

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, or 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.

Contents

1	Introduction	
1.1	General	9
1.2	ÄKTAprime plus user documentation	10
1.3	ÄKTAprime plus User Manual	11
1.4	Safety	12
1.5	Typographical conventions	13
2	Installation	
2.1	General	15
2.2	Pre-requisites	16
2.3	Unpacking the system	16
2.4	Installing the system	17
2.5	Connecting the mains cable	18
2.6	Connecting a computer for PrimeView to the system	19
2.7	System self-test	20
3	Making your first run	
3.1	General	21
3.2	Purification work flow	21
3.3	Pre-requisites	22
3.4	Starting ÄKTAprime plus	22
3.5	Starting PrimeView	22
3.6	Buffer preparation	23
3.7	Sample preparation	23
3.8	Purification setup	23
3.9	Selecting template and starting the run	27
3.10	Viewing the run	28
3.11	Viewing the result	29
3.12	Creating and printing a simple report	31
3.13	Cleaning after a run	33
3.14	Making further runs	34
4	System overview	
4.1	General	35
4.2	System components	35
4.3	System flow path	36
4.4	Functional description	37
4.5	Columns and tubing	38
4.6	Operator interface	39
4.7	Main menu overview	40

5	Making further runs	
5.1	General	41
5.2	Preparing the system further	42
5.3	Calibrations schedule	44
5.4	Applying the sample	45
5.5	Collecting fractions	52
5.6	Starting a run	53
5.7	During a run	56
5.8	Running a method template	59
5.9	Running a stored method	62
5.10	Running the system manually	63
5.11	Cleaning after a run and storage	66
5.12	Cold room operation	68
6	Method programming	
6.1	General	69
6.2	Programming using method templates	69
6.3	Programming line-by-line	72
6.4	Editing a stored method	78
6.5	Copying a method	80
7	Template description	
7.1	General	81
7.2	Application templates	81
7.3	Method templates	96
8	Installing and modifying components	
8.1	General	99
8.2	Optical unit	100
8.3	Conductivity cell	104
8.4	Fraction collector	104
8.5	pH flow cell and electrode (optional)	109
8.6	Recorder REC 112	110
9	Maintenance	
9.1	Periodic maintenance	119
9.2	Cleaning the system	121
9.3	Cleaning the system flow path	122
9.4	Moving the system	124
9.5	Checking the UV monitor	124
9.6	Checking the pump	125
9.7	Checking the fraction collector	126
9.8	Checking the rotary valves	126
9.9	Cleaning the UV flow cell in-place	126
9.10	Cleaning the UV flow cell off-line	126
9.11	Cleaning the conductivity flow cell off-line	127

9.12	Replacing the conductivity cell	127
9.13	Replacing plates in the rotary valves	128
9.14	Removing and assembling the pump	129
9.15	Replacing O-rings in the pump	130
9.16	Cleaning and replacing check valves in the pump	130
9.17	Replacing mixer chamber	133
9.18	Cleaning the pH electrode (optional)	134
9.19	Replacing the pH electrode (optional)	134
9.20	Calibrations	135
10	Troubleshooting	
10.1	Faults and actions	143
10.2	System	144
10.3	Pressure curve	144
10.4	UV curve	145
10.5	Conductivity curve	146
10.6	pH curve (optional)	147
10.7	Mixer	148
10.8	Pump	148
10.9	Fraction collector	149
10.10	Buffer valve and injection valve	149
10.11	Error messages	150
10.12	Adjusting the spring tension of the delivery arm	154
10.13	Removing trapped air bubbles	154
10.14	Restart after power failure	154
11	Reference information	
11.1	System description	155
11.2	Menus	167
11.3	Technical specifications	181
11.4	Chemical resistance guide and chemical compatibility	187
11.5	Ordering information	188

Index

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.

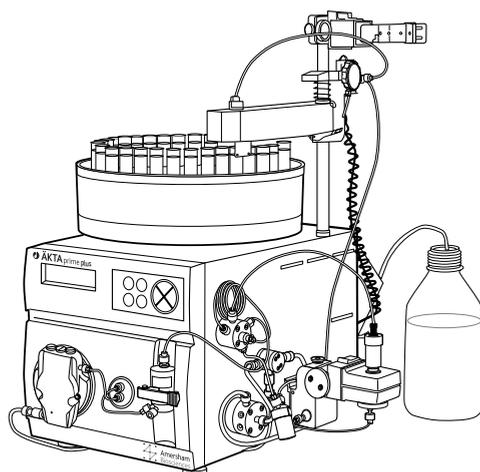
System description and instructions for installation, maintenance and troubleshooting are also included in this manual.

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

- Preparing for installation..... 16
- Unpacking ÄKTAprime plus..... 16
- Installing the system 17
- Connecting the mains power cabling..... 18
- Connecting a computer for using PrimeView..... 19
- Running the system self-test..... 20

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.

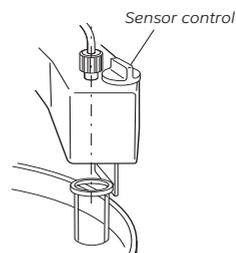
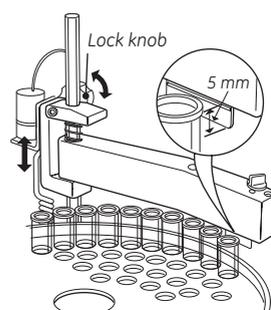
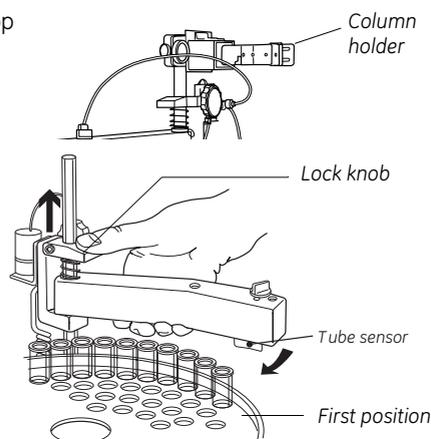
- 3 Loosen the lock knob holding the delivery arm and raise the arm.

- 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

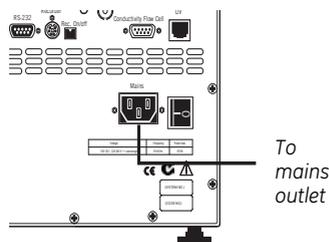


WARNING! ÄKTAprime plus must be connected to a grounded mains socket to prevent system parts from becoming live.



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.



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.

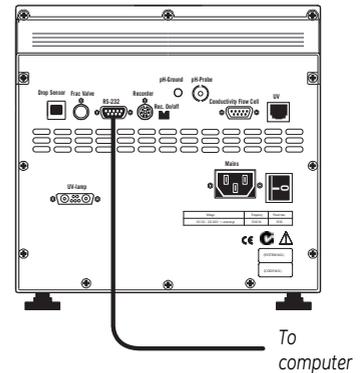


WARNING! The computer should be installed and used according to the instructions provided by the manufacturer of the computer.

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.

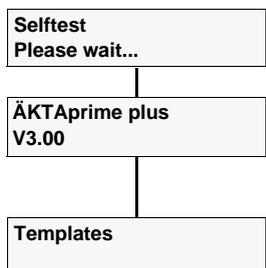
Note: Data is sent through pin 3 and received through pin 2.
Ground is on pin 5.



- 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.

Press OK to start run

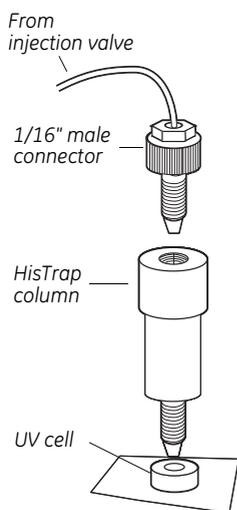
7 Scroll to **OK** and press the **OK** button.

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.

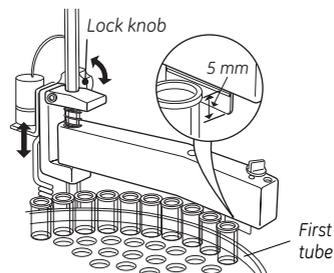
**3.8.2 Preparing the tubing and column**

- 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.

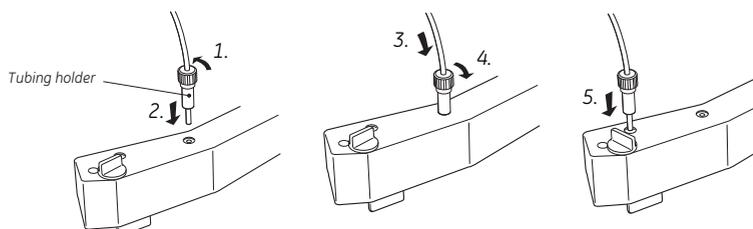
Note: Other unions and connectors might be required for other columns.

3.8.3 Preparing the fraction collector

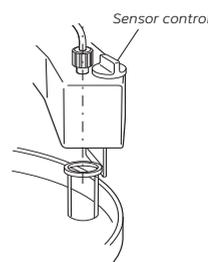
- 1 Fill the fraction collector rack with 18 mm tubes (minimum 40 pcs.).
- 2 Adjust the height of the delivery arm using the lock so that the bottom of the tube sensor is about 5 mm below the top of the tubes. The tubes should always be below the horizontal mark on the tube sensor.



- 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

- 7 Make sure that drop synchronization is turned on.

Set Drop Sync Active
(yes)

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.

Templates

- 2 Select **Templates** in the main menu using the Δ and ∇ buttons and press **OK**.

Application template

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

System Wash Method

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

Select Buffer V. Pos

B,A: _ , _ , _ , _ , _ , _ OK

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

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

Press OK to start run

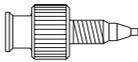
- 7 Press **OK** to start the method.

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

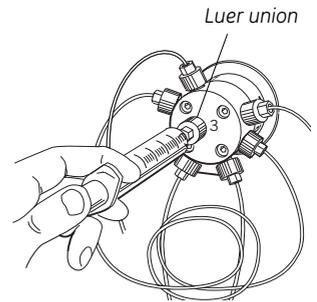
3.8.6 Filling the sample loop

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.



Luer union



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**.

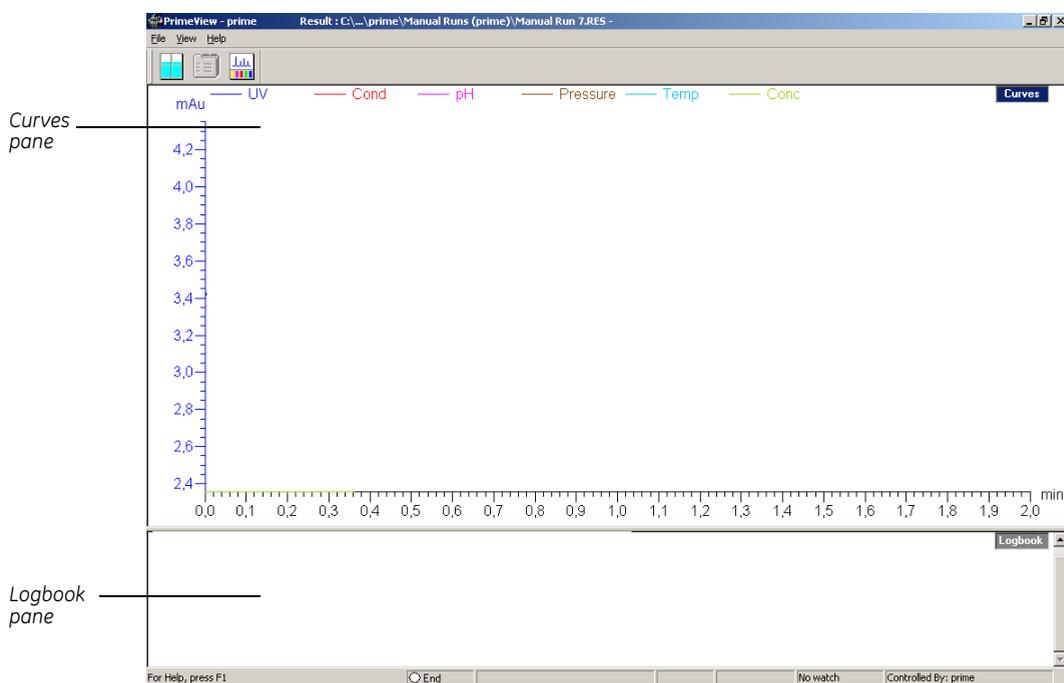
Press **OK** to
start run

- 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*.

Method Complete
Press OK to continue

3.10.2 Finishing the run

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

End method?
(yes) yes no

3.11 Viewing the result

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



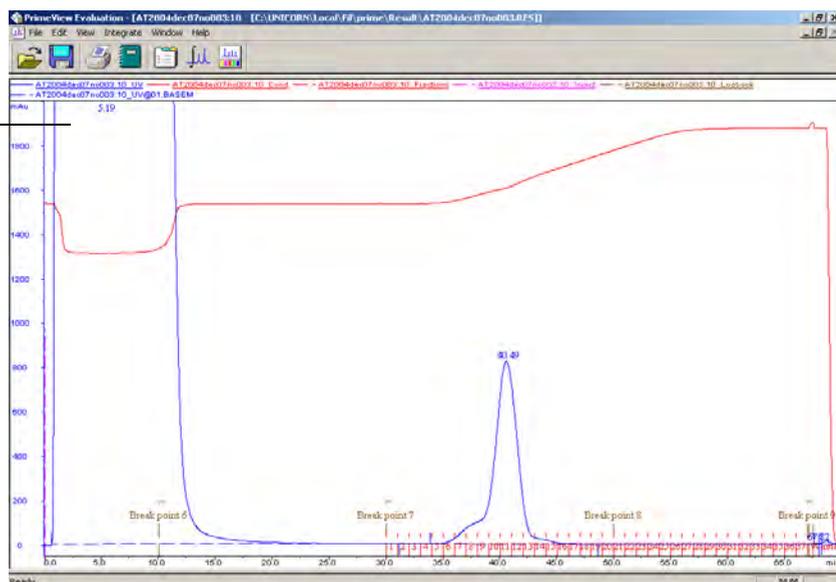
- 1 To start PrimeView Evaluation module, click **PrimeView Evaluation** icon on the Windows desktop.

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.

Curves
pane



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**.

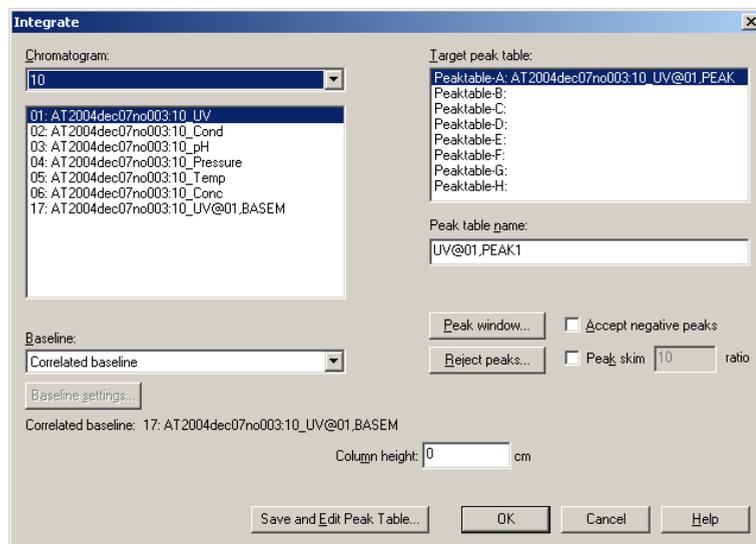
The Chromatogram Layout dialog is opened.

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

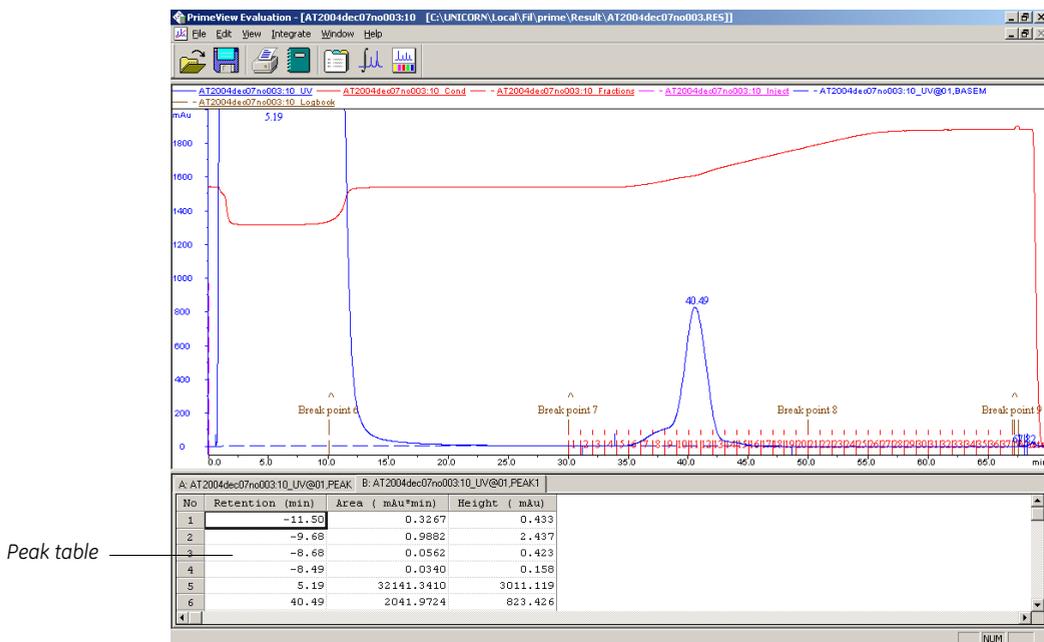
3.11.3 Integrating peaks

To make a simple integration of the UV curve peaks:

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



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

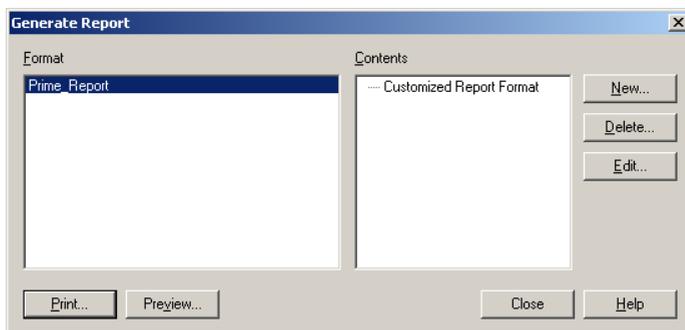


3.12 Creating and printing a simple report

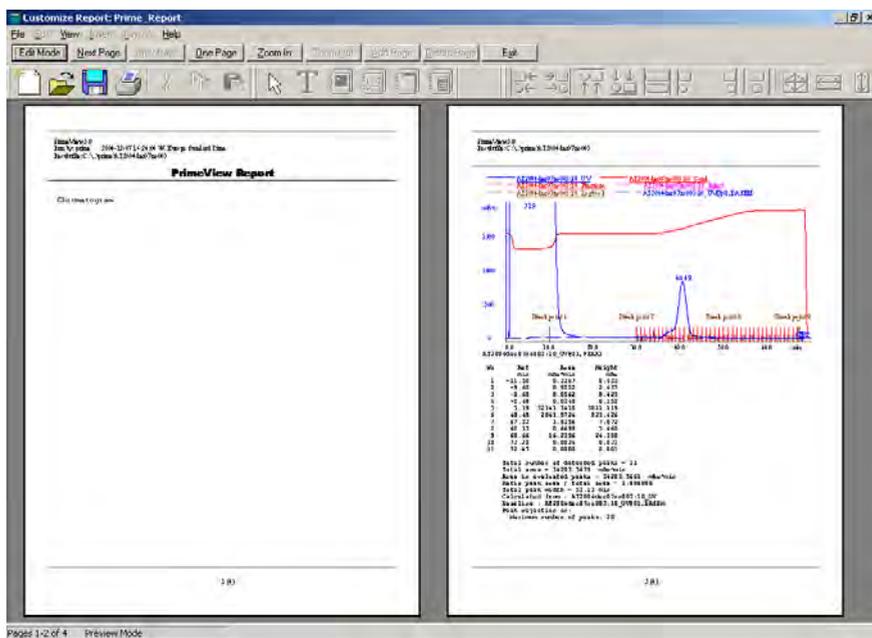
This description describes how create and print a simple report. However, it is possible choose from a variety of objects to include in a report, including chromatograms, methods, documentation, free text and more in the customized report interface.

To create and print a report:

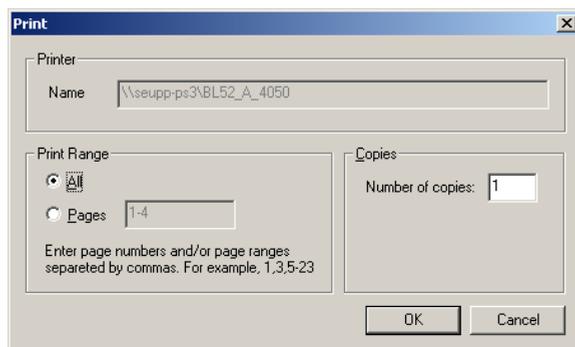
- Select **File:Report** to open the Generate Report dialog.



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



- Click **Exit** to return to the Generate Report dialog.
- Click **Print** to open the Print dialog.



- 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).

If a buffer containing salt has been used, flush the flow path with deionized water. This is especially important if an organic solvent will be used in the next run.

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:

Topic	Page
Preparing the system for a run	42
Purging the inlet tubing	43
Scheduling calibrations	44
Applying sample of all sizes using a sample loop, Superloop or the buffer valve	45
Collecting fractions.	52
Starting a run using an application template	53
Viewing and changing parameters during a run.	56
Performing a run using a method template	59
Performing a run using a stored method	62
Running the system manually	63
Cleaning after a run. Storage	66
Operating the system in a cold room.	68

Other topics:

Topic	Page
Connecting and using a recorder.	110
Calibrating the flow, pressure and monitors	135
Injection valve positions and flow paths	158
Using the flow restrictor.	161
Chemical compatibility.	187

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.

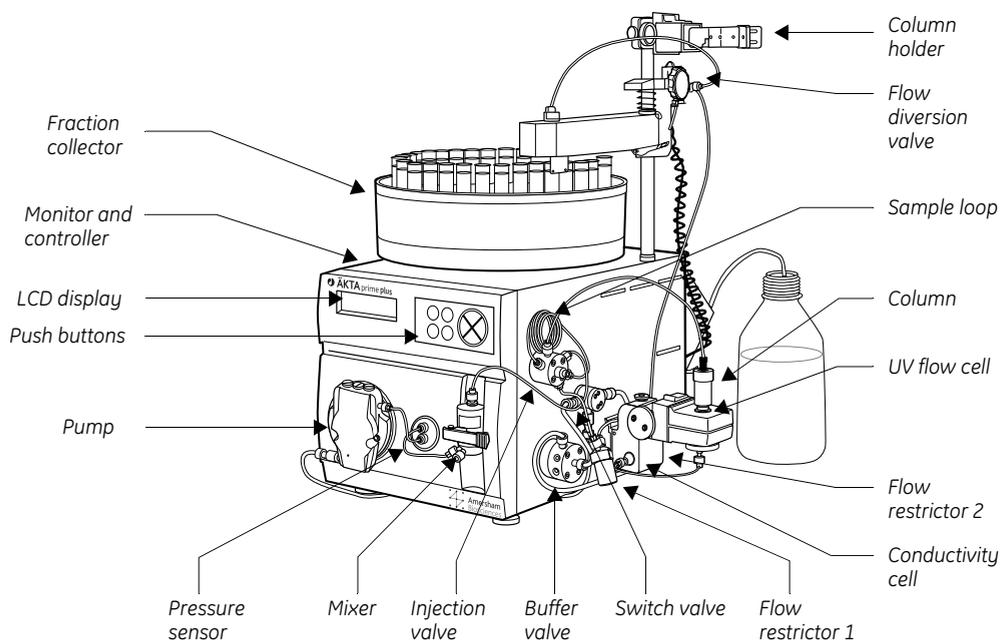
4.1 General

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.

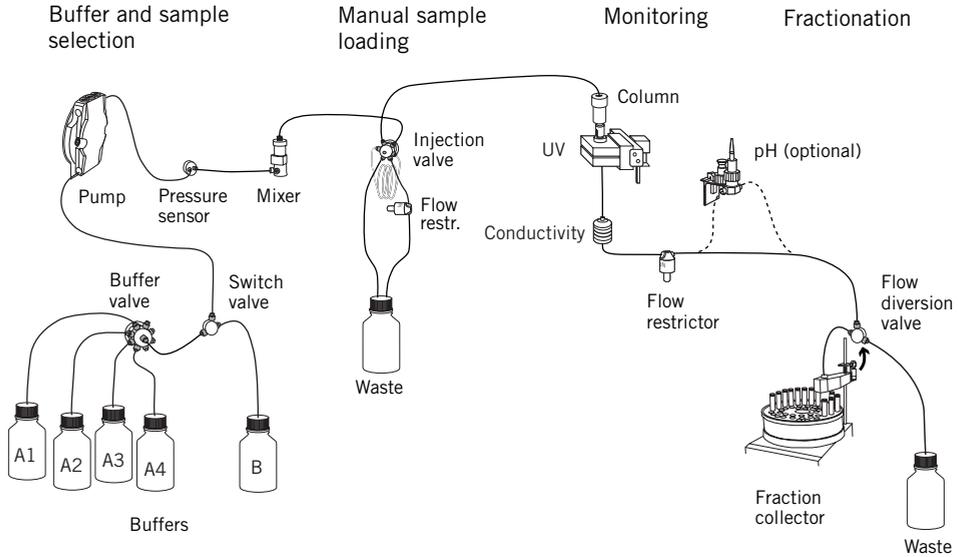
4.2 System components

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 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.

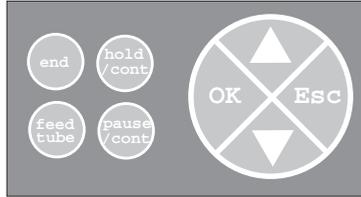
On delivery, the system is equipped with i.d. 1.6 mm inlet tubing, i.d. 0.75 mm tubing from the pump to the outlet, and i.d. 1.0 mm waste tubings.

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.

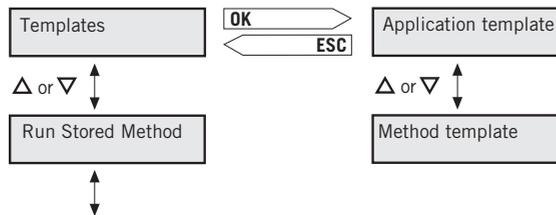
Note: *If tubings are changed, the delay volume to the fraction collector must be changed.*

4.6 Operator interface

4.6.1 Menu navigation



- Δ 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.

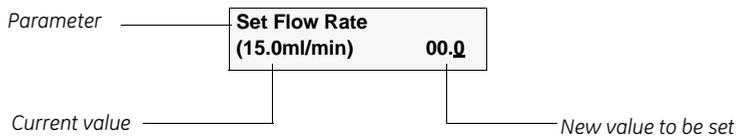
pause /cont

- 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:

Templates	<ul style="list-style-type: none"> • 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.
Run Stored Method	<ul style="list-style-type: none"> • Used to run methods that are programmed by the user. See section 5.9 Running a stored method.
Manual Run	<ul style="list-style-type: none"> • Used to run the system manually without using methods. See section 5.10 Running the system manually.
Program Method	<ul style="list-style-type: none"> • Used to program user-specific methods. See chapter 6 Method programming.
Copy Method	<ul style="list-style-type: none"> • 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.
Set Parameters	<ul style="list-style-type: none"> • 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 menu.
Check	<ul style="list-style-type: none"> • 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:

Topic	Page
Preparing the system further	42
Purging the inlet tubing	43
Scheduling calibrations	44
Applying sample of all sizes using a sample loop, Superloop or the buffer valve	45
Collecting fractions	52
Starting a run using an application template	53
Viewing and changing parameters during a run	56
Performing a run using a method template	59
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Other topics:

Topic	Page
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Chemical compatibility	187

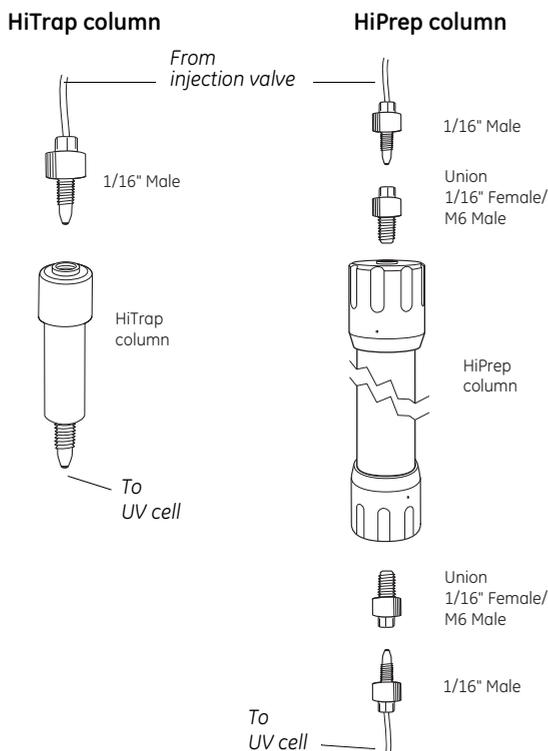
5.2 Preparing the system further

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).



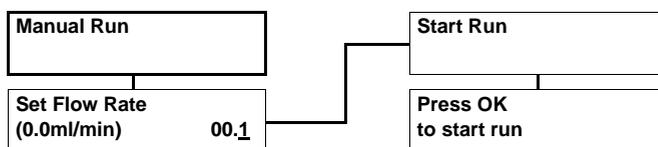
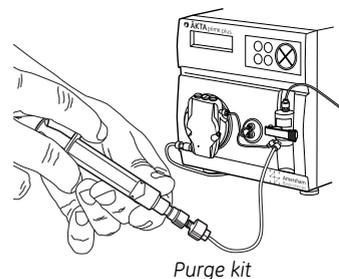
Note: More information regarding columns, buffer solutions, etc. for the provided applications is found on the cue cards supplied.

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% methanol 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 mixer.
- 2 Connect the Purge kit to the mixer.
- 3 Put the used inlet tubing in the appropriate buffers.
- 4 Run the pump at 0.1 ml/min.



Filling inlet tubing A1–A8:

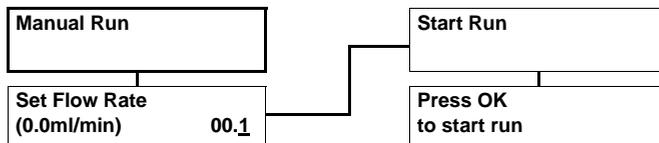
- 1 Go to **Set Buffer Valve** using the arrow buttons.
- 2 Set the chosen A inlet and click **OK**. The valve switches to the selected port.
- 3 Draw buffer with the purge syringe until liquid enters the syringe.
- 4 Repeat step 1–3 until all chosen A inlet tubing is filled.

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% methanol

- 1 Put inlet tubing A1 in 100% methanol.
- 2 Run the pump at 40 ml/min for 10–20 s and press **pause/cont.**



- 3 Set the flow rate to 5 ml/min using the arrow buttons.
- 4 Press **pause/cont.**, run the pump for at least 30 s and press **pause/cont.**
- 5 Move inlet tubing A1 to deionized water.
- 6 Press **pause/cont.** and run the pump for 1 min.
- 7 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.

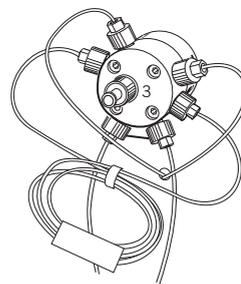
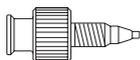
Partial filling is used when high recovery is required. The sample volume loaded should be, at maximum, 50% of the loop volume. The volumetric accuracy is that of the syringe. Partial filling allows the injected volume to be changed without changing the loop and does not waste sample. The sample loop 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 μ l, 25 MPa	18-1113-98
Loop 500 μ l, 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

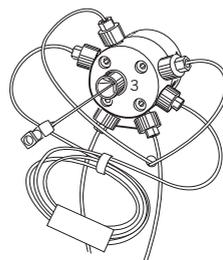
An *injection fill port* can be used instead of the luer union connector. Then prepare the injection valve as follows:



- 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

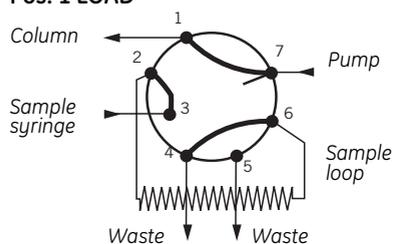
Partial filling

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.
- 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.

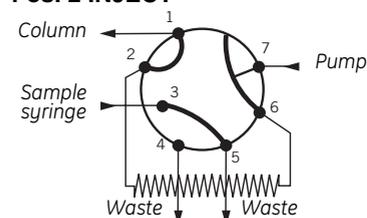
Pos. 1 LOAD



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.

- 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.

Pos. 2 INJECT



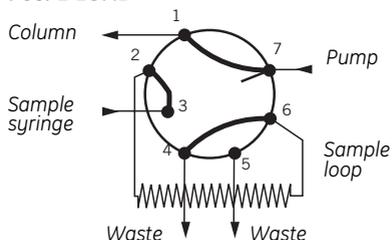
Complete filling

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.

Pos. 1 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.

- 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.

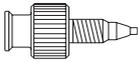
Superloop is an accessory available in three sizes:

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.

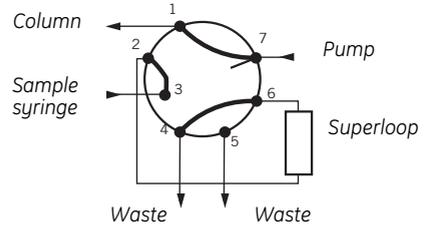
Preparation

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.

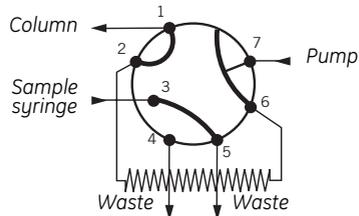
Pos. 1 LOAD



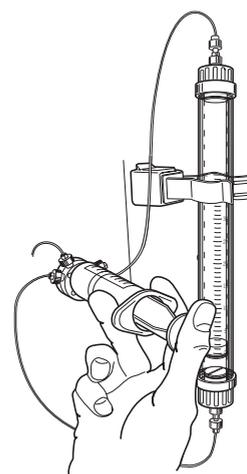
Filling Superloop

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.**
- 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.

Pos. 2 INJECT

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



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

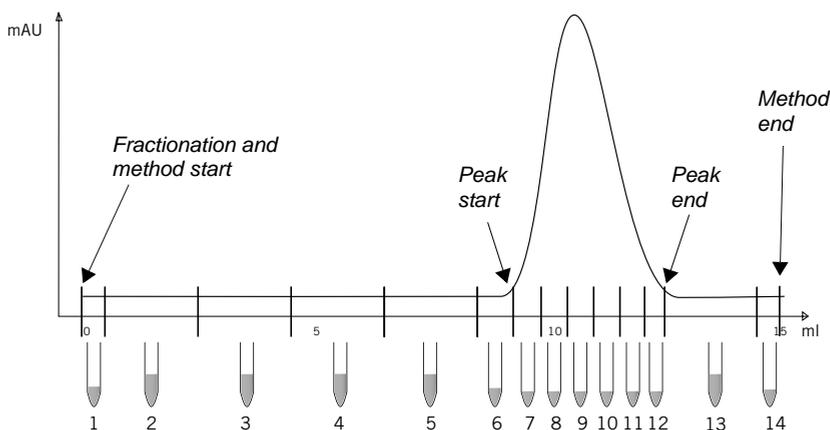
Peak fractionation allows you to collect peaks during the elution, besides fixed volume fractions. In this case, the slope of the curve decides when the actual fractionation should start and end.

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.

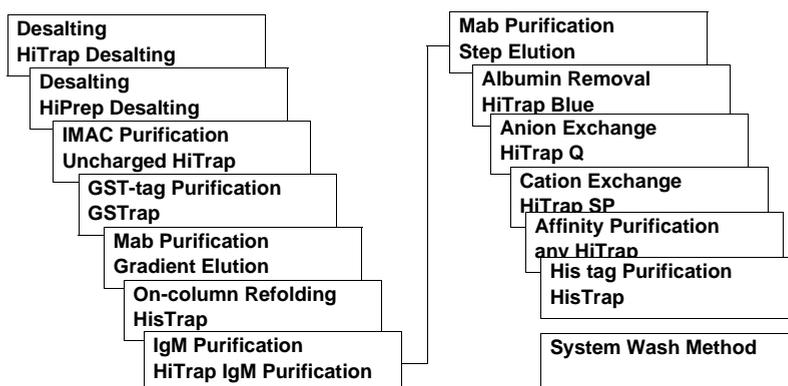
To use an application template:

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 Δ or ∇ .

M RUN	10.0 ml
20.0 ml/min	1.10 MPa

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

The available running modes are:

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

0.00002AU	pH 8.50
20%B	22.90mS/cm

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

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

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

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

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

Printing progress

The process parameters can be printed directly during the run 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**.

Set Concentration %B (20 %B)	<u>30</u>
---------------------------------	-----------

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**.

Set Flow Rate (0.1 ml/min)	<u>0.8</u>
-------------------------------	------------

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**.

Set Fraction Size (00.0 ml)	<u>0.2</u>
--------------------------------	------------

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.

Set Buffer Valve Pos (Pos 1)	<u>1</u>
---------------------------------	----------

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**.

Set Inject Valve Pos (Load) Waste <u>Load</u> Inject	2
---	---

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

Autozero on the recorder

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

Event Mark

Setting an 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)	y	n
-----------------------------	----------	----------

- 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)	y	n
----------------------------------	----------	----------

- 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.

Templates

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

Method Template

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

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

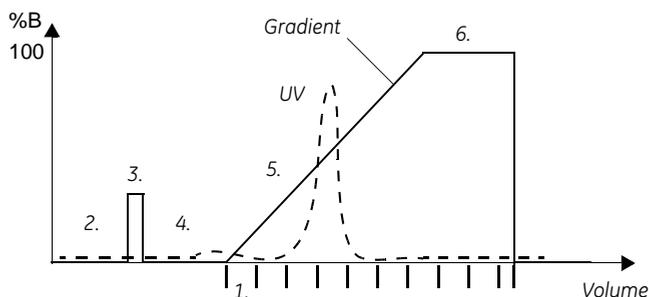
Gelfiltration/
Buffer Exchange

Ion Exchange/
Gradient elution

HIC
Gradient elution

Affinity
Step Gradient

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 Equibr. 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.

- | |
|---|
| Set Wash 1 Volume
(0.0 ml) <u>0.0</u> |
|---|
- 7 Set the wash 1 volume (4.) and press **OK**.
This setting does NOT apply to the Gel filtration method template.
- | |
|---|
| Set Elution. Volume
(0.0 ml) <u>0.0</u> |
|---|
- 8 Set the elution volume (5.) and press **OK**.
- | |
|---|
| Set Wash 2 Volume
(0.0 ml) <u>0.0</u> |
|---|
- 9 Set the wash 2 volume (6.) and press **OK**.
This setting does NOT apply to the Gel filtration method template.
- | |
|---|
| Method ready?
(yes) <u>yes</u> no |
|---|
- 10 Select **yes** at the **Method ready?** prompt and press **OK**.

5.8.3 Storing the method

- | |
|---|
| Save Method
(yes) <u>yes</u> no |
|---|
- 1 Select **yes** to store the method, then press **OK**.
Otherwise, select **no** and press **OK**.
- | |
|-------------------------------------|
| Free Methods 25 |
| Sel. Method (Free) <u>16</u> |
- 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.
- | |
|-------------------------------------|
| Free Methods 25 |
| Sel. Method (Used) <u>16</u> |
- 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

- | |
|------------------------------|
| Press OK to start 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

- | |
|---|
| Method Complete
Press OK to continue |
|---|
- 1 Press **OK** at the **Method Complete** prompt to finish the run.
- To abort the run before it is finished, press **End**. Confirm the following message by selecting **yes**, then press **OK**.
- 2 When the run is finished, the curves obtained can be printed on the chart recorder 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.

Run Stored Method

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

Run Stored Method
Number 13

- 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**.

Run Stored Method
From System PC

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

Run Stored Method
From PC No: 73

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

or

Run Stored Method
From System No: 13

5.9.2 Starting the run

Press OK to
start 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

Method Complete
Press OK to continue

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

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

- 2 When the run is finished, the curves obtained can be printed 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).

Manual Run

- 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**.

Set Method Base (ml) <u>min</u> ml
--

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**.

Set Concentration %B (20 %B) <u>30</u>
--

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).

Set Gradient (off)

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

Set Length (0.00 ml) <u>6.50</u>
--

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

Set Target (00 %B) <u>50</u>
--

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

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

Setting the flow rate

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

Set Flow Rate (0.1 ml/min)	<u>0.8</u>
-------------------------------	------------

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

Setting the fraction base

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

Set Fraction Base (ml)	min	<u>m</u>	drp
---------------------------	-----	----------	-----

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

Setting the fraction size

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

Set Fraction Size (00.0 ml)	<u>0.2</u>
--------------------------------	------------

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

Setting the pressure limit

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

Set Pressure Limit (1.00 MPa)	<u>1.00</u>
----------------------------------	-------------

- 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**.

Set Buffer Valve Pos (Pos 1)	<u>1</u>
---------------------------------	----------

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

Setting the injection valve position

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

Set Inject Valve Pos (Load) Waste	<u>L</u> oad	Inject
--------------------------------------	--------------	--------

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

Waste – the flow is diverted to waste (ports 4 and 5).

Load – the sample loop is loaded (between ports 2 and 6) when injecting the sample through port 3.

Inject – the sample loop is emptied through port 1 and the flow directed to the column.

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

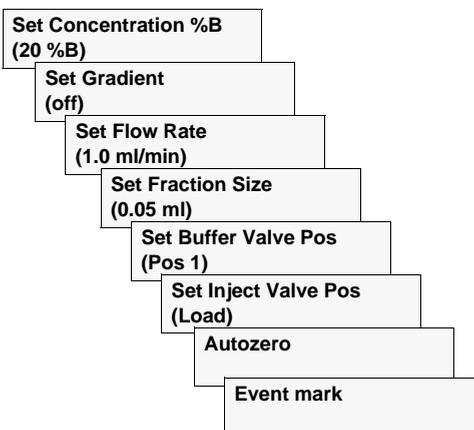
Viewing parameters

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).

If a buffer containing salt has been used, the flow path should be flushed with deionized water. This is especially important if an organic solvent will be used in the next run.

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.

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.

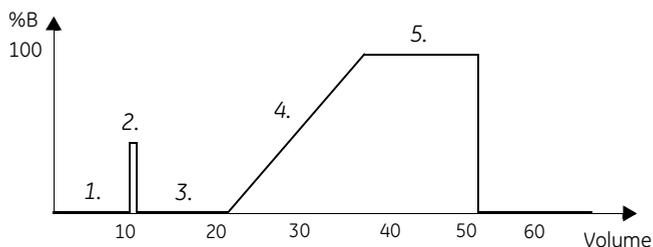
6.2 Programming using method templates

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
<i>Re-equilibration (hidden in template)</i>	20	0	F	0	pos 1	LOAD

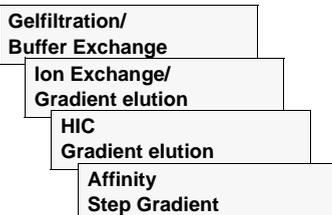
6.2.1 Selecting method template

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: <i>When using the system pump for sample application, the sample tubing should be connected to port 8 on the Buffer valve.</i> |
| Set Pressure Limit
(1.00 MPa) 1.00 | 2 Set the pressure limit and press OK .

Note: <i>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.</i> |
| 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: <i>15 ml of buffer is automatically added to Wash 1 when using the system pump for the sample application.</i> |
| 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) <u>yes</u> no | 10 Select yes at the Method ready? prompt and press OK . |

Save Method (yes)	yes	no
----------------------	------------	----

Free Methods	25
Sel. Method (Free)	16

Free Methods	25
Sel. Method (Used)	16

6.2.3 Storing the method

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

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.

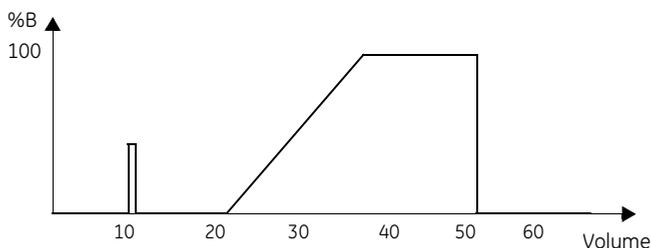
Select a free method number and press **OK**. Alternatively, press **OK** to clear the number in the **Clear Method** menu.

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.



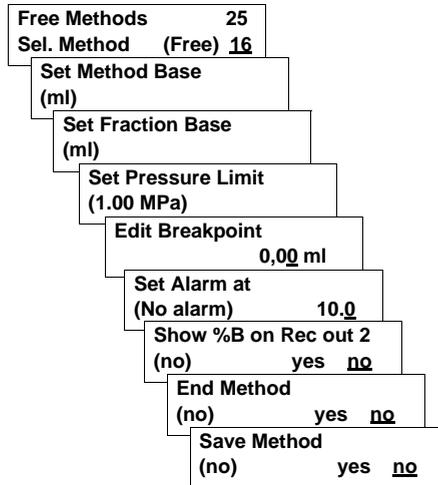
Breakpoint [ml]	Parameter	Conc %B	Flow	Fract.	Buffer V	Inject V
0	Equilibration	0	1	0	pos 1	LOAD
10	Sample applic.	0	1	0	pos 1	INJECT
10+S	Elution delay/wash	0	1	0	pos 1	LOAD
20+S	Elution, start fraction, start gradient	0	1	1	pos 1	LOAD
35+S	Column wash	100	1	1	pos 1	LOAD
49.9+S	End column wash, end 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)	<u>16</u>

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)	<u>16</u>

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 (edit)	Occupied	25
	<u>edit</u>	clear

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

Clear Method 09 (yes)	25
	<u>yes</u> no

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

6.3.2 General parameter set-up

Setting the method base

Set Method Base (ml)	<u>min</u> ml
-------------------------	---------------

- 1 Select menu **Set Method Base** and press **OK**.
- 2 Select time (**min**) or volume (**ml**) and press **OK**.

Setting the fraction base

Set Fraction Base (ml)	min <u>m</u> l drp
---------------------------	--------------------

- 1 Select menu **Set Fraction Base** and press **OK**.
- 2 Choose time (**min**), volume (**ml**) or drops (**drp**). Press **OK**.

Setting the pressure limit

Set Pressure Limit (1.00 MPa)	1.0 <u>0</u>
----------------------------------	--------------

- 1 Select menu **Set Pressure Limit** and 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.

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	0.0 <u>0</u> 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:

Set Concentration %B (20 %B)	30
---	-----------

- 1 Select menu **Set Concentration %B** and press **OK**.
- 2 Set the desired concentration and press **OK**.
 - To create a linear gradient, set two breakpoints with different values for concentration of B. This creates a linear gradient from the first to the second value over the interval between the breakpoints.
 - To create a step gradient, set two breakpoints separated by 0.1 ml or min with different values for the buffer B concentration. This creates an immediate change in the B concentration between the breakpoints; a step gradient.

Setting the flow rate

Set Flow Rate (0.1 ml/min)	0.8
---	------------

- 1 Select menu **Set Flow Rate** and press **OK**.
- 2 Set the flow rate (cannot be 0.0) and press **OK**.

Setting the fraction size

Set Fraction Size (00.0 ml)	0.2
--	------------

- 1 Select menu **Set Fraction Size** and press **OK**.
- 2 Set the fraction size and press **OK**.

Setting the buffer valve position

Set Buffer Valve Pos (Pos 1)	1
---	----------

- 1 Select menu **Set Buffer Valve Pos** and press **OK**.
- 2 Set the position and press **OK**. Refer to the number printed on the buffer valve.

Setting the injection valve position

Set Inject Valve Pos (Load) Waste Load Inject	2
--	----------

- 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.

Set Peak Collect (no)

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

Set Slope (0.00 mAU/min)	0.00
-----------------------------	------

- 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

Autozero (no)	yes	<u>no</u>
------------------	-----	-----------

- 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

Event Mark (no)	yes	<u>no</u>
--------------------	-----	-----------

- 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

Edit time/volume (12.7ml)

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

Edit time/volume <u>Change</u> Replace

- 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.

New time/volume (12.7ml)	15.0
------------------------------	------

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

Saving the breakpoint

Save Breakpoint (0.00 min)

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

Delete Breakpoint (0.00 min)

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:

Set Alarm at (No alarm)	26.00
----------------------------	-------

- 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:

Show %B on Rec out 2 (no)	<u>yes</u> no
------------------------------	---------------

- 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 is 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.

End Method (yes)	<u>yes</u> no
---------------------	---------------

- 1 Go to the **End Method** menu.
- 2 Select **yes** and press **OK**.

Edit Breakpoint	0.00 ml
-----------------	---------

- 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:

Save Method (yes)	<u>yes</u> no
----------------------	---------------

- 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

Program Method

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

Free Methods	25
Sel. Method	Used) 08

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

Method	Occupied
(edit)	<u>edit</u> clear

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

Edit Breakpoint
0.00 ml

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.

Save Breakpoint
(0.00 ml)

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

6.4.3 Editing time/volume of a breakpoint

Edit Breakpoint
0.00 ml

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.

Edit time/volume
(12.7ml)

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

Edit time/volume
<u>Change</u> Replace

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.

New time/volume
(12.7ml) 15.0

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

Edit Breakpoint
0.00 ml

Edit Breakpoint
NEW

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**.

Save Breakpoint
(0.00 ml)

Edit Breakpoint
0.00 ml

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.

Delete Breakpoint
(0.00 ml)

Delete Breakpoint
(0.00 ml) **yes**

6.4.6 Printing the method

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

Show %B on Rec out 2
(no) **yes** no

- 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.

Save Method
(yes) **yes** no

- 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.

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

Copy Method
- 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 PC
- 3 Enter the number of the method and press **OK**.

Copy Method From
System No: 25
- 4 At the **Copy Method To ?** sub menu, select to which unit you want to copy the method. Press **OK**.

Copy Method To ?
System PC
- 5 Enter the new number of the method and press **OK**.

Copy Method To
System (free) No: 29
- 6 If the **Method occupied** display appears, select to clear the number or not (**yes** or **no**). Press **OK**.

Copy Method To
PC (used) No: 285
- 7 Confirm the selected procedure by pressing **OK**.

Method occupied
Clear it? yes no

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 entered.

- 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

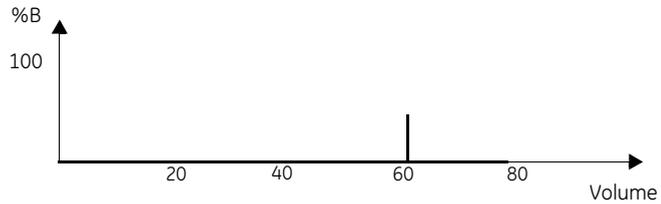
The HiTrap desalting application template is used for desalting a sample solution.

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

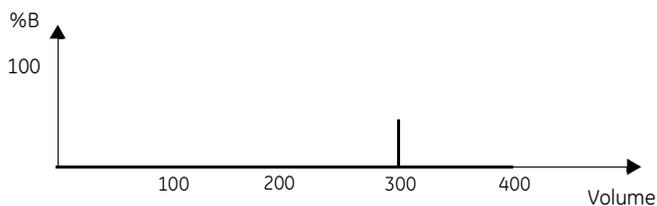
The HiPrep desalting application template is used for desalting a sample solution.

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

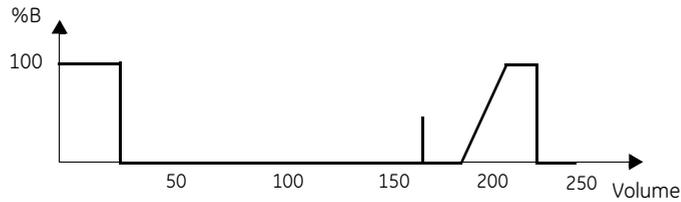
The IMAC purification application template is used for purification of Histidine-tagged proteins.

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

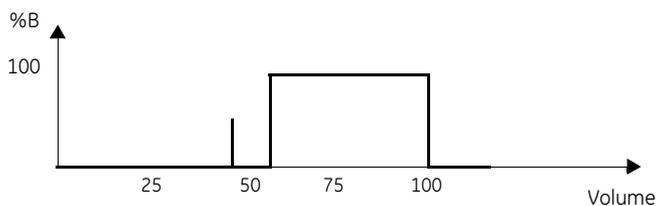
The GST-tag purification application template is used for purification of GST-tagged proteins.

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)

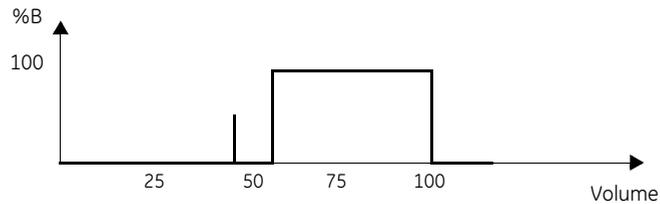
The Mab purification (step elution) application template is used for purification of monoclonal antibodies by step elution.

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

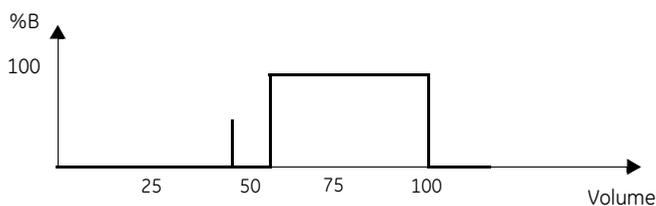
The Albumin removal application template is used for removing albumin.

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)

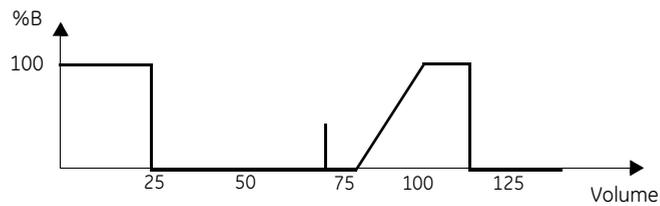
The Mab purification (gradient elution) application template is used for purification of monoclonal antibodies using a pH gradient

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

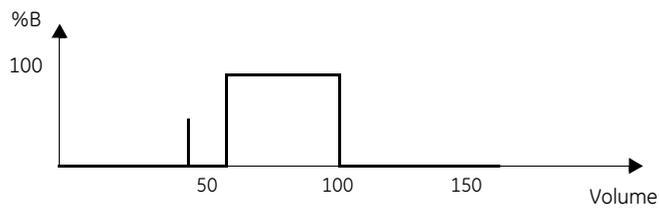
The IgM purification application template is used for purification of monoclonal antibody IgM.

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

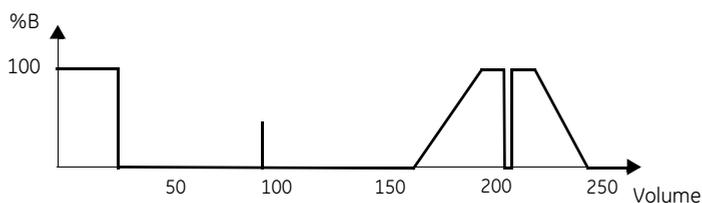
The On-column Refolding application template is used for protein renaturation on a column.

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

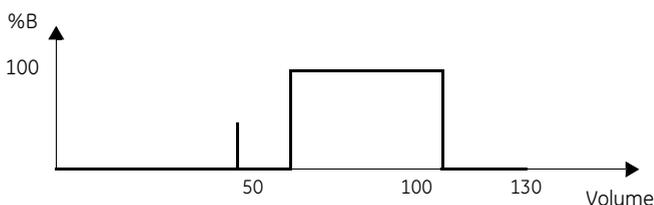
The Affinity purification application template is used for purification of, for example benzamidine.

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

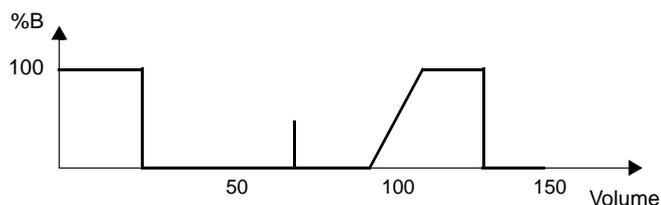
The Histidine-tag purification application template is used for purification of Histidine-tagged proteins.

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).

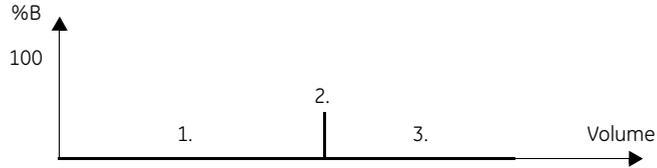
“V” represents the fractionation volume.

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

**Gelfiltration/
Buffer Exchange**

7.3.2 Gel filtration/buffer exchange

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



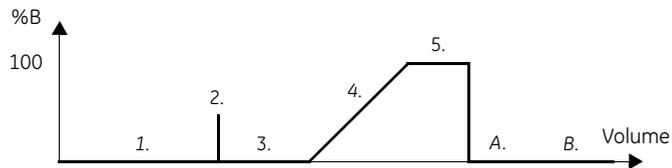
Action	Step	Conc %B	Flow	Fract.	Buffer V	Inject V
Equilibration	1	0	F	0	pos 1	LOAD
Sample application	2	0	F	V	pos 1 ¹	INJECT ¹
Elution	3	0	F	V	pos 1	LOAD

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

**Ion Exchange
Gradient elution**

7.3.3 Ion exchange

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.

HIC Gradient elution

7.3.4 HIC (hydrophobic interaction chromatography)

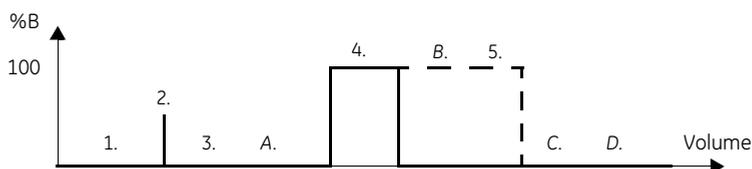
To access the template, select **HIC Gradient elution** and press **OK**.

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

Affinity Step Gradient

7.3.5 Affinity

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 (= equilbr. 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).