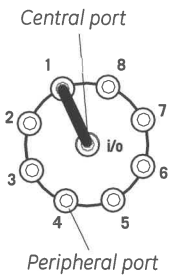
The following illustration shows the location of the components in the system.





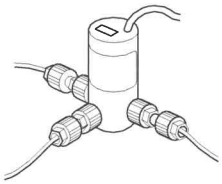
Buffer valve

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.



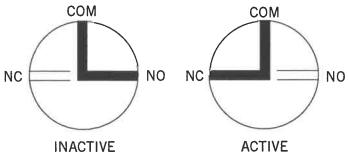
The buffer valve is used on the low pressure side in the flow path (before the pump). It it used for switching between sample and buffer solutions.

The switching parts are made of PEEK, which ensures long mechanical and chemical lifetime.

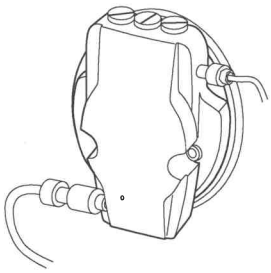


Switch valve/ Flow diversion valve

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.



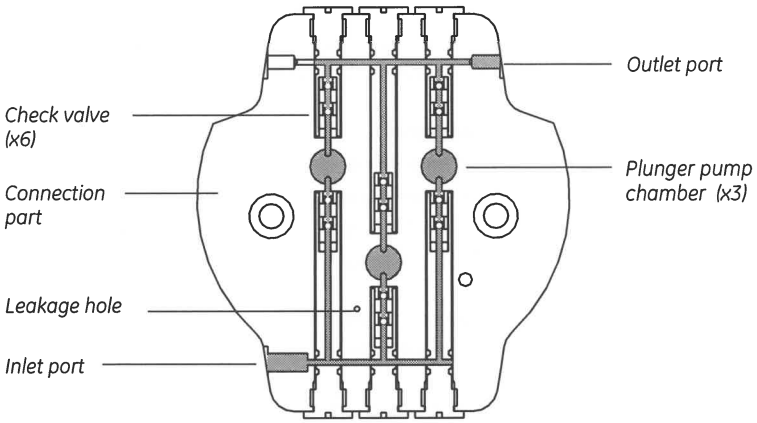
There are two switch valves in the system; one is used for gradient formation and the other one for flow diversion during fractionation.



Pump

The pump is equipped with a single input and double outputs (only one is used). It contains three internal plunger pumps which in combination with check valves create a smooth flow from the pump. The pump delivers flows up to 50 ml/min and pressures up to 1.0 MPa.

In the connection part, the inlet and outlet flow paths are split into three separate flow paths – one to/from each plunger pump chamber. All six flow paths are equipped with non-return check valves.



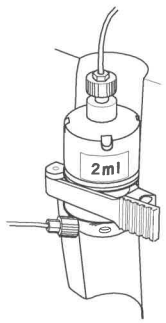
The plungers pump the liquid through the chambers while the check valves prevent the liquid from flowing backwards. The pump phases of three plunger pumps are displaced by 120° which results in a sequential motion of the plunger pumps, and thereby a smooth liquid delivery.

Leakage between the connection part and the pump chambers is prevented by O-rings. Any leakage behind the plungers is diverted through the drainage hole in the front of the connection part. The check valves are also equipped with O-rings.

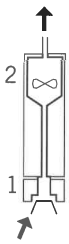
The pump is equipped with automatic speed control to reduce pulsation and with pressure compensation.

Pressure sensor
The pressure in the system is continuously measured by the pressure sensor located next to the pump. The pressure is shown on the display during the run. A maximum pressure limit can be set to protect the column.

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).

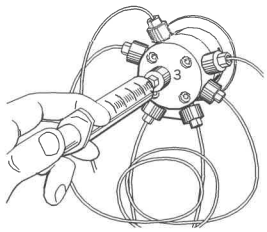
2 Dynamic mixing in a chamber with a rotating stirrer.

A mixer motor inside the system spins a magnet at 600 rpm, which causes the stirrer in the mixing chamber to rotate.

For optimal gradients at high flow rates, a larger mixer chamber is required. Other mixer chambers with 0.6, 5 and 12 ml mixer volumes are available as accessories.

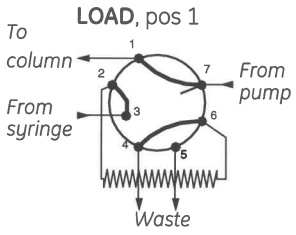
When using eluents that are more difficult to mix, such as isopropanol and water, or if the pH and conductivity readings indicate uneven mixing of your buffers (unstable readings) a larger mixer volume will give better mixing.

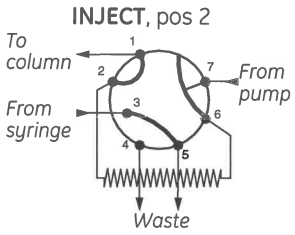
Injection valve
A 7-port motorized rotary valve is used as sample injection valve. It has a valve body with a rotating central core, the channel plate. As the channel plate is rotated by the motor, different ports are connected.

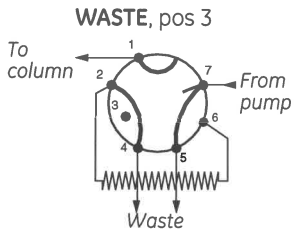


The valve has three different operating positions which make it possible to:

- 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.



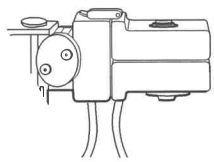




The geometry of the valves ensures that the flow path is completely swept so that solvent or sample "memory effect" is virtually non-existing. The switching parts are made of PEEK, which ensures both long mechanical and chemical lifetime.

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.



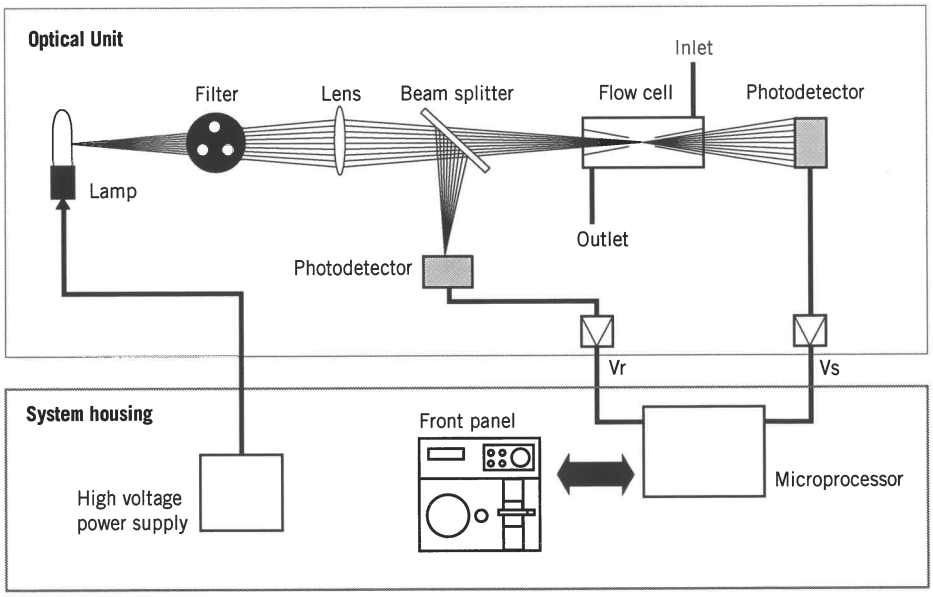
UV optical unit

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.

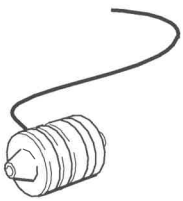


The Hg lamp emits light only at certain wavelengths. It does not emit light at 280 nm, so for this wavelength, the light is converted at a fluorescent surface before it passes the filter. On the lamp housing, there is a special exit for 280 nm light, which means that the lamp position needs to be changed 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.

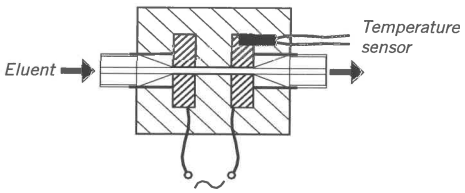
The lamp connectors are keyed to inform the monitor software which lamp type is connected.

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.

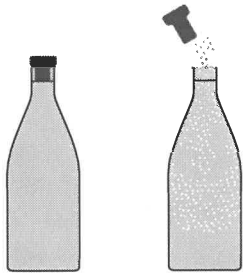
The conductivity is automatically calculated by multiplying the measured conductance by the flow cell's cell constant. The cell constant is pre-calibrated on delivery but can be measured with a separate calibration procedure. This procedure is described in section 9.20.3 Calibrating conductivity.



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.

Flow restrictor FR-902

The flow restrictor is connected after the conductivity cell. It prevents air bubbles being formed after the column when the pressure created in the column is released. Compare to a cork in a bottle of champagne.



This must be allowed for when setting the maximum pressure limit in the methods. For example, when using a column with a pressure limit of 0.3 MPa, the pressure limit in the method should be set to 0.5 MPa. This is described below in detail.

Two types of pressure limits

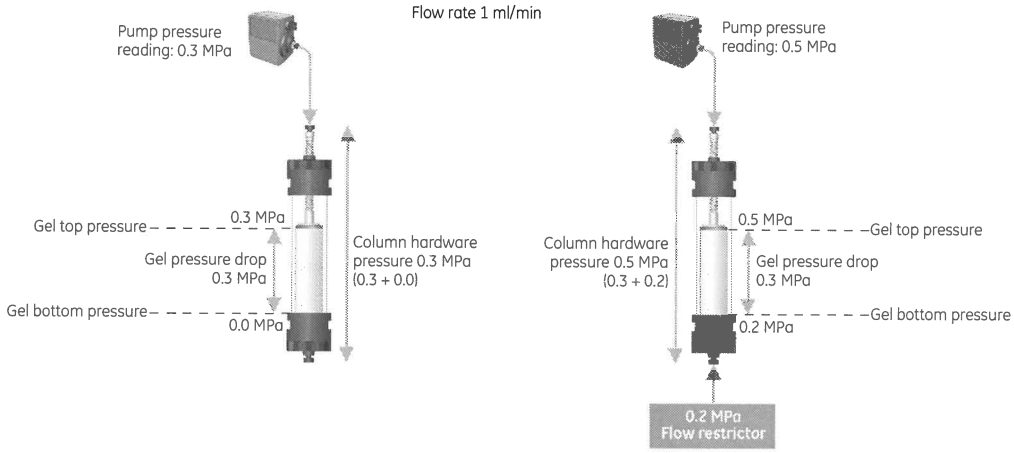
All chromatography columns have two types of pressure limits, one for the column hardware and one limit for the gel packing.

The pressure affecting the column hardware depends on the backpressure generated after the column by, for example flow cells, flow restrictors and tubing. When the pressure limit for the column hardware is exceeded, the column will start leaking. Empty chromatography columns from GE Healthcare are all supplied with data on the column hardware pressure limit.

The pressure affecting the gel packing is dependent on the flow rate and viscosity of the buffer. When the flow rate is too high and/or a high viscosity buffer is used, the pressure limit for the packed gel bed can be exceeded (the gel pressure limit is the maximum allowable pressure drop over the packed gel bed). When the pressure limit is exceeded, the gel particles are forced to the bottom of the column and cause the backpressure to increase. This leads to a collapse of the packed bed and the risk of poor chromatographic performance. The pressure limit data referring to the gel packing is found in the column instructions delivered with all prepacked columns from GE Healthcare.

How the flow restrictor affects the pressure limits

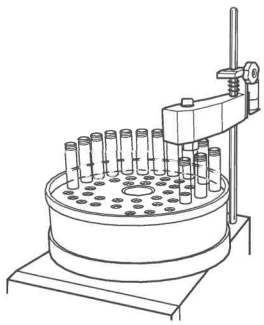
The flow restrictor only affects the column hardware pressure limit. The gel packing pressure limit is always unaffected by a flow restrictor. The figure below shows an example of how a flow restrictor affects the packed column at a hypothetical flow rate of 1 ml/min. The flow rate generates a pump pressure reading of 0.3 MPa. With no restrictor mounted, this pressure equals the pressure drop over both the gel and the column hardware.



When a flow restrictor generating a backpressure of 0.2 MPa is mounted after the column and the same flow rate is used, the pressure over the column hardware is affected and will be 0.5 MPa. Hence, the pump pressure gauge reading will be 0.5 MPa. However, the pressure drop over the packed gel bed is still 0.3 MPa.

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 and up to 95 fractions in 10–18 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.

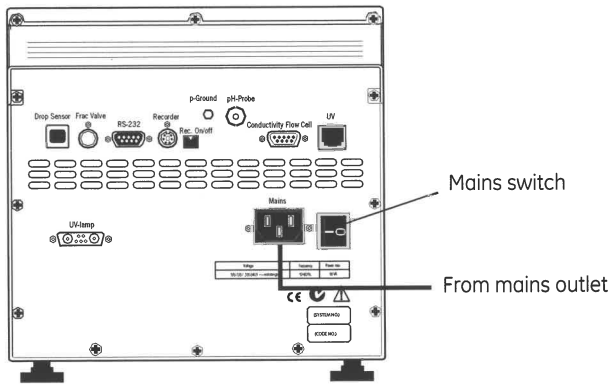
The flow diversion valve mounted on the fraction collector is used as follows:

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

11.1.2 Electrical connections

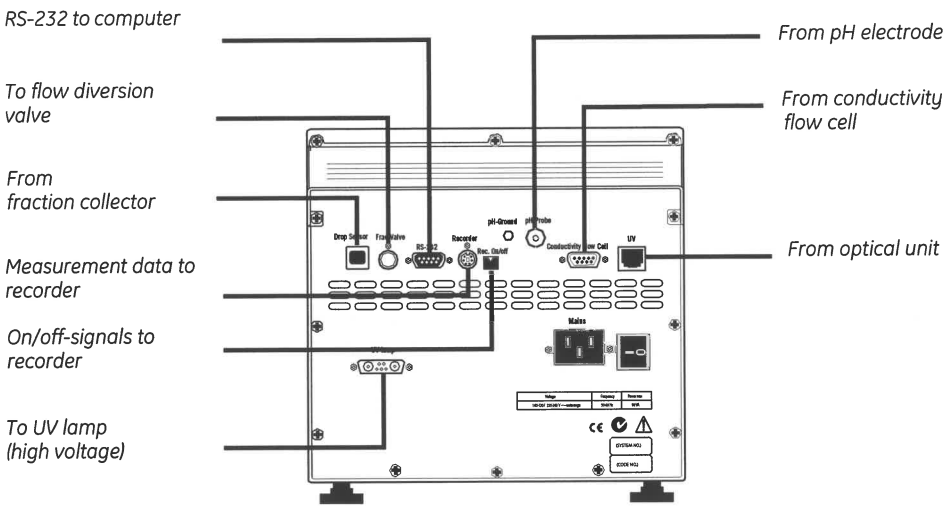
All electrical connections for ÄKTAprime plus are located at the rear of the system.

Mains cable



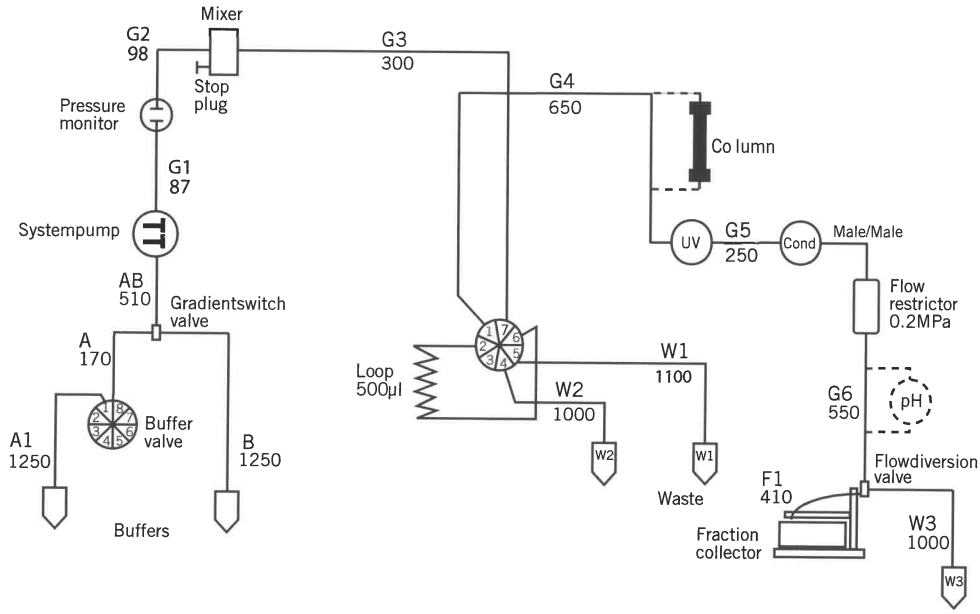
One mains input is required for the system.

Communication cables



11.1.3 Fluid handling path

The following flow diagram shows the positions of the components in ÄKTAprime plus fluid handling path.



All capillaries are fitted at the factory. The figure states the length in millimeters of the pre-fabricated capillaries.

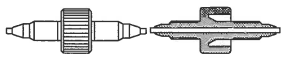
Tubing

The table below lists the tubing fitted at delivery of ÄKTAprime plus.

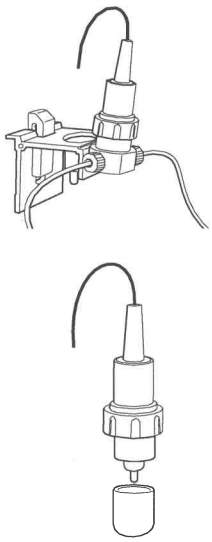
Tubing designation	Tubing i.d., o.d.	Material	Color	Max. pressure	Volume of 10 cm	Connected
A1, A, B	3.2 mm, 3/16"	Teflon	Clear	3.4 MPa	660.5 µl	All inlet tubings from buffer vessels to switch valve.
AB	1.6 mm, 1/8"	Teflon	Clear	2 MPa	201.1 µl	From switch valve to pump.
W1–W3	1.0 mm, 1/16"	PEEK	Brown	4.1 MPa	78.5 µl	Waste tubing.
G1–G6, F1	0.75 mm, 1/16"	PEEK	Green	2.6 MPa	44.2 µl	From pump to fraction collector.
Union, male/male	i.d. 1/16"	PEEK	Black (orange tubing)	25 MPa	–	Between conductivity flow cell and flow restrictor.
Stop plug	i.d. 1/16"	PEEK	Black	–	–	Mixer inlet.

Tubing connectors

The table below lists the connectors and unions used.

Tubing designation	Connector
A1, A, B	Nut and ferrule for o.d. 3/16" tubing (blue ferrule) 
AB	Nut and ferrule for o.d. 1/8" tubing (yellow ferrule) 
W1–W3, G1–G6, F1	Fingertight connector, for o.d. 1/16" tubing. 
Union male/male	Union, 1/16" male/ 1/16" male 
Stop plug	Stop plug, 1/16", 

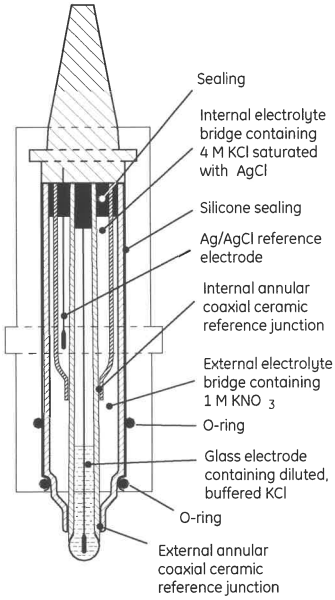
11.1.4 Optional components



pH flow cell with electrode

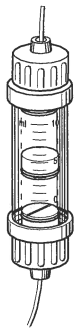
The pH electrode is of the sealed combination double junction type. It contains a sealed Ag/AgCl reference which cannot be refilled, an internal electrolyte bridge of 4 M KCl saturated with Ag/AgCl, an outer electrolyte bridge of 1 M KNO_3, an annular ceramic reference junction and a low profile pH membrane. 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.



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.

11.2 Menus

This section describes the Set parameters and Check menus. It also contains an complete overview of the menus in ÄKTAprime plus.

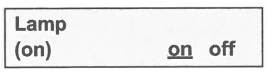
11.2.1 Set Parameters menus

Turning the lamp on/off

We recommend the lamp be turned 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.



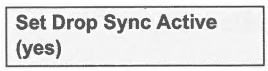
3 Switch the lamp on/off with the up and down buttons, and then press **OK**.

Setting drop synchronization

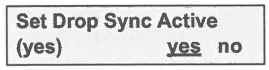
If drop synchronization is active, tube changes will only occur directly after a drop is registered by the drop counter to minimize spillage between tubes. Drop synchronization operates in all fraction collection modes; time, volume and drop.

Drop synchronization is only possible at flow rates up to 3 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**.

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**.

Print out to? **Recorder Computer**	3 Select printing to a recorder or a computer and press **OK**.
Set Rec Out 1 (UV)	4 The current setting for channel 1 is displayed. Press **OK** to access the setting menu.
Set Rec Out 1 (UV) **UV pH Cond %B Tmp Pr**	5 Select the parameter to be printed on channel 1 and press **OK**.
	6 Repeat steps 4 and 5 for channels 2 and 3.
Autoscale UV (no)	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.
Autoscale UV (no) **yes no**	8 Select the desired setting and press **OK**. The three selected curves are now printed.

Setting analog outputs
See section 8.6.5 Setting analog outputs.

Calibrating the flow rate
See section 9.20.1 Calibrating the pump.

Calibrating the pressure offset
See section 9.20.2 Calibrating the pressure sensor.

Set up show pH
Normally, the pH is displayed in the running window. If not required, the pH display can be set to off.

Set Parameters	1 From the main menu, select sub menu **Set Parameters** and press **OK**.
Setup and calibration	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**.

Set up pH calibration
Set up pH temperature compensation
See section *9.20.5 Calibrating the pH electrode (optional).*

Set up 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**.

Setting the conductivity cell constant
Calibrating the conductivity
Setting the conductivity temperature compensation
Setting the conductivity reference temperature
See section *9.20.3 Calibrating conductivity.*

Set up 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**.

Set up 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

Set up 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.

Set up 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**.

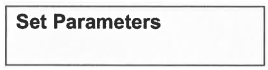
Set up Delay UV to Frac

The delay volume (in µl) is the volume of the flow path between the UV flow cell and the fraction collector. If the set delay volume is correct, the event marks on the UV absorbance curve will be synchronized with the tube changes.

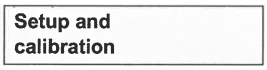
Calculate the volume of the tubing from the UV flow cell to the very end of the tubing. The volume of 0.75 mm i.d. tubing is 44.2 µl/10 cm.

The default delay volume is 380 µl.

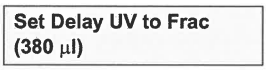
If using a pH electrode, 100 µl should be added to the delay volume (480 µl) provided that the tubing lengths remain unchanged.



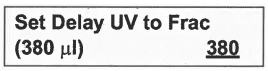
1 From the main menu, select menu **Set Parameters** and press **OK**.



2 Select sub menu **Setup and calibration** and press **OK**.



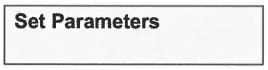
3 Select sub menu **Set Delay UV to Frac**. The current delay volume is shown. Press **OK** to change the volume.



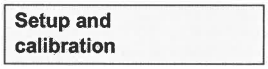
4 Change the setting as desired and press **OK**.

Set up mixer chamber volume

If the mixer chamber is exchanged for a chamber with another volume, the new chamber volume must be set. On delivery, the chamber volume is set to 2.0 ml. If a larger chamber volume is used, the wash and priming volumes must be increased accordingly.

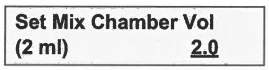


1 From the main menu, select menu **Set Parameters** and press **OK**.



2 Select sub menu **Setup and calibration** and press **OK**.

3 Select sub menu **Set Mix Chamber Vol**. The current chamber volume is shown. Press **OK** to change the volume.



4 Change the setting as desired and press **OK**. Possible values are 0.6, 2.0, 5.0 and 12.0 ml.

11.2.2 Check menus

Checking communication

The communication between the ÄKTAprime plus system and a computer can be checked.

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

2 The system immediately checks the communication with the computer.

PC App. Connected! The communication is OK.

PC Driver Connected! PrimeView software is not properly installed.

Not Connected The serial cable between the system and the computer is not properly connected , or PrimeView software is not installed on the computer.

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.

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**.

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**.

Checking pump run time

The pump run time can be checked to determine the need for maintenance.

Check Pump Run Time
246h

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

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

Checking pumped volume

The volume delivered by the pump can be checked to determine the need for maintenance.

Check Pumped Volume
3567 ml

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

2 Select sub menu **Check Pumped Volume**.

Checking tube shifts

The number of tube shifts done by the fraction collector can be checked to determine the need for maintenance.

Check Tube Shifts
3592

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

2 Select sub menu **Check Tube Shifts**.

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**.

Checking the recorder

The function of a connected chart recorder can be tested.

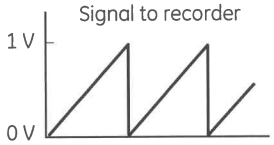
1 Select menu **Check** and press **OK**.

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**.

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 **V3.00**

6 The system name and software version are displayed. Press **OK**.

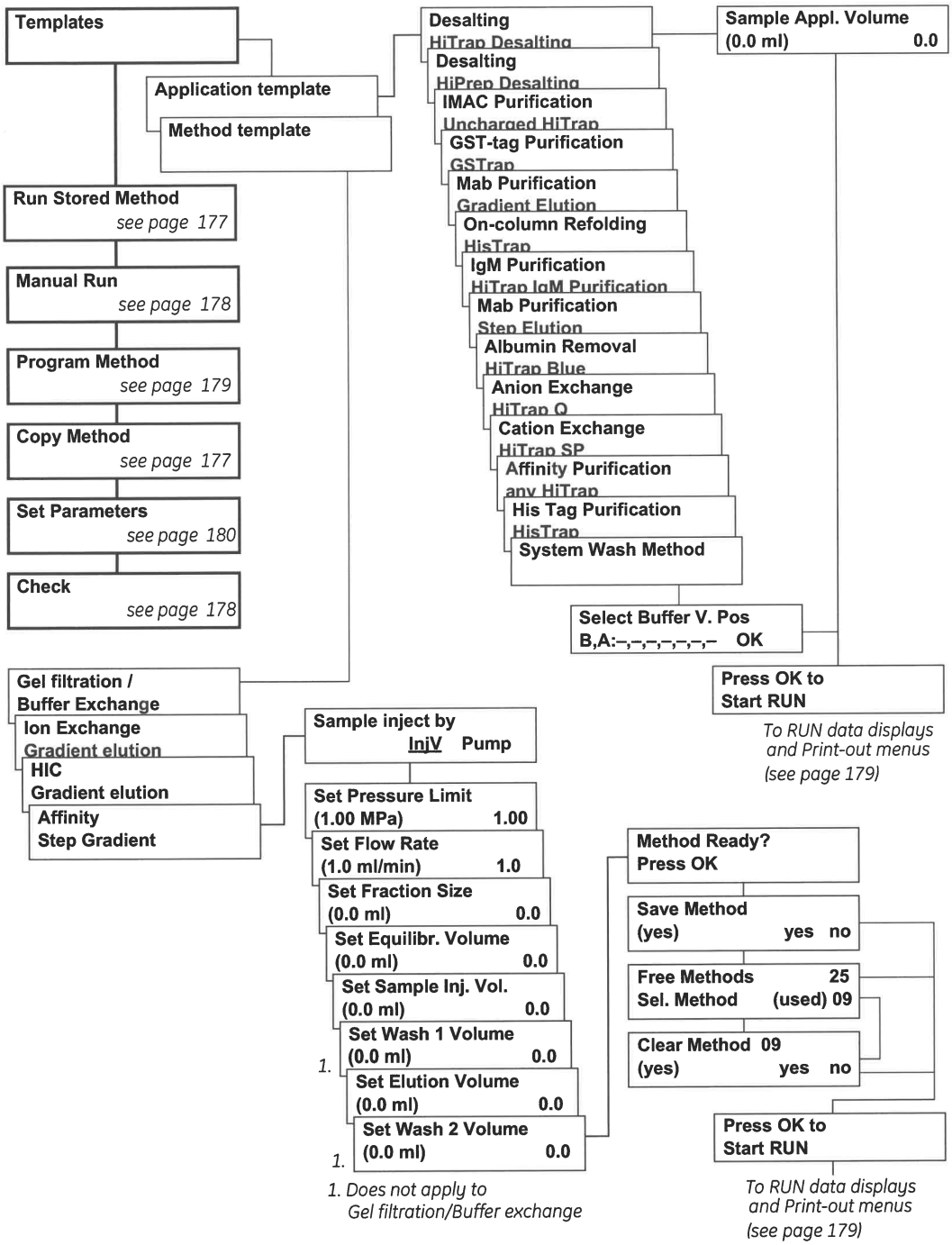
Date of maintenance: **DD MMMM YYYY**

7 The date of the last service is displayed. Press **OK**.

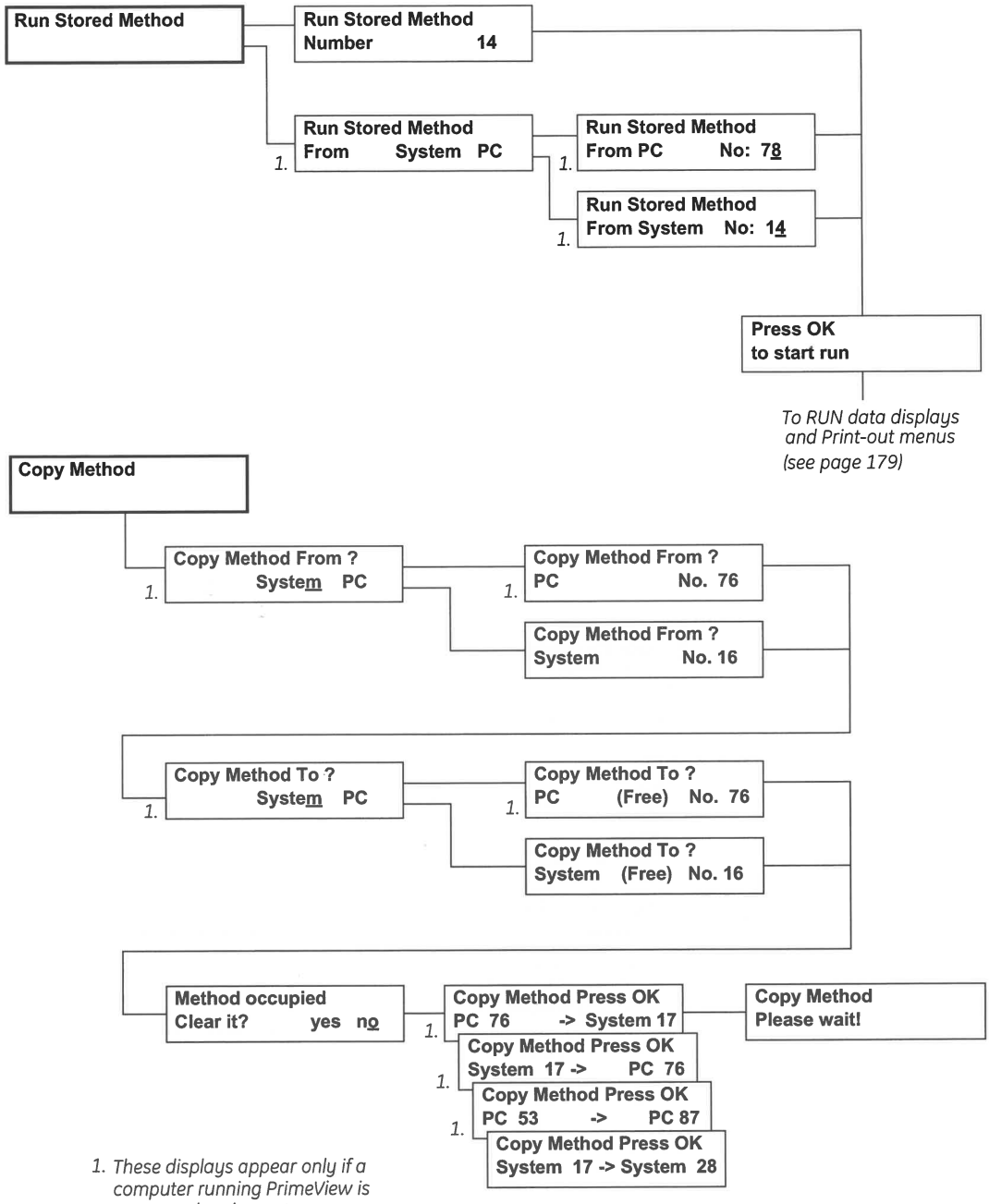
Buzzer Test

8 A test of the system buzzer is performed. Press **OK**.

11.2.3 Menu overview

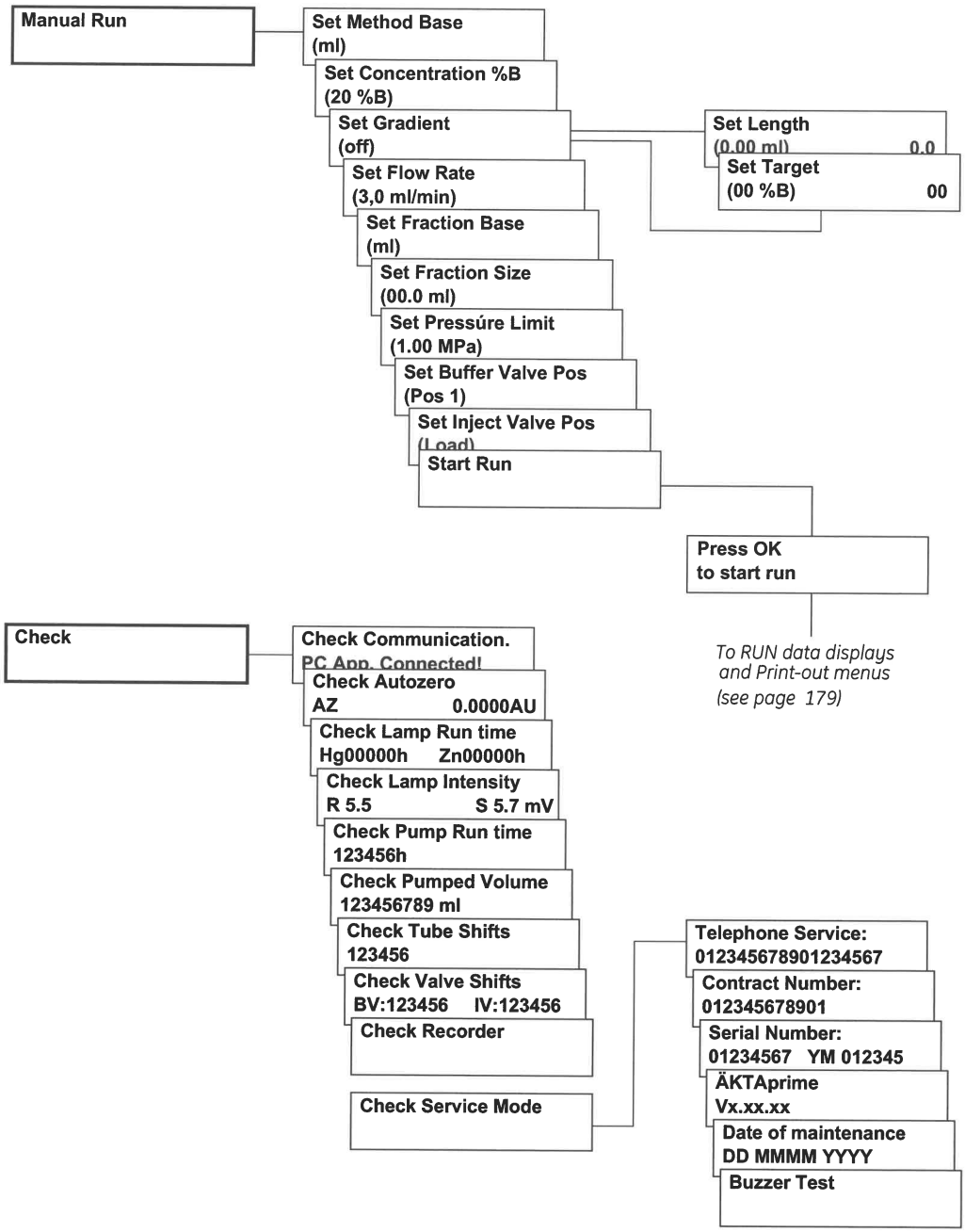


Menu overview (cont.) 2

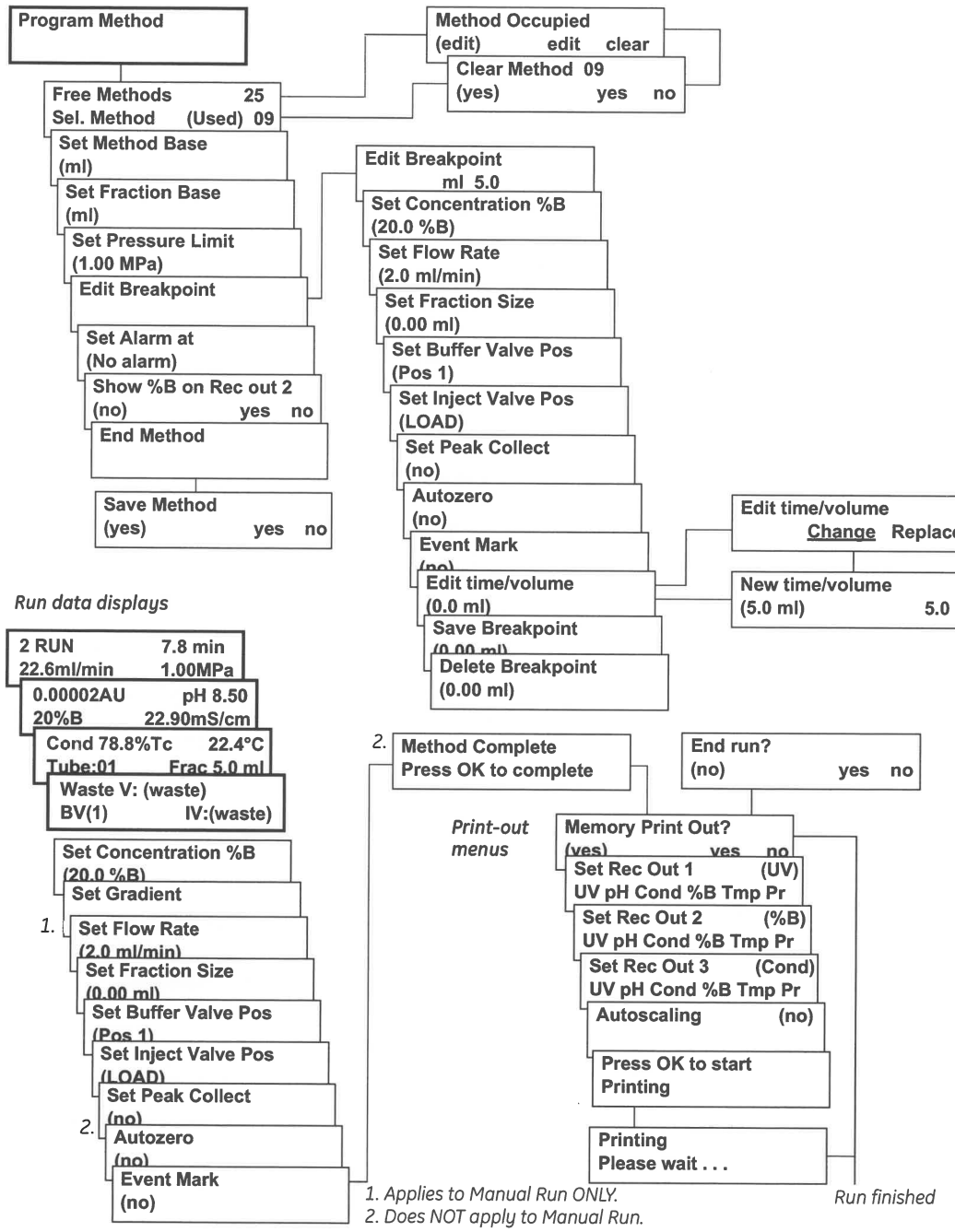


1. *These displays appear only if a computer running PrimeView is connected to the system.*

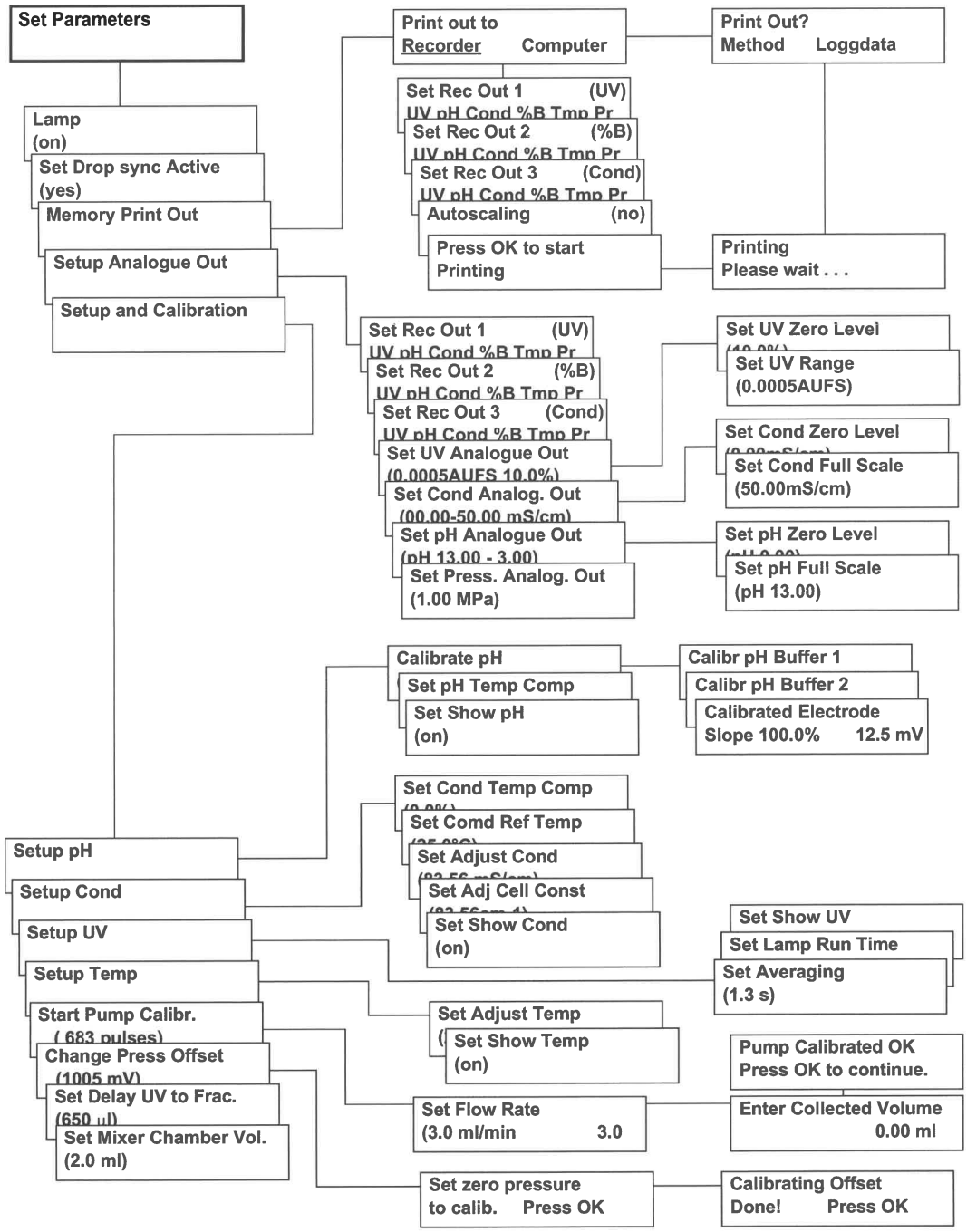
Menu overview (cont.) 3



Menu overview (cont.) 4



Menu overview (cont.) 5



11.3 Technical specifications

Relevant system and component specifications are listed below.

11.3.1 Operating data

Pump	
Flow rate range	0.1–50 ml/min in steps of 0.1 ml/min
Operating pressure range	0–1.0 MPa (10 bar, 145 psi)
Pressure pulsation	≤ ±10% of mean value in entire range
pH stability range	1–14 (spec. valid between pH 2–12)
Viscosity *≤10 ml/min* *> 10 ml/min*	 Max. 10 cP Max. 5 cP
Flow rate reproducibility	rsd ≤ 1% during one day (pressure ≥ 0.1 MPa, flow rate ≥ 0.4 ml/min)
Flow rate accuracy	-5%– +3% of setting (pressure ≥ 0.1 MPa, flow rate ≥ 0.4 ml/min)
Gradient composition *accuracy* *reproducibility*	 ±3% at 0.1–50 ml/min (5–95%B) ±1.0% at 0.1–50 ml/min (5–95%B)
Leakage	< 1.0 µl/min (complete system)
Pressure sensor *range* *scale error* *offset error*	 0–1.1 MPa ±2% ±0.02 MPa
UV measurement	
Wavelengths *Hg lamp, fixed* *by changing filter (option)* *Zn lamp (optional)*	 254 and 280 nm 313, 365, 405, 436 and 546 nm 214 nm
Absorbance range	0.001–5.0 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% at other wavelengths except 280 nm
Static noise *short term* *long term*	 40×10^{-6} AU at 254 nm 40×10^{-6} AU at 254 nm
Static drift	$\pm 100 \times 10^{-6}$ AU/hour at 254 nm
Flow sensitivity	2×10^{-4} AU min/ml

UV flow cell, 2 mm	
Flow rate	0–100 ml/min
Max. pressure	4.0 MPa
Max. backpressure	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. backpressure	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* *long term*	 Max. ±1% or ±5 µS/cm whichever is greater 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* *drift*	 ±2.0 °C ±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. backpressure	0.01 MPa at 100 ml/min
Fraction collection	
Tube capacity	95 in tube rack 18 mm 175 in tube rack 12 mm (optional)

pH measurement	
pH range	0 to 14 (spec. valid between 2 and 12)
Accuracy *temperature compensated* *not compensated*	 ±0.1 pH within +4 to +40 °C ±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. backpressure	0.02 MPa at 100 ml/min

11.3.2 Physical data

Control	Via membrane keyboard and display (2x20 characters)
Degree of protection *housing* *flow cells*	 IP 20 IP 44
Power requirement	100–120/220–240 V ~ autorange, 50–60 Hz
Power consumption	Max. 90 VA
Fuse specification	T 1.0 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).
Compliance with standards	The declaration of conformity is valid for the instrument only if it is: • used in laboratory locations • used in the same state as it was delivered from GE Healthcare except for alterations described in the User Manual • connected to other CE labelled GE Healthcare modules or other products as recommended.
Safety standards	This product meets the requirement of the Low Voltage Directive (LVD) 73/23/EEC through the following harmonized standards: • EN 61010-1 • IEC 61010-1 • CAN/CSA-C22.2 No. 61010-1 • UL61010-1

EMC standards	This device meets the requirements of the EMC Directive 89/336/EEC through the following harmonized standards: • EN 61326 (emission and immunity) • EN 55011, GR 2, Class A (emission) • This device complies with part 15 of the FCC rules (emission). Operation is subject to the following two conditions: 1 This device may not cause harmful interference. 2 This device must accept any interference received, including interference that may cause undesired operation.

11.3.3 Wetted materials

The wetted materials in ÄKTAprime plus are listed below:

	FFKM	PEEK	PTFE	FEP	ETFE	ECTFE	PP	PE	Titanium alloy	Elgiloy	UHMWPE	Sapphire	Quartz	Glass	Gold	Simriz	Ceramic
Pump		X							X	X	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															
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
UHMWPE = Ultra-high molecular weight polyethylene
PE = polyethene
PP = polypropylene

11.4 Chemical resistance guide and chemical compatibility

The chemical resistance of ÄKTAprime plus 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	

11.5 Ordering information

11.5.1 Recommended columns

Ion Exchange Columns

Column name	Pack size	Code no.
HiTrap™ Q HP, 1 ml	5 × 1 ml	17-1153-01
HiTrap Q HP, 5 ml	5 × 5 ml	17-1154-01
HiTrap SP HP, 1 ml	5 × 1 ml	17-1151-01
HiTrap SP HP, 5 ml	5 × 5 ml	17-1152-01
HiTrap IEX Selection kit	7 × 1 ml	17-6002-33
HiTrap Q FF, 1 ml	5 × 1 ml	17-5053-01
HiTrap Q FF, 5 ml	5 × 5 ml	17-5156-01
HiTrap SP FF, 1 ml	5 × 1 ml	17-5054-01
HiTrap SP FF, 5 ml	5 × 5 ml	17-5157-01
HiTrap DEAE FF, 1 ml	5 × 1 ml	17-5055-01
HiTrap DEAE FF, 5 ml	5 × 5 ml	17-5154-01
HiTrap CM FF, 1 ml	5 × 1 ml	17-5056-01
HiTrap CM FF, 5 ml	5 × 5 ml	17-5155-01
HiTrap ANX FF (high sub), 1 ml	5 × 1 ml	17-5162-01
HiTrap ANX FF (high sub), 5 ml	5 × 5 ml	17-5163-01
HiTrap Q XL, 1 ml	5 × 1 ml	17-5158-01
HiTrap Q XL, 5 ml	5 × 5 ml	17-5159-01
HiTrap SP XL, 1 ml	5 × 1 ml	17-5160-01
HiTrap SP XL, 5 ml	5 × 5 ml	17-5161-01
HiTrap Capto Q, 1 ml	5 x 1 ml	11-0013-02
HiTrap Capto Q, 5 ml	5 x 5 ml	11-0013-03
HiTrap Capto ViralQ, 5 ml	5 x 5 ml	28-9078-09
HiTrap Capto MMC, 1 ml	5 x 1 ml	11-0032-73
HiTrap Capto MMC, 5 ml	5 x 5 ml	11-0032-75
HiTrap Capto S, 1 ml	5 x 1 ml	17-5441-22
HiTrap Capto S, 5 ml	5 x 5 ml	17-5441-23
HiPrep™ 16/10 Q XL	1 × 20 ml	17-5092-01
HiPrep 16/10 SP XL	1 × 20 ml	17-5093-01
HiPrep 16/10 CM FF	1 × 20 ml	17-5091-01
HiPrep 16/10 DEAE FF	1 × 20 ml	17-5090-01
HiPrep 16/10 Q FF	1 × 20 ml	17-5190-01
HiPrep 16/10 ANX FF (high sub)	1 × 20 ml	17-5191-01
HiPrep 16/10 SP FF	1 × 20 ml	17-5192-01

Buffer Exchange/Desalting Columns

Column name	Pack size	Code no.
HiTrap Desalting, 5 ml	5 × 5 ml	17-1408-01
HiTrap Desalting, 5 ml	100 × 5 ml[1]	11-0003-29
HiPrep 26/10 Desalting	1 × 53 ml	17-5087-01
HiPrep 26/10 Desalting	4 × 53 ml	17-5087-02

[1] Pack size available on special order.

Size Exclusion (Gel filtration) Columns

Column name	Pack size	Code no.
HiPrep 16/60 Sephacryl™ S-100 HR	1 × 120 ml	17-1165-01
HiPrep 26/60 Sephacryl S-100 HR	1 × 320 ml	17-1194-01
HiPrep 16/60 Sephacryl S-200 HR	1 × 120 ml	17-1166-01
HiPrep 26/60 Sephacryl S-200 HR	1 × 320 ml	17-1195-01
HiPrep 16/60 Sephacryl S-300 HR	1 × 120 ml	17-1167-01
HiPrep 26/60 Sephacryl S-300 HR	1 × 320 ml	17-1196-01

Chelating Columns

Column name	Pack size	Code no.
HiTrap Chelating HP, 1 ml	5 × 1 ml	17-0408-01
HiTrap Chelating HP, 5 ml	1 × 5 ml	17-0409-01
HiTrap Chelating HP, 5 ml	5 × 5 ml	17-0409-03
HiTrap Chelating HP, 5 ml	100 × 5 ml[1]	17-0409-05
HiTrap IMAC HP, 1 ml	5 x 1 ml	17-0920-03
HiTrap IMAC HP, 5 ml	5 x 5 ml	17-0920-05
HiTrap IMAC FF, 1 ml	5 x 1 ml	17-0921-02
HiTrap IMAC FF, 5 ml	5 x 5 ml	17-0921-04
HiPrep IMAC FF 16/10	1 x 20 ml	17-0921-06

[1] Pack size available on special order.

Affinity Columns

Column name	Pack size	Code no.
HiTrap MabSelect SuRe, 1 ml	5 x 1 ml	11-0034-93
HiTrap MabSelect SuRe, 5 ml	1 x 5 ml	11-0034-94
HiTrap MabSelect SuRe, 5 ml	5 x 5 ml	11-0034-95
HiTrap MabSelect, 1 ml	5 x 1 ml	28-4082-53
HiTrap MabSelect, 5 ml	1 x 5 ml	28-4082-55
HiTrap MabSelect, 5 ml	5 x 5 ml	28-4082-56

Column name	Pack size	Code no.
HiTrap MabSelect Xtra, 1 ml	5 x 1 ml	28-4082-58
HiTrap MabSelect Xtra, 5 ml	1 x 5 ml	28-4082-60
HiTrap MabSelect Xtra, 5 ml	5 x 5 ml	28-4082-61
HiTrap Protein A HP, 1 ml	5 × 1 ml	17-0402-01
HiTrap Protein A HP, 1 ml	2 × 1 ml	17-0402-03
HiTrap Protein A HP, 5 ml	1 × 5 ml	17-0403-01
HiTrap Protein A HP, 5 ml	5 x 5 ml	17-0403-03
HiTrap Protein G HP, 1 ml	5 × 1 ml	17-0404-01
HiTrap Protein G HP, 1 ml	2 × 1 ml	17-0404-03
HiTrap Protein G HP, 5 ml	1 × 5 ml	17-0405-01
HiTrap Protein G HP, 5 ml	5 x 5 ml	17-0405-03
HiTrap Heparin HP, 1 ml	5 × 1 ml	17-0406-01
HiTrap Heparin HP, 5 ml	1 × 5 ml	17-0407-01
HiTrap Heparin HP, 5 ml	5 x 5 ml	17-0407-03
HiPrep 16/10 Heparin FF	1 × 20 ml	17-5189-01
HiTrap rProtein A HP, 1 ml	5 × 1 ml	17-5079-01
HiTrap rProtein A HP, 1 ml	2 × 1 ml	17-5079-02
HiTrap rProtein A HP, 5 ml	1 × 5 ml	17-5080-01
HiTrap rProtein A FF, 5 ml	5 x 5 ml	17-5080-02
HiTrap Blue HP, 1 ml	5 × 1 ml	17-0412-01
HiTrap Blue HP, 5 ml	1 × 5 ml	17-0413-01
HiTrap NHS-activated HP, 1 ml	5 × 1 ml	17-0716-01
HiTrap NHS-activated HP, 5 ml	1 × 5 ml	17-0717-01
HiTrap IgM Purification HP, 1 ml	5 × 1 ml	17-5110-01
HiTrap IgY Purification HP, 5 ml	1 × 5 ml	17-5111-01
HiTrap Streptavidin HP, 1 ml	5 × 1 ml	17-5112-01
HisTrap™ HP, 1 ml	5 × 1 ml	17-5247-01
HisTrap HP, 1 ml	100 × 1 ml[1]	17-5247-05
HisTrap HP, 5 ml	1 × 5 ml	17-5248-01
HisTrap HP, 5 ml	5 × 5 ml	17-5248-02
HisTrap HP, 5 ml	100 × 5 ml[1]	17-5248-05
HisTrap FF, 1 ml	5 × 1 ml	17-5319-01
HisTrap FF, 1 ml	100 × 1 ml[1]	17-5319-02
HisTrap FF, 5 ml	5 × 5 ml	17-5255-01
HisTrap FF, 5 ml	100 × 5 ml[1]	17-5255-02
HisPrep™ FF 16/10	1 × 20 ml	17-5256-01
HisTrap FF crude, 1 ml	5 × 1 ml	11-0004-58
HisTrap FF crude, 1 ml	100 × 1 ml[1]	11-0004-59

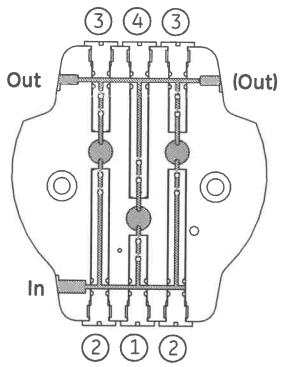
Column name	Pack size	Code no.
HisTrap FF crude, 5 ml	5 × 5 ml	17-5286-01
HisTrap FF crude, 5 ml	100 × 5 ml[1]	17-5286-02
GSTrap™ HP, 1 ml	5 × 1 ml	17-5281-01
GSTrap HP, 1 ml	100 × 1 ml[1]	17-5281-02
GSTrap HP, 5 ml	1 × 5 ml	17-5282-01
GSTrap HP, 5 ml	5 × 5 ml	17-5282-02
GSTrap HP, 5 ml	100 × 5 ml[1]	17-5282-05
GSTrap FF, 1 ml	5 × 1 ml	17-5130-01
GSTrap FF, 1 ml	2 × 1 ml	17-5130-02
GSTrap FF, 1 ml	100 × 1 ml[1]	17-5130-05
GSTrap FF, 5 ml	1 × 5 ml	17-5131-01
GSTrap FF, 5 ml	5 × 5 ml	17-5131-02
GSTrap FF, 5 ml	100 × 5 ml[1]	17-5131-05
GSTPrep™ FF 16/10	1 × 20 ml	17-5234-01
GSTrap 4B, 1 ml	5 x 1 ml	28-4017-45
GSTrap 4B, 1 ml	100 × 1 ml[1]	28-4017-46
GSTrap 4B, 5 ml	1 x 5 ml	28-4017-47
GSTrap 4B, 5 ml	5 x 5 ml	28-4017-48
GSTrap 4B, 5 ml	100 × 5 ml[1]	28-4017-49
HiTrap Benzamidine FF (high sub), 1 ml	2 × 1 ml	17-5143-02
HiTrap Benzamidine FF (high sub), 1 ml	5 × 1 ml	17-5143-01
HiTrap Benzamidine FF (high sub), 5 ml	1 × 5 ml	17-5144-01

[1] Pack size available on special order.

Hydrophobic Interaction Columns

Column name	Pack size	Code no.
HiTrap HIC Selection kit	6 × 1 ml	11-0034-53
HiTrap Phenyl FF (high sub), 1 ml	5 × 1 ml	17-1355-01
HiTrap Phenyl FF (high sub), 5 ml	5 × 5 ml	17-5193-01
HiTrap Phenyl FF (low sub), 1 ml	5 × 1 ml	17-1353-01
HiTrap Phenyl FF (low sub), 5 ml	5 × 5 ml	17-5194-01
HiTrap Phenyl HP, 1 ml	5 × 1 ml	17-1351-01
HiTrap Phenyl HP, 5 ml	5 × 5 ml	17-5195-01
HiTrap Octyl FF, 1 ml	5 × 1 ml	17-1359-01
HiTrap Octyl FF, 5 ml	5 × 5 ml	17-5196-01
HiTrap Butyl FF, 1 ml	5 × 1 ml	17-1357-01
HiTrap Butyl FF, 5 ml	5 × 5 ml	17-5197-01
HiTrap Butyl-S FF, 1 ml	5 × 1 ml	17-0978-13

Column name	Pack size	Code no.
HiTrap Butyl-S FF, 5 ml	5 × 5 ml	17-0978-14
HiPrep 16/60 Phenyl FF (high sub)	1 × 20 ml	17-5095-01
HiPrep 16/60 Phenyl FF (low sub)	1 × 20 ml	17-5094-01
HiPrep 16/60 Octyl FF	1 × 20 ml	17-5097-01
HiPrep 16/60 Butyl FF	1 × 20 ml	17-5096-01

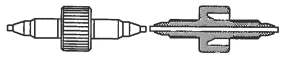
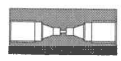


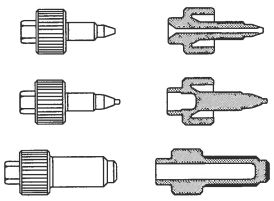
Connection part, (schematic drawing)

11.5.2 Consumables and accessories

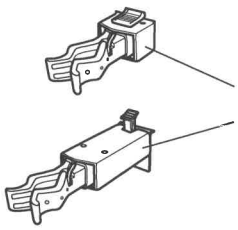
Item	Quant./ pack	A/C*	Code no.
ÄKTAprime plus incl. REC 112	1	C	11-0013-12
ÄKTAprime plus without REC 112	1	C	11-0013-13
PrimeView software kit incl. CD, manual and serial cable	1	A	11-0003-59
Pump			
Inlet check valve, short (1)	1	C	18-1172-43
Inlet check valve, long (2)	1	C	18-1172-44
Outlet check valve, short (3)	1	C	18-1172-45
Outlet check valve, long (4)	1	C	18-1172-46
O-ring kit, P-960, incl.		C	18-1172-53
1.42 × 1.52 mm (connection part)	7		
4 × 1.2 mm (check valves)	12		
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
Conductivity flow cell			
Conductivity flow cell	1	C	18-1111-05
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	18-1112-92
Dummy electrode, round tip	1	A	18-1111-03

Item	Quant./ pack	A/C*	Code no.
Injection valve			
Repair kit INV-907	1	C	18-1109-05
Injection fill port 0.7 mm	1	C	18-1127-66
Buffer valve			
Repair kit IV-908	1	C	18-1109-07
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
Fraction collector			
Tube racks, complete with bowl, tube support, holder and guide:			
12 mm	1	A	19-8684-03
18 mm	1	A	18-3050-03
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
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
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
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

	Item	Quant./ pack	A/C*	Code no.
	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
	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
	Signal cable, ÄKTAprime plus	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
	Tubing connector, inlet nut for o.d. 1/8", PEEK	10	A	18-1121-17
	Ferrule, for 1/8" o.d. tubing, PEEK	10	A	18-1121-18
	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, 5/16" female/M6 male, PEEK	3	A	18-1127-76
	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



Item	Quant./ pack	A/C*	Code no.
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
Purge kit	1	A	18-1153-28
Miscellaneous			
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
Screw lid GL45, incl. cap membrane	1	A	11-0004-10
Flow restrictor, FR-902	1	A	18-1121-35
Flow restrictor, FR-904	1	A	18-1119-63
Cramp, for column holder, ÄKTAprime plus	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
Tubing cutter	1	A	18-1112-46
U-wrench, M6	1	A	19-7481-01
U-wrench, 1/4"	1	A	18-1112-45
Hex wrench, 2.5 mm	1	A	19-4442-01
Chart recorder REC 112, 2 channel	1	A	18-1132-33
Computer, laptop	1	A	18-3535-35



Item	Quant./ pack	A/C*	Code no.
User Documentation			
ÄKTAprime plus User Manual	1		11-0026-44
ÄKTAprime plus Cue Cards	1		11-0027-48
Related literature			
Protein Purification Handbook	1		18-1132-29
Affinity Chromatography, Principles and Methods	1		18-1022-29
Gel Filtration, Principles and Methods	1		18-1022-18
Hydrophobic Interaction Chromatography, Principles and Methods	1		18-1020-90
Ion Exchange Chromatgraphy & Chromatofocusing, Principles and Methods	1		11-0004-21
Antibody Purification Handbook	1		18-1037-46

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