

ÄKTApurifier

User Guide



Important user information

All users must read this entire manual to fully understand the safe use of ÄKTApurifier™.

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

WARNING!



The Warning sign highlights an instruction that must be strictly followed in order to avoid personal injury. Be sure not to proceed until the instructions are clearly understood and all stated conditions are met.

CAUTION!

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

Note!

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

Recycling



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

WARNING!

This is a class A product. In a domestic environment, it may cause radio interference, in which case the user may be required to take suitable measures.

WARNING!

All repairs should be done by personnel authorized by GE Healthcare. Do not open any covers or replace parts unless specifically stated in the instructions.

CE Certification

This product complies with the European directives listed below, by fulfilling corresponding standards.

A copy of the Declaration of Conformity is available on request.

- 2006/95/EC, Low Voltage Directive
- 2004/108/EC, EMC Directive

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

- 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.

Note: The Declaration of conformity is valid only for systems that are marked with the CE logo::



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About this manual

This manual describes the operation of ÄKTApurifier™ and ÄKTApurifier UPC, 10 and 100. The description is common for all the systems, unless otherwise clearly stated in the contents. The differences are few and indicated with divergent typeface.

Installation description, system description, system maintenance and troubleshooting are also found in this manual.

1 Introduction

1.1 General

ÄKTApurifier™ is an automated liquid chromatography system designed for method development and research applications. The system simplifies the transition from laboratory to full scale production. Scale-up to production is predictable and trouble-free. Two versions are available; ÄKTApurifier with monitor UV-900 for multiple wavelengths detection and ÄKTApurifier UPC with monitor UPC-900 for combined measurement of UV absorption, pH and conductivity.

Note: When instructions or specifications are similar for both the models only ÄKTApurifier is mentioned.

ÄKTApurifier features:

- Flow rates up to:
 - ÄKTApurifier 100 (P-901): 100 ml/min at pressures up to 10 MPa.
 - ÄKTApurifier 10 (P-903): 10 ml/min at pressures up to 25 MPa.
- One working platform for most liquid chromatography techniques from micro-gram to gram scale.

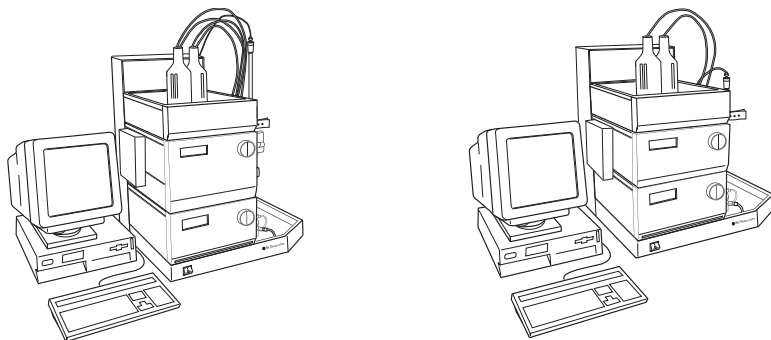


Fig 1-1. ÄKTApurifier to the left and ÄKTApurifier UPC to the right

ÄKTApurifier consists of a compact separation unit, a personal computer running UNICORN™ control system version 5.10 or higher, and as options:

- a fraction collector, and
- a monitor for pH and conductivity measurement (included for ÄKTApurifier UPC).

1 Introduction

1.1 General

The chromatography system is described in detail in Section 8.1 in "*System description*". Brief descriptions of the individual components are given in Section 8.2 in "*Component description*". Detailed information on the components can be found in their respective User Manuals and Instructions. The UNICORN control system is described in the separate UNICORN User Manuals.

The location of the modules and components in the separation units are shown in the following illustrations.

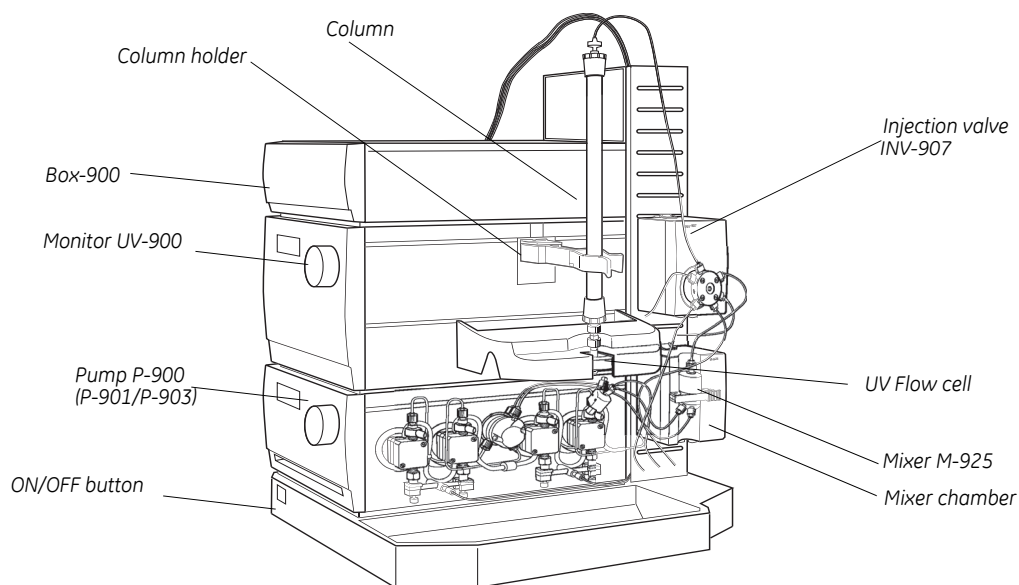


Fig 1-2. ÄKTApurifier

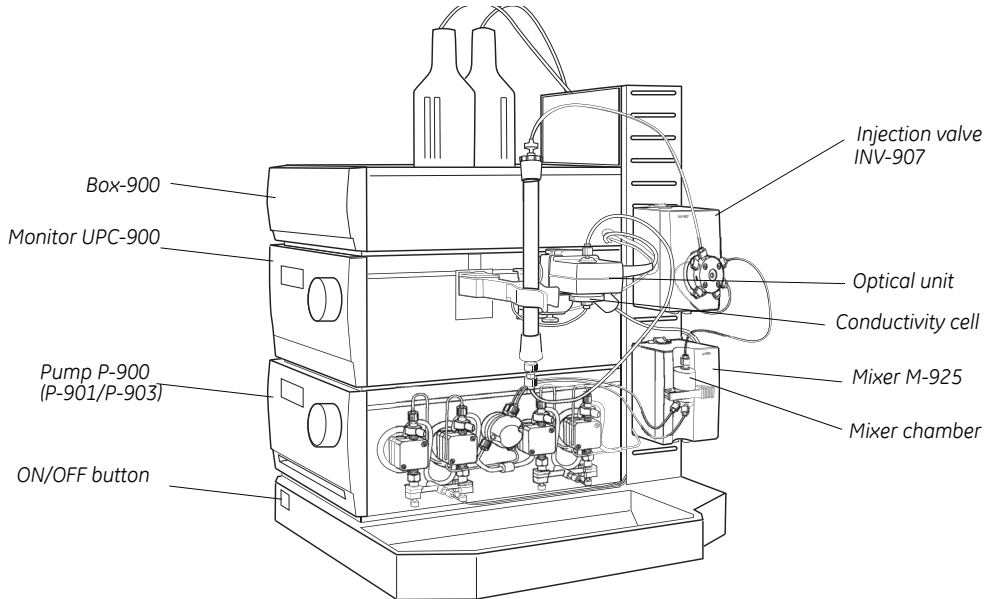


Fig 1-3. ÄKTApurifier UPC

1.2 Labels

The instrument has labels attached to the inside of the frame at the rear of the system. The rating label is on the left side and identification labels are on the right viewed from the rear.

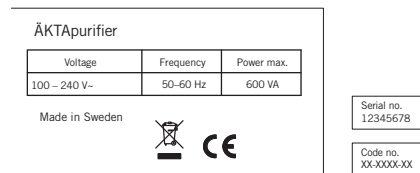


Fig 1-4. Rating label and identification labels on ÄKTApurifier

1.3 Safety

- The system is designed for indoor use only.
- Do not use in a dusty atmosphere or close to spraying water.
- Operate in accordance with local safety instructions.
- Do not block the air inlet on the front panels or the outlets on the rear of the instrument.



WARNING! This instrument is intended for laboratory use only, not for clinical or *in vitro* purposes.



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



WARNING! The covers of the modules and components must not be removed by the user. The modules and components contain high voltage circuits that can give a lethal electric shock.



WARNING! The optical units of Monitor UPC-900 and Monitor UV-900 uses high intensity ultra-violet light. Do not disassemble the optical unit while the lamp is ON.



WARNING! Incorrectly fitted tubing may loosen, causing a jet of liquid to spray out. This is especially dangerous if hazardous chemicals are used. Connect the tubing by first inserting the tubing fully, then tightening the connector finger-tight. PEEK tubing should be tightened a further 1/4 turn using the key supplied. Do not tighten Teflon™ tubing further as this will damage the end of the tubing.



WARNING! Never place waste containers on top of the system. If they become full and overflow, liquid might penetrate the system causing a short-circuit.



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



WARNING! In case of an emergency situation, the system mains switch must always be easy to access, and the mains plug must always be easy to disconnect.



WARNING! The computer must be placed in a dry location and according to the environmental requirements stated in the manufacturer's instructions.



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! Only use spare parts that are approved or supplied by GE Healthcare for maintaining or servicing the system.



WARNING! Only use tubing supplied by GE Healthcare to make sure that the pressure specifications of the tubing are fulfilled.



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



WARNING! For continued protection against risk of fire, replace only with a fuse of the specified type and rating. Refer to "Reference information" for fuse data.



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



WARNING! There must always be a sample loop or Superloop™ 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.

When using a sample pump for sample application directly onto the column, a tubing must be connected between ports 3 and 6 on the Injection valve.



WARNING! Superloop 10 ml and Superloop 50 ml must not be used at pressures above 4 MPa (40bar, 580 psi). Superloop 150 ml must not be used above 2 MPa (20 bar, 290 psi). At higher pressures, the glass tube might shatter.

1 Introduction

1.4 Typographical conventions



WARNING! Never use i.d. 0.25 mm tubing or smaller with columns that can only withstand a low maximum pressure and that allow high flow rates, as the columns might rupture, resulting in injury.



WARNING! In ÄKTApurifier 100, never use i.d. 0.50 or 0.75 mm tubing or smaller with columns that can only withstand a low maximum pressure and that allow high flow rates, as the columns might rupture, resulting in injury.



WARNING! Never attempt to remove the mains fuse while mains voltage is applied to the system. For continued protection against risk of fire, replace only with fuse of the specified type and rating.

Please, refer to the Technical specifications for fuse data.

1.4 *Typographical conventions*

Menu commands, field names and dialog box prompts are identified in the text by ***bold italic*** text. A colon separates menu levels, thus ***File:Open*** refers to the ***Open*** command in the ***File*** menu.

Hardware items, for example, keyboard key names, key combinations, key sequences, hard key names on the equipment and connector port names located on the equipment are identified in the text by **bold** text.

2 Installation

2.1 General

ÄKTApurifier is assembled and fully tested before delivery. For safe transportation, however, some components have been secured and thus need to be detached before the system can be tested and used. Cables, capillaries, accessories, column holder, etc. are enclosed in paper boxes.

This chapter describes how to install ÄKTApurifier. It is divided into two parts, one describing the installation and one describing how to run the installation test. After the installation procedure has been performed, your ÄKTApurifier is ready for use.

Note: *Two versions are available; ÄKTApurifier with monitor UV-900 for multiple wavelength detection and ÄKTApurifier UPC with monitor UPC-900 for combined measurement of UV absorption, pH and conductivity. When instructions or specifications are common to both models only ÄKTApurifier is mentioned.*

The installation test can be performed at regular intervals, for example, to verify the solvent delivery and UV monitoring of your ÄKTApurifier chromatography system.

For full details of specifications, methods, maintenance etc., refer to the User Manuals and Instructions for the components.

2.1.1 Installation procedure overview

- Unpack ÄKTApurifier.page 16
- Detach secured items and install items enclosed 16
- Unpack and install the computer. 22
- Install pH/C-900. 18
- Install Frac-920 or Frac-950 22
- Connect UniNet™-1 data communication chain cabling 26
- Connect mains power cabling 27
- Complete the two first Sections of the Installation record. 28
- Prepare ÄKTApurifier for the installation test. 30
- Run the installation test method 33
- Evaluate the gradient 36
- Evaluate the step response 37
- Evaluate the UV response. 39
- Complete the Test record 39
- Complete the Registration form. 40
- Complete the last Section of the Installation record 28
- Store photocopies of all records and forms in the System Logbook

2.2 Pre-requisites



WARNING! ÄKTApurifier must be connected to a grounded mains socket outlet.

- Two people are required to lift ÄKTApurifier onto a workbench.
- The system should be installed on a stable laboratory bench providing a suitable working area. To install ÄKTApurifier, a working area of about 200 x 80 cm is required. The mains power switch must always be easy to access, and the mains plug must always be easy to disconnect.
- To maintain correct ventilation, the system requires an appropriate amount of free space. Do not block the ventilation inlet on the front panels and the outlets on the rear of the instrument!
- ÄKTApurifier requires 100-120/220-240 V~, 50-60 Hz electrical supply with protective grounding.
- Pliers are recommended for cutting plastic straps.
- A waste flask.
- The installation test requires the following solutions:
 - 1000 ml of distilled water for priming and purging the pump.
 - 200 ml of distilled water.
 - 200 ml of 0.4% acetone in distilled water.
 - 100 ml of 20% ethanol in distilled water.

2.3 Installation of ÄKTApurifier

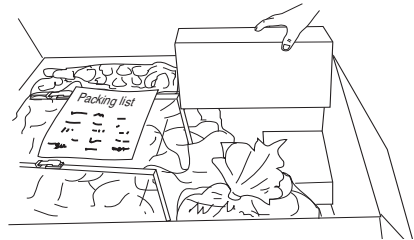
Create a clean and dry working area of 200 x 80 cm that allows easy access. Follow the step-by-step instructions below and check the "Installation Record." as you go along, see page 29.

Note: Some items are packed in separate boxes delivered with the system.

Note: Some packing lists are included in the boxes.

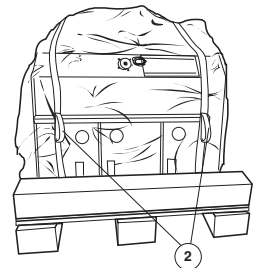
2.3.1 Unpacking the system

- 1 After removing the cardboard hood and other packing material, check the contents against the enclosed packing list. Also check all enclosed boxes. Store all the boxes and plastic bags in a convenient nearby place.



- 2 Check the system for obvious damage which might have occurred during transportation. If unpacking ÄKTApurifier UPC, continue with "ÄKTApurifier UPC specific".

- 3 Lift ÄKTApurifier onto the work area using the four strap handles (2). Two people are required to lift the system. Do not raise to upright position yet. Lay the system on the same side as on the pallet.

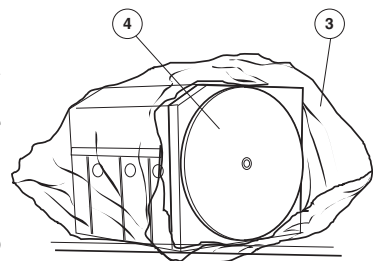


- 4 Release the two red straps with the strap handles. Allow the straps to remain taped to the plastic cover.

- 5 Pull back the plastic cover (3) to uncover the swivel platform (4) and raise the system to an upright position.

- 6 Pull off the plastic cover to uncover the system.

- 7 Save all the original packing material. If, for any reason, the equipment has to be repacked, for transportation or otherwise, it is important that the system can be safely packed using the original packing material.

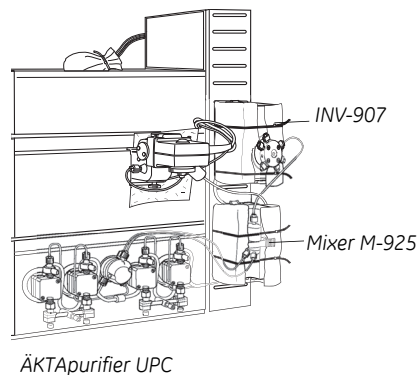


2.3.2 Unpacking the fluid handling components

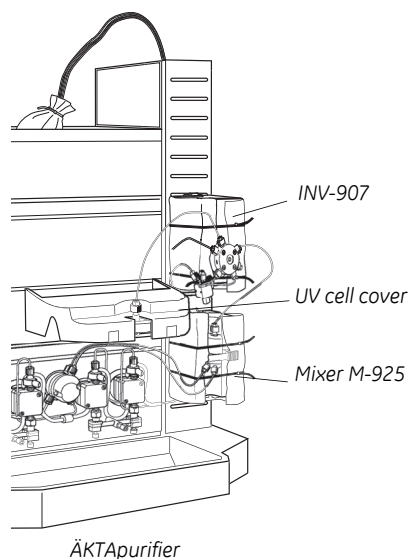
- 1 Turn the system to access the fluid component side of the system.
- 2 Detach the following items by cutting straps and removing red tape. Also remove the desiccant bags.

Note: Use pliers. Be careful not to cut any capillaries by accident. Do not lose any of the capillary marking tags.

- **Valve INV-907**, located at the top right side. Lift off the valve to remove the protective foam. Refit the valve into the top slot in the attachment bracket.
- **Mixer M-925**, located below valve INV-907 in ÄKTApurifier. Lift off the mixer to remove the protective foam, and then refit.



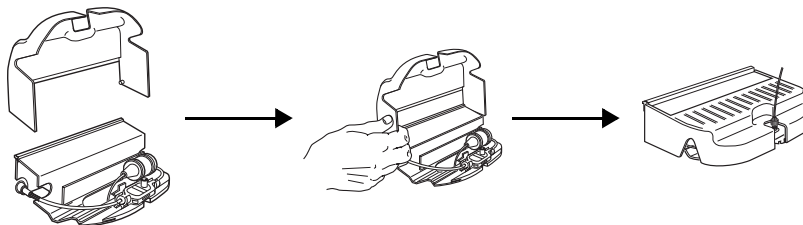
- **Optical unit.** Lift the optical unit gently to remove the plastic foam packing.
- **Capillary loops.** Remove all red tape holding capillaries and loops attached to valve INV-907.
- **UV cell cover** (ÄKTApurifier), located above the UV flow cell. Remove the red tapes.



2 Installation

2.3 Installation of ÄKTApurifier

Should the cover over the UV cell compartment come loose, it is refitted in the following way:



- 1 The cover is a simple push fit onto the cell holder. Two small lugs on the cover locate in holes at the front and rear of the cell holder.
- 2 The cover is then lowered over the cell holder.
 - **Waste tubings.** Place the waste tubings marked W1, W2 and W3 in a waste bottle and place the bottle in a convenient place.
 - **Inlet tubing.** Unpack the inlet tubing contained in a plastic bag taped to the buffer tray on top of Box-900.
 - Make sure all items are securely fitted and that no capillary connection has worked loose or been tangled.
 - **Column holder:**

ÄKTApurifier: Attach the column holder enclosed in Box-900 above the UV cell. Select a slot to suite the height of the column to be used.

ÄKTApurifier UPC: Select a position that minimizes the distance between the column and the optical unit.

2.3.3 Installing pH/C-900 (optional)

Monitor pH/C-900 is a high precision on-line monitor for measurement of pH and conductivity in liquid chromatography. Monitor pH/C-900 is an option for ÄKTApurifier, for detailed descriptions refer to *Monitor pH/C-900 User Manual*.

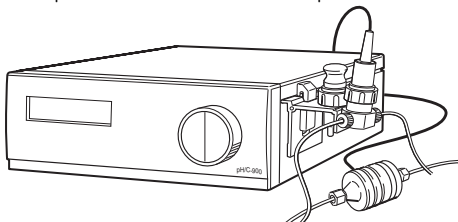


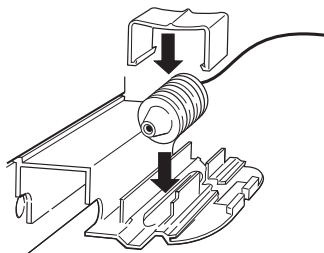
Fig 2-1. Monitor pH/C-900

Unpacking pH/C-900

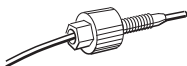
- 1 Unpack and assemble pH/C-900 according to the instructions given in the *Monitor pH/C-900 User Manual*.
- 2 Place pH/C-900 in ÄKTApurifier.

Installing the conductivity cell

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



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



- 3 Connect the tubing with the "Fingertight" connectors. The flow cell itself does not have a recommended flow direction. In conjunction with the pH electrode, place the conductivity flow cell and select its flow direction so that the screw head end of the flow cell faces the pH flow cell (in ÄKTApurifier systems, the pH electrode is optional).

2 Installation

2.3 Installation of ÄKTApurifier

2.3.4 Installing a pH electrode (optional)

The pH electrode is to be mounted in a separate pH flow cell, housing the pH electrode itself, as well as a dummy pH electrode. The dummy pH electrode replaces the pH electrode whenever the pH electrode is to be removed from the system, e.g. for storage.

The pH electrode contains a sealed Ag/AgCl reference which cannot be refilled, an internal electrolyte bridge of 4 M KCl saturated with Ag/AgCl, an outer electrolyte bridge of 1 M KNO₃, an annular ceramic reference junction and a low profile pH membrane. This is commonly known as a sealed combination double junction type. The pH electrode is delivered with a transparent cover.

Note: The flow cell should not be used with any other pH electrode.

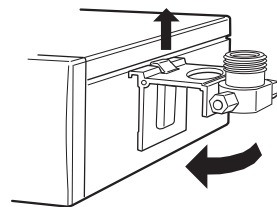
Note: Always use the 0.50 tubing kit and flow restrictor FR-902 when the flow cell is mounted.

Installation

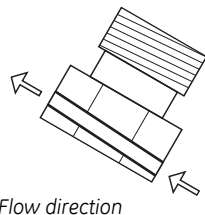
CAUTION! Never use 0.25 mm i.d. tubing (blue) when the pH flow cell is mounted in the system. The electrode might rupture due to high backpressure.

To install the pH flow cell and pH electrode between the conductivity cell and flow restrictor (for more details, refer to *Monitor UPC-900/Monitor pH/C-900 User Manual*):

- 1 Remove the flow restrictor and the male/male union from the conductivity flow cell.
- 2 Hook the flow cell holder directly to the right of the optical unit. Secure it with the slide clamp. The optical unit might have to be moved slightly to the left.



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

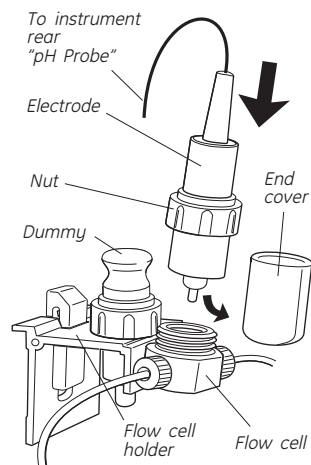


- 3 Unpack the pH electrode. Ensure that it is not broken or dry.
- 4 Prior to first using the electrode, remove the electrode end cover and immerse the glass bulb in buffer for 30 minutes.

- 5 Remove the dummy electrode from the flow cell and store it in the flow cell holder.
- 6 Carefully insert the electrode in the flow cell. Tighten the nut by hand to secure the electrode.

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

- 7 Cut a new capillary of 90 mm (i.d. 0.50 mm) and connect the outlet (the screw head end) from the conductivity flow cell to the inlet on the pH flow cell (lower connection marked IN).



- 8 Connect the outlet from the pH flow cell (marked OUT) to the inlet on the FR-902 (marked IN) using the male/male union.
- 9 Connect the pH electrode BNC connector to the **pH-Probe** connector on the rear of Monitor p/HC-900/UPC-900. Should interference from static fields occur, connect the pH flow cell to the **pH-Ground** connection as well using a standard laboratory 4 mm "banana plug" cable.

Note: *When using the pH electrode, the flow restrictor FR-904 must be replaced with the supplied flow restrictor FR-902. Otherwise, the long term stability and lifetime of the pH electrode will be reduced.*

2 Installation

2.3 Installation of ÄKTApurifier

2.3.5 Installing the computer and printer

Unpack and install the computer and printer according to the manufacturer's instructions. Place them to the left of the system.

Note: Do not turn them on yet!



WARNING! In case of an emergency situation, the system mains switch must always be easy to access, and the mains plug must always be easy to disconnect.



WARNING! The computer must be placed in a dry location and according to the environmental requirements stated in the manufacturer's instructions.



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

2.3.6 Installing Fraction collector Frac-920 (optional)

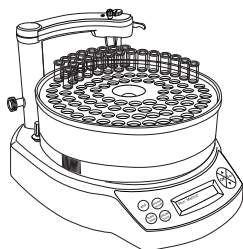
Frac-920 can be used for both small scale and preparative scale purification with ÄKTApurifier. It collects up to 175 fractions in 12 mm diameter tubes, up to 95 fractions in 10–18 mm diameter tubes, and up to 40 fractions in 30 mm diameter tubes. An optional flow diversion valve, FV-923, allows flow diversion to waste.

Frac-920 allows fixed volume fractionation and/or automatic peak fractionation. The latter function is based on peak detection using slope or level sensing. Fraction marks and fraction numbers make it easy to identify fractions and peaks. Fast tube change minimizes spill between tubes, eliminating it entirely below flow rates of 5 ml/min.

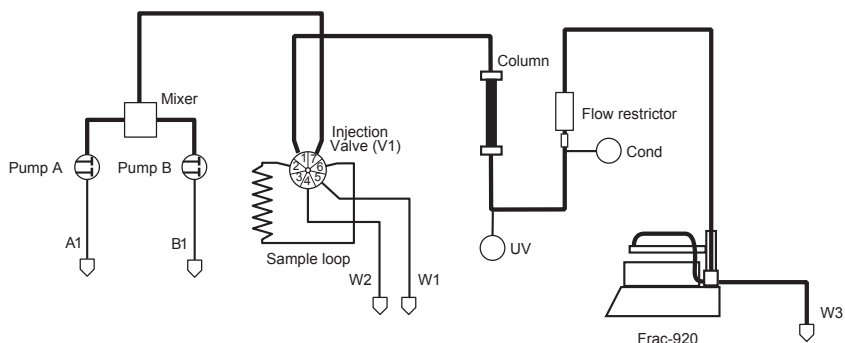
Drop synchronization eliminates sample loss during tube change.

Installation

- 1 Unpack and assemble Frac-920 according to the instructions in the *Fraction Collector Frac-920 User Manual*.

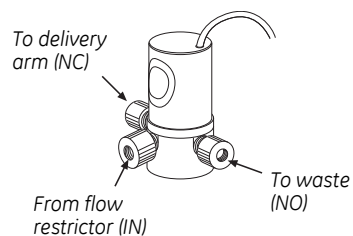


- 2 Place Frac-920 to the right of the system.

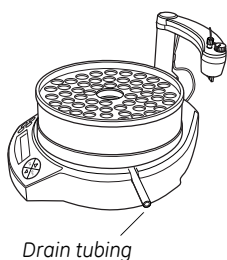


- 3 Connect a tubing from the outlet of the flow restrictor to the IN port on the flow diversion valve.

Note: Use tubing with the same inner diameter as G5 to connect to the IN and NC ports.



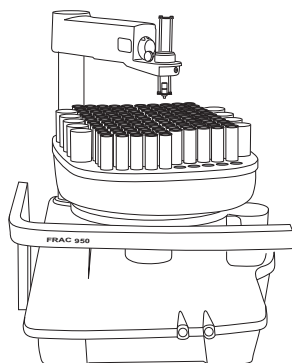
- 4 Connect tubing from the NC (normally closed) port on the flow diversion valve to the delivery arm on Frac-920. Follow the instructions given in the *Frac-920 User Manual*.
- 5 Connect tubing (W3) from the NO (normally open) port on the flow diversion valve to waste.
- 6 Place the valve in the hole behind the bowl.
- 7 Extend the drain tubing from Frac-920 (34) to a suitable waste bottle.
- 8 Set the delay volume in UNCORN, see Section 8.1.6 "Setting the delay volume"



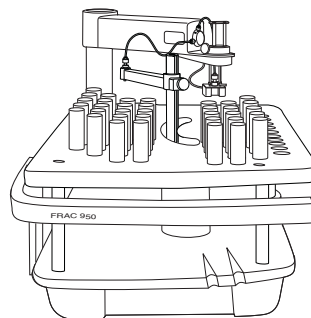
2 Installation

2.3 Installation of ÄKTApurifier

2.3.7 Installing Fraction collector Frac-950 (optional)



Standard mode



Prep mode

Frac-950 can be used for both small scale (standard mode) and preparative scale (prep mode) purification with ÄKTApurifier. Up to 392 fractions can be collected in time or volume base, as fixed volume and/or peak fractionation.

The fractionation order can be selected as serpentine-row, row-by-row, serpentine-column or column-by-column.

For **standard mode**, four sizes of tube racks for tube diameters of 12, 18 and 30 mm tubes, and 96-well microplates are available.

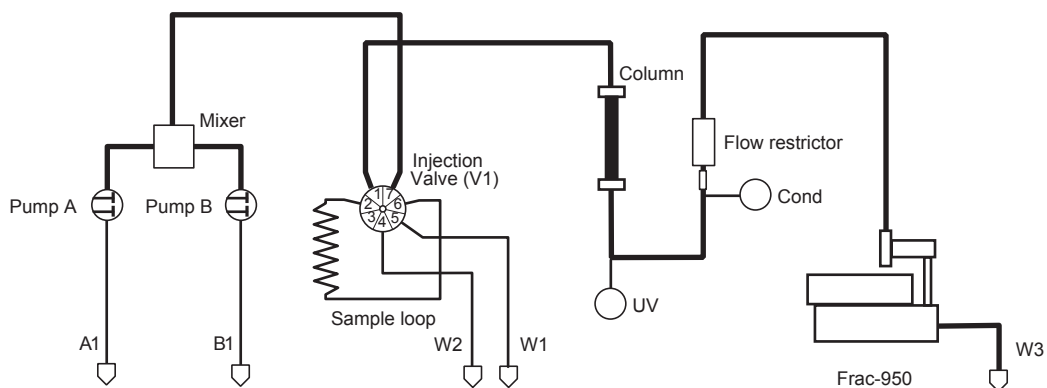
For **prep mode**, two sizes of racks for tube diameter of 30 mm tubes, and 250 ml bottles are available. A Funnel-to-flask kit for transporting the fractions to other vessels is also available. Using Frac-950 in prep mode requires a dispenser arm specially made for the prep mode racks.

Sample loss during tube change can be eliminated by using drop synchronisation, or by using a built-in accumulator for intermediate liquid storage between tube change.

Installation

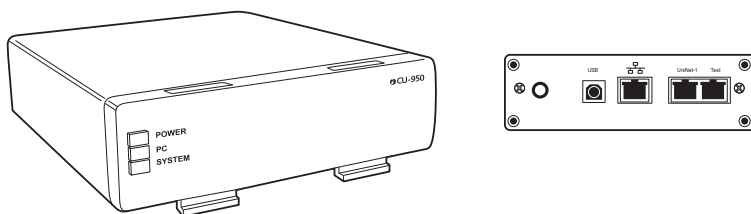
- 1 Unpack and assemble Frac-950 according to the instructions given in the *Fraction collector Frac-950 User Manual*.
- 2 Put Frac-950 on the workbench to the right of ÄKTApurifier.
- 3 Locate the capillary kit included with Frac-950 and select if you want to include the accumulator in Frac-950 or not.

- 4 Connect the tubing from the flow restrictor to Frac-950 as described in the *Frac-950 User Manual*.



- 5 Connect the included waste tubing (W3) to the waste outlet on Frac-950 and route it to a suitable waste container.
- 6 Set the delay volume in UNCORN, see Section 8.1.6 "Setting the delay volume".

2.3.8 Installing Controller unit CU-950



- 1 Unpack CU-950.
- 2 Hang the CU-950 on the **left side** of the system by inserting the hooks on the front of CU-950 into the channel on the side of the UV-900 and rotating into position.

2.3.9 Connecting the UniNet cables

CAUTION! The mains power to ÄKTApurifier must be turned OFF before installing the UniNet-1 cables.

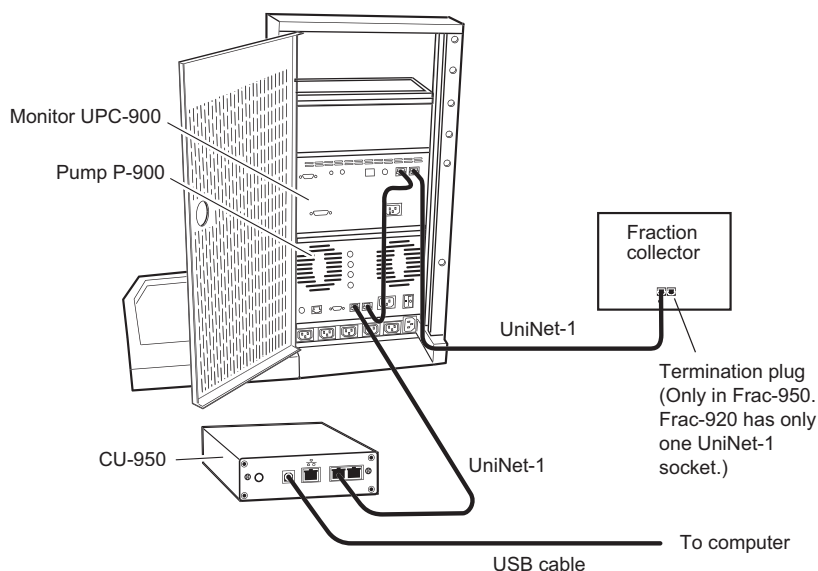


Fig 2-2. Illustration of ÄKTApurifier UPC connections. ÄKTApurifier connections are similar.

- 1 Connect a UniNet-1 cable between the **UniNet-1** port on CU-950 and Pump P-900 in the system.
- 2 Connect a UniNet-1 cable between Pump P-900 and Monitor UPC-900/
Monitor UV-900
- 3 When using a fraction collector:
 - connect a UniNet-1 cable between Monitor UPC-900/Monitor UV-900 and the fraction collector.
 - connect a termination plug to the empty **UniNet-1** socket (Frac-950 only).
- 4 When not using a fraction collector, connect a termination plug to the empty **UniNet-1** socket in Monitor UPC-900/Monitor UV-900.
- 5 Connect the USB cable to the **USB** port on CU-950.
Note that the CU-950 should be attached to the left side of the system.

- 6 Connect the other end of the USB cable to the rear of the computer. For connection to and installation on the computer, please refer to *UNICORN Administration and technical manual*.
- 7 All other UniNet-1 cables are connected at delivery.

2.3.10 Connecting the mains cables



WARNING! ÄKTApurifier must always be used with the protective earth lead of the power cord correctly grounded to earth at the mains outlet.



WARNING! In case of an emergency situation, the system mains switch must always be easy to access and the mains plug must always be easy to disconnect.



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

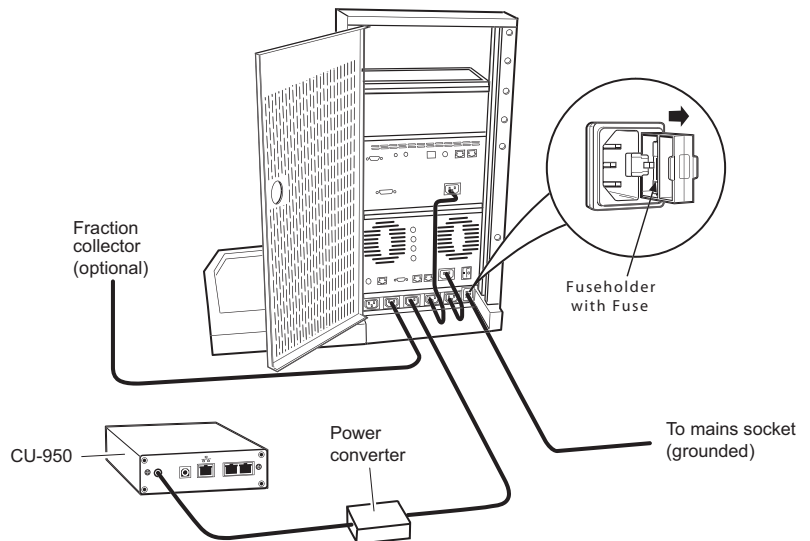


Fig 2-3. ÄKTApurifier UPC, the connections on ÄKTApurifier are similar

- 1 Connect the power converter cable to the **Input** port on CU-950. The CU-950 should be attached to the left side of the system.
- 2 Connect a mains cable supplied between the power converter and the mains supply socket on the system.
- 3 If a fraction collector is used, connect a mains cable supplied between the fraction collector and the mains supply socket on the system.

2 Installation

2.3 Installation of ÄKTApurifier

- 4 Connect a mains cable supplied between ÄKTApurifier and a properly grounded mains socket according to *Figure 2-3*.
Do not switch on!
- 5 All other mains cables are connected at delivery.

Complete the two first Sections of the Installation record.

The unpacking and installation phase of ÄKTApurifier is now completed.

2.3.11 Settings and calibrations

- 1 If fraction collection should be used, remember to change the delay volume. This setting is found in **System Control**, in **System:Settings:Specials**. To calculate the new delay volume see Section 8.1.6 "*Setting the delay volume*".
- 2 Calibrate the pH electrode and set up the additional monitor functions desired for pH measurement. These procedures are described in the *Monitor UPC-900/Monitor pH/C-900 User Manual* and in *UNICORN User Manuals*.

Check	Sign	Remarks
1 Unpacking		
Contents according to packing lists.		
All packing material removed.		
No visible damage.		
2 Installation		
Injection valve waste tubings (port 4 and 5, marked W1 and W2) to waste reservoir.		
Fraction collector installed (optional).		
Waste tubing (marked W3) extended to waste reservoir.		
Monitor pH/C-900 installed (optional).		
pH electrode holder installed (optional).		
Computer and printer installed.		
UniNet-1 (and UniNet-2, optional) cabling installed.		
Mains power cabling installed.		
Column holder installed.		
Computer and printer installed.		

3 Installation test		
Solutions prepared.		
Tubings to piston seal rinsing system in 20% ethanol.		
ÄKTApurifier prepared.		
Installation Test method run.		
Installation Test results evaluated.		
Test Record completed.		
Registration Form completed.		
Test Record and copy of Registration form stored in System Logbook.		
Registration form posted to Service Administration.		
Installation Guide stored in User Manual box for future use.		

Table 2-1. Installation Record.

2.4 Installation test

The installation test checks the function of the liquid delivery and the UV monitoring system of ÄKTApurifier. The installation test can also be used at any time to check the condition of the system, for example, after a prolonged stop.

Correct gradient formation is tested by producing a linear gradient and a series of concentration steps of acetone.

Correct UV monitoring is tested by monitoring the acetone concentration at:

- 254 nm (Monitor UPC-900).
- 265, 254 and 280 nm and calculating the absorbance ratios 265 nm/254 nm and 265 nm/280 nm (Monitor UV-900).



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.

2.4.1 Preparing ÄKTApurifier

Starting the separation unit

Turn on the separation unit using the mains switch located to the left on the base platform.

Starting the computer

Turn on the computer, the display and the printer according to the instructions in the manufacturer manuals.

Starting and logging on to UNICORN control software

Start UNICORN as described in Section 3.3 "UNICORN software".

Checking the optical unit

- 1 Check that the correct UV cell is fitted:

Monitor UV-900

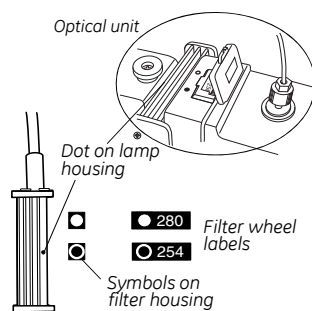
ÄKTApurifier 10 is delivered with the 10 mm cell fitted. A 2 mm cell (optical path length 2 mm, internal volume 2 µl) is available as an accessory. If a lower detection sensitivity is desired, due to output signal limitation, the 2 mm flow cell should be used.

ÄKTApurifier 100 is delivered with the 2 mm cell fitted. A 10 mm cell (optical path length 10 mm, internal volume 8 µl) is available as an accessory. For higher detection sensitivity, the 10 mm flow cell should be used.

- 2 **Monitor UPC-900**

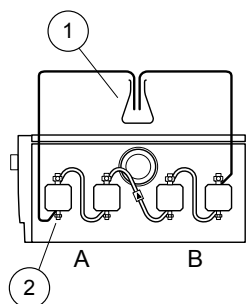
Check on the optical unit that the correct UV wavelength is set.

If not, set the wavelength by selecting lamp position (indicated by a dot on the lamp housing) in combination with the appropriate filter (the dot on the lamp housing should be adjacent to the symbol on the filter wheel corresponding to the symbol on the filter wheel for the desired filter). A click will indicate that the filter is in position. The illustration to the right shows setting of the wavelength 254 nm.



Priming the piston seal rinsing system of Pump P-900

- 1 Immerse the rinsing tubing in a flask (1) containing 20% ethanol in distilled water.
- 2 Connect a syringe to the rinsing tubing that is connected underneath the left pump head (2) on pump A. Slowly draw rinsing solution into the syringe.



When rinsing solution starts to enter the syringe, continue to draw a few milliliters.

- 3 Loosen the syringe and immerse the tubing in the rinsing solution (1). Both ends should now be in the flask (1).

Note: For more detailed information, refer to Pump P-900 User Manual.

Purging Pump P-900

- 1 Immerse the inlet tubing of all pump modules, with filters, in the distilled water.

Note: Never place the reservoir flask below the level of the pump inlet.

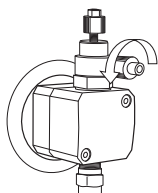
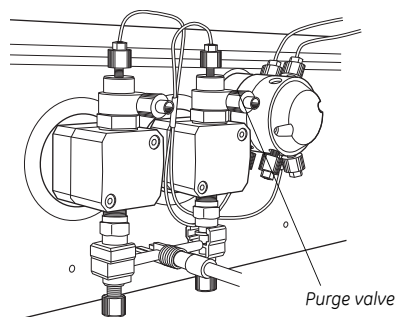
- 2 Connect a male Luer syringe of at least 30 ml to the purge tubing.

- 3 Connect the other end of the purge tubing to the left purge valve at pump module A.

- 4 Turn the purge valve counter-clockwise half a turn to open it and slowly draw eluent into the syringe.

- 5 When fluid starts to enter the syringe, continue to draw a few milliliters before closing the purge valve. Check that there is no air left in the inlet tubing.

- 6 Repeat steps 3 to 5 for the other pump heads.



Testing pressure stability

Perform a pressure test to establish that all air has disappeared from the pump heads:

- 1 Connect a column bypass capillary between the injection valve, port 1, and the top of the UV cell.

ÄKTApurifier 10:

Manually select pump flow 0.2 ml/min and run at 0%B (distilled water). Check on the pump display that the pressure reading is stable (variation less than ± 0.02 MPa).

ÄKTApurifier 100:

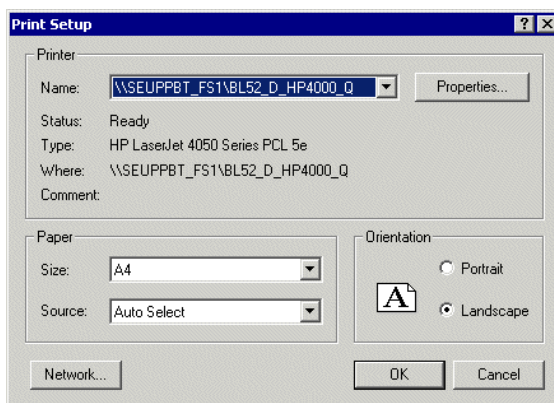
Manually select pump flow 10 ml/min and run at 0%B (distilled water). Check on the pump display that the pressure reading is stable (variation less than ± 0.2 MPa).

- 3 **ÄKTApurifier 10:**
Manually select pump gradient 100%B, length 0 and run with 0.2 ml/min (distilled water). Check on the pump display that the pressure reading is stable (variation less than ± 0.02 MPa).
- ÄKTApurifier 100:**
Manually select pump gradient 100%B, length 0 and run with 10 ml/min (distilled water). Check on the pump display that the pressure reading is stable (variation less than ± 0.2 MPa).
- 4 Proceed to step 5 if the pressure is stable. If not, consult the *Pump P-900 User Manual* for troubleshooting instructions.
- 5 Click **END**.
- 6 Check that the inlet filters are submerged in the corresponding buffers A and B according to the table below.

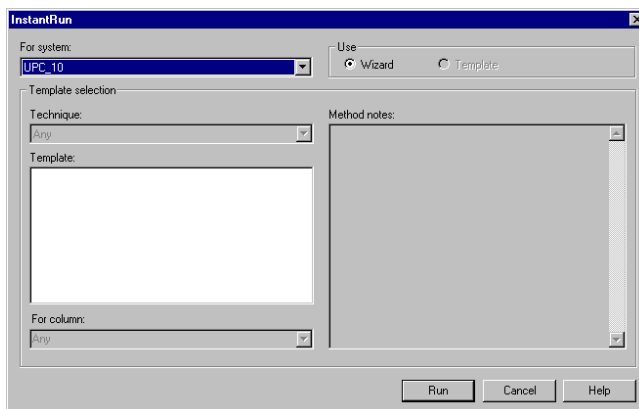
Installation Test Method Guide	
Buffer A:	Distilled water (200 ml)
Buffer B:	1.0% acetone in distilled water (200 ml)
Test flow rate:	5 ml/min
Test run time:	Approximately 30 minutes

2.4.2 Running the installation test method

- 1 In **UNICORN Manager** module, select **File:Printer Setup....** Select the appropriate printer from the list and select **Landscape**. Then click **OK** to acknowledge the printer chosen.

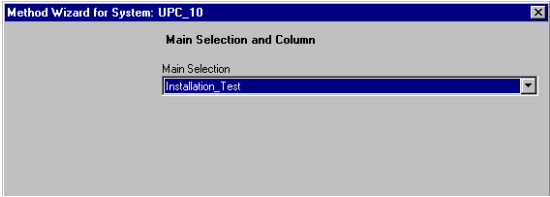


- 2 In **System Control** module, select **File:Instant Run**. The **Instant Run** window opens.



- 3 Select the appropriate system from the **For system** list.
- 4 Click **Run**. The Method Wizard is started.

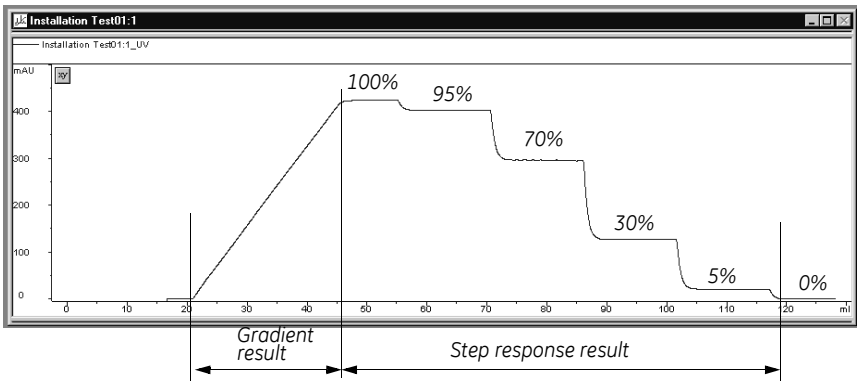
- 5 Select **Installation_Test** from the **Main Selection** list and click **Run**.



- 6 In the **Evaluation Procedures** window, select the procedure for your system, for example:
- **InstPurifier_UPC**, for ÄKTApurifier
 - **InstBasic_UPC**, for ÄKTApurifier UPC
 - Click **Next**.
- 7 Click **Next** in the **Method Information** window.
- 8 Click **START** in the **Result Name** window to start the installation test.

The progress of the test is monitored in the **System Control** module. The installation test run time is approximately 30 min.

When the test is finished, the printer automatically prints the chromatogram and the test results.

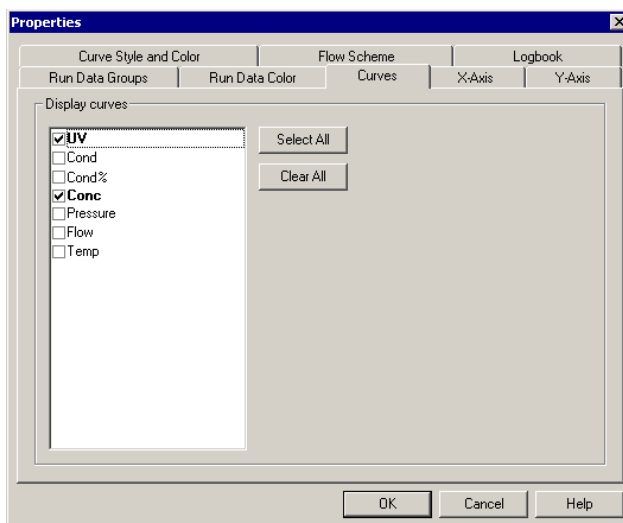


Customizing the Curves pane

To customize the Curves pane:

- 1 Right-click in the **Curves** pane and select **Properties**.

- 2 Click the **Curves** tab.



- 3 Select the following curves to be displayed:

ÄKTApurifier	ÄKTApurifier UPC
<i>UV1_215nm</i>	<i>UV</i>
<i>Conc</i>	<i>Conc</i>

Clear all other curves. Click **OK**.

2.4.3 Automatic evaluation of the installation test results

The system automatically prints the test result when the test is finished. The print-out consists of a chromatogram and an evaluation of the test result.

- If the gradient test result is OK, the print-out says "Gradient linearity accepted".
- If the step response test result is OK, the print-out says "Step response accepted".
- If the UV response test result is OK, the print-out says "UV response accepted".

If any of the evaluated values falls outside the specified range, go to Section 2.4.5 "Correcting faulty evaluation results".

2.4.4 Manual evaluation of the installation test results

If the chromatography system deviates from the standard configuration, for example, if optional components have been installed, the automatic evaluation will not give a reliable result. A manual evaluation is required.

- 1 Select the **UNICORN Manager** module.
- 2 Click on  in the **Results** window and then double-click on the **Installation Test01** icon to open the result file.
- 3 Right-click in the **Curves** pane and select **Properties**.
- 4 Click the **Curves** tab and select the following curves to be displayed:

ÄKTApurifier	ÄKTApurifier UPC
Installation Test01:1_UV1_215nm@01,SMTH	Installation Test01:1_UV@01,SMTH
Installation Test01:1_Conc	Installation Test01:1_Conc

- 5 Click **OK**.
- 6 Right-click in the chromatogram window and select **Marker**.
- 7 Read the absorbance for the steps corresponding to

ÄKTApurifier	ÄKTApurifier UPC
Installation Test01:1_UV1_215nm@01,SMTH	Installation Test01:1_UV@01,SMTH

Move the vertical bar to the constant Section of each plateau by dragging it. Enter the absorbance values (in mAU) in column 2 in the Step response table of the Test record (see page 39), leaving out the decimals.

- 8 Click **Print** under **File** to print the chromatogram.
- 9 (**ÄKTApurifier**) Read the absorbance for the plateaus corresponding to 0%B and 100%B for the curve **Installation Test01:1_UV1_215nm@01,SMTH**, and enter the values in column 2 in the UV response table of the Test record.

Evaluating the gradient

Place a ruler along the gradient part of curve

ÄKTApurifier	ÄKTApurifier UPC
Installation Test01:1_UV1_215nm@01,SMTH	Installation Test01:1_UV@01,SMTH

in the printed report.

The curve should be linear between 10% B and 90% B and void of discontinuities.

Evaluating the step response

Calculate the relative adsorption plateau heights for curve

ÄKTApurifier	ÄKTApurifier UPC
<i>Installation Test01:1_UV1_215nm@01,SMTH</i>	<i>Installation Test01:1_UV@01,SMTH</i>

as follows:

- Subtract the base line value (0%B) from each of the values in column 2 in the Step response table of the Test record (see page 39) and enter the results in column 3.
- Divide each value in column 3 by the base line corrected value corresponding to 100%B, multiply by 100 and enter the results in column 4.

The values of column 4 should all fall within the intervals given in column 5.

ÄKTA purifier: Evaluating the UV response

Calculate the UV response ratios in the following way:

- 1 Subtract the base line values (0% B) corresponding to each UV curve from the values in column 2 of the UV response table of the Test record (see page 39) and enter the results in column 3.
- 2 Calculate the absorbance ratios 265 nm/254 nm and 265 nm/280 nm using the values of column 3 and enter the results in column 4.

The ratios obtained should all fall within the intervals given in column 5.

2.4.5 Correcting faulty evaluation results

Should any of the evaluated values fall outside the specified range, proceed as follows:

- If the system differs from the standard configuration, evaluate the result manually.
- If the faulty evaluation result remains, continue below.

Faulty gradient

- The gradient is linear but the interval is too small - the mixer chamber is too large, or the mixer is faulty.
- Disturbances - may arise from air in the pump, pump valves or bad sealings in the pump. Refer to the *Pump P-900 User Manual*.

Faulty step response

- If all values are faulty – air in the pump or a faulty pump.
- 5% and 95% faulty – bad sealing in the pumps (5% faulty = pump module B, 95% faulty = pump module A).

2.5 Test record

Date:.....

ÄKTApurifier serial no.:.....

2.5.1 Gradient test result

Gradient linear from.....%B to.....%B. (10 - 90%)

2.5.2 Step response test result

Step response table:

1 Programmed Conc.%B	2 Value read	3 Baseline cor- rected value	4 Normalized value	5 Allowed interval
100				
95				94 - 96
70				69 - 71
30				29 - 31
5				4 - 6
0				

2.5.3 UV response test result

UV response table:

1 Wavelength (nm)	2 Value read		3 Baseline corrected value	4 Absorbance ratio	5 Allowed interval
	100% B	0% B			
254					
265/254					1.11 - 1.26
265					
265/280					1.26 - 1.53
280					

2.6 **Registration form**

IMPORTANT

WARRANTY REGISTRATION INFORMATION

Please ensure that this form is completed and returned to Service Administration to register the users' equipment under warranty.

Name:.....

Institute/company:.....

Address:.....

Department/location:.....

Post Code:.....

Phone Number:..... Tax Number:.....

End Users:..... E-mail:.....

Date of Installation:..... Quote No:.....

Customer Order No:..... Invoice No:.....

Support Agreement purchased with the instrument: Y / N

If YES give details:.....

Installer (name):.....

Signature of Installer:.....

Installation Accepted:..... Date:.....

Note: *Fill in serial numbers overleaf.*

2.6.1 Components

ÄKTApurifier system serial numbers:.....

Qty	Part Number	Description	Serial Number
		System rack	
		Mixer M-925	
		Monitor UV-900	
		Monitor UPC-900	
		Pump P-900	
		INV-907	
		Computer	
		Monitor	

2 Installation

2.6 Registration form

3 System overview

This chapter gives a brief overview of ÄKTApurifier with the separation unit and UNICORN software.

Note: Two versions are available; ÄKTApurifier with monitor UV-900 for multiple wavelengths detection and ÄKTApurifier UPC with monitor UPC-900 for combined measurement of UV absorption, pH and conductivity. When instructions or specifications are similar for both the models only ÄKTApurifier is mentioned.

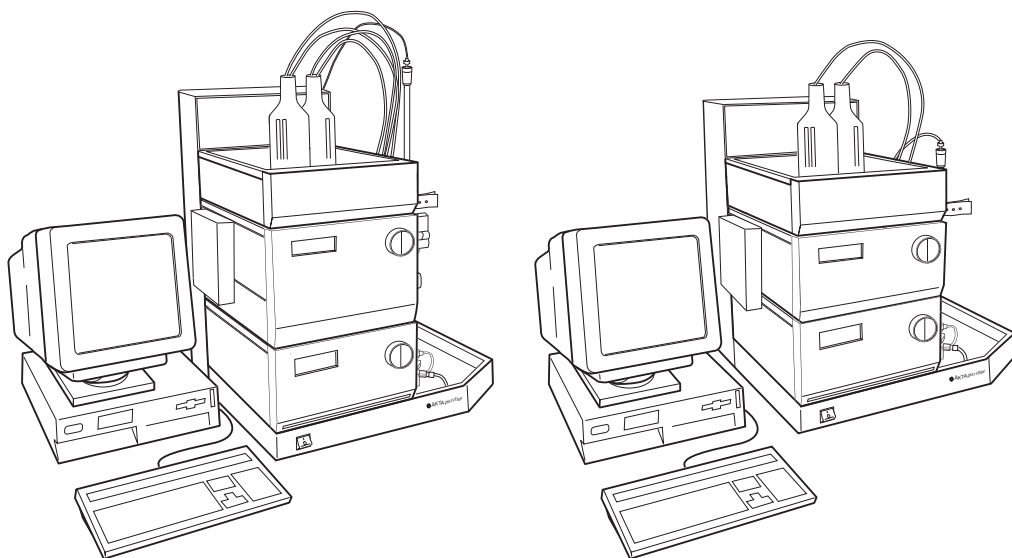


Fig 3-1. ÄKTApurifier to the left and ÄKTApurifier UPC to the right

3.1 Separation unit

ÄKTApurifier is an automated liquid chromatography system designed for method development and research applications. The separation unit of the chromatography system has two main modules which are stacked on each other.

The modules are:

- Pump P-900, a binary high performance gradient pump, and
- **ÄKTApurifier:** Monitor UV-900, a multi-wavelength UV-Vis monitor for measuring up to three wavelengths simultaneously.

3 System overview

3.1 Separation unit

- **ÄKTApurifier UPC:** Monitor UPC-900, a high precision on-line combined monitor for measuring UV absorption, conductivity and pH (optional).

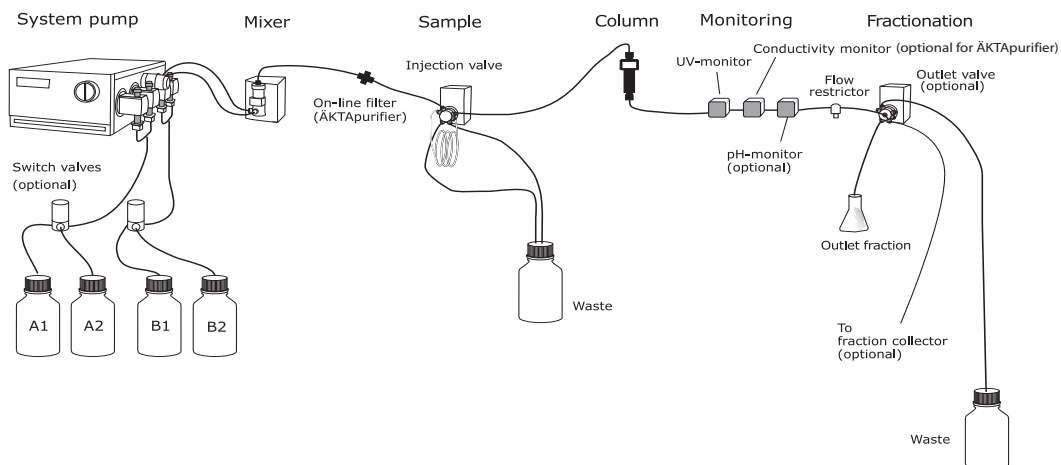
Components, such as the mixer, column and injection valve, are mounted to the right. Columns are snapped in place in column holders.

The separation unit is controlled from UNICORN software.

Pump P-900, Monitor UV-900 and Monitor UPC-900 can also be controlled individually from the modules without UNICORN software. This is described in the respective component manuals.

3.1.1 Liquid flow path

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



- 1 The pump has four pump heads, two for pump module A and two for pump module B. Pump module A is the one closest to the front.
- 2 Pump inlets A and B are immersed in buffer A and B respectively. The buffer solutions are pumped to a mixer by the pump. Inlet A1 and B1 are placed in buffer A and B respectively. Inlet A2 and B2 are used when buffers are prepared automatically by BufferPrep. They can also be used for changing buffer during the method run.
- 3 The flow path continues from the mixer to the injection valve. (For ÄKTApurifier, via an online filter.)

- 4 A sample loop is connected between ports 2 and 6 on the injection valve. The sample loop is filled manually using a syringe and a fill port connected to port 3 in the injection valve.
- 5 After the injection valve, the flow is directed through the column, the UV cell in the optical unit and the conductivity cell located below the optical unit.

If a pH flow cell (optional) is included in the flow path, it will be located after the conductivity cell.
- 6 The flow path then continues through the flow restrictor. The flow restrictor generates a constant backpressure to eliminate the risk of air bubble formation.
- 7 The flow continues to the outlet valve (optional), which is used to switch the outlet flow to waste, fraction collection or outlet fraction.

3.2 Columns and tubing

A wide range of pre-packed columns for ion exchange, size exclusion, hydrophobic interaction, reversed phase and affinity chromatography are suitable for use with ÄKTApurifier A comprehensive list of the recommended pre-packed columns is given in Section 8.4 in "Recommended tubing and columns" together with information on the recommended tubing for each column.

It is very important to use the correct tubing. Consider the maximum allowed pressure for the column and the size of the column.

For low pressure columns, such as HiTrap™ and HiLoad™, it is necessary to compensate for the pre-column pressure as described in Section 5.4 in "Extra system pressure measurement".

3.2.1 ÄKTApurifier10 tubing



WARNING! Never use i.d. 0.25 mm tubing or smaller with columns that only withstand low maximum pressures and allow high flow rates, as the columns might rupture, resulting in injury.

On delivery, the system is equipped with i.d. 0.50 mm tubing (PEEK, orange) from the pump to the injection valve, and i.d. 0.25 mm tubing (PEEK, blue) from the injection valve to the conductivity cell.

I.d. 0.50 mm tubing can also be used from the injection valve to the conductivity cell. This diameter should be used with columns that have a low maximum pressure limit and allow high flow rates. If tubing with smaller inner diameter is used, there is a risk that the back-pressure will be too high and the columns might rupture.

The i.d. 0.25 mm tubing should also be changed to i.d. 0.50 mm tubing when a pH electrode (optional) is used.

Note: Use only a maximum of 80% acetonitrile eluent at pressures above 10 MPa. Otherwise, the lifetime of the tubing will deteriorate.

3.2.2 ÄKTApurifier 100 tubing

On delivery, the system is equipped with i.d. 0.75 mm tubing (PEEK, green) from the pump to the conductivity cell.

3.3 UNICORN software

UNICORN is a software package for control and supervision of the ÄKTAdesign chromatography system. It runs on an IBM-compatible PC under Microsoft™ Windows™ operating system and includes hardware for interfacing the controlling PC to the chromatography liquid handling parts of ÄKTApurifier.

3.3.1 Starting UNICORN

To start UNICORN:



- 1 Switch on the computer. Log on to Windows by first pressing **Ctrl-Alt-Del** and then clicking **OK**. After a while the Windows desktop appears.

- 2 Start UNICORN by double-clicking the UNICORN icon.

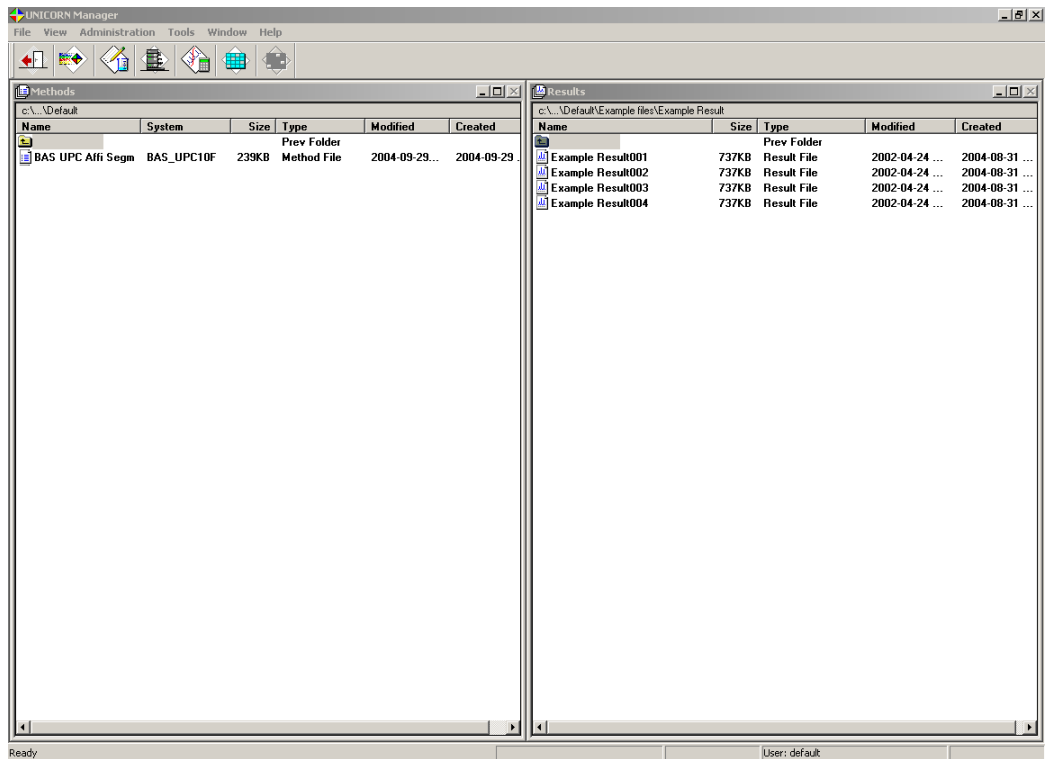
An information window appears during start-up.

- 3 In the **Logon** dialog, select a user from the **Users** list and enter the password. If you log on for the very first time, select user **default** and enter the password **default**. Click **OK**.

Note: You should enter users and individual passwords before starting using ÄKTApurifier on a regular basis. See UNICORN User manuals.



4 Eventually, the **UNICORN Manager** module appears on the screen.



5 **UNICORN Manager** module is the central part of the UNICORN displays. It is mainly used for file handling and administration. From this module you navigate through the control system.

In the **Methods** pane to the left, all method files that you create are displayed. A method file contains a series of instructions for controlling a run.

In the **Results** pane to the right, all result files are displayed. A result file is the result from a run, including all documentation (e.g. the method used) and the generated chromatogram.

3.3.2 UNICORN modules and buttons

In general, UNICORN consists of 4 different modules of which **UNICORN Manager** is one. The other modules are represented by icons in the toolbar. These modules are:



- Method Editor Opens a dialog window for creating new methods.



- System control Opens a dialog window for controlling the system and running your methods.



- Evaluation Opens a dialog window for evaluating your results.

To swap between the module, click their respective button in the task bar at the bottom of the screen.



Additional buttons are provided in the toolbar. These are:



- Instant run Opens a dialog window where you directly can create a method to run. This is handy for starting routine runs instantly.



- Logon/Logoff Opens a dialog window to control the logon/logoff process.



- Method Queue* Opens a dialog window for defining a new Method Queue.



- Running Method Queues Opens a dialog window for showing a Method Queue that is running.

* Method Queues are used to link several methods together.

3.3.3 Help

Comprehensive on-line help is available.

To get help about an instruction or module:

- Place the cursor on the instruction/module and press the **F1** key, or
- Click on the **Help** menu in the upper right corner of each module and select **Help for.....** to get general help about the current instruction or module and find new help topics, or **Index** for a specific topic.

In any dialog, click on the **Help** button to get help with using the current active dialog.

4 Operation

In this chapter you will learn how to operate ÄKTApurifier from UNICORN.

Note: Two versions are available; ÄKTApurifier with monitor UV-900 for multiple wavelengths detection and ÄKTApurifier UPC with monitor UPC-900 for combined measurement of UV absorption, pH and conductivity. When instructions or specifications are similar for both the models only ÄKTApurifier is mentioned.

The following topics are described:

- Starting the system.
- Creating methods.
- Preparing the system for a run.
- Performing and monitoring a run.
- Performing simple result evaluation.
- Viewing and printing results.
- Performing automatic method optimization (Scouting).
- Preparing automatically buffers of any pH (BufferPrep).

The chapter also contains information about fraction collection, cold room operation, mixing gradients and feed back tuning.



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.

4.1 Starting the system

IMPORTANT! Before using ÄKTApurifier, read all the safety information in Section 1.3 *Safety*.

4.1.1 Pre-requisites

Before starting a run, make sure that:

- the system and software are installed and functioning
- the monitors and pumps are tested.

This is described in Chapter 2 *Installation*.

4.1.2 Starting the system

To start the system:

- 1 turn on the separation unit with the mains switch located to the left on the base platform.
- 2 turn on the computer, display and printer.
- 3 start UNICORN. See section 3.3.1 "*Starting UNICORN*".

4.2 Creating a method

UNICORN control software is supplied with a *Method Wizard* used for creating new methods. The Method Wizard consists of a number of dialogs in which you answer questions and receive instructions on how to create your method. The options in subsequent dialogs depend on the choices you have made in the previous dialogs.

The Method Wizard covers the most commonly used chromatographic techniques.

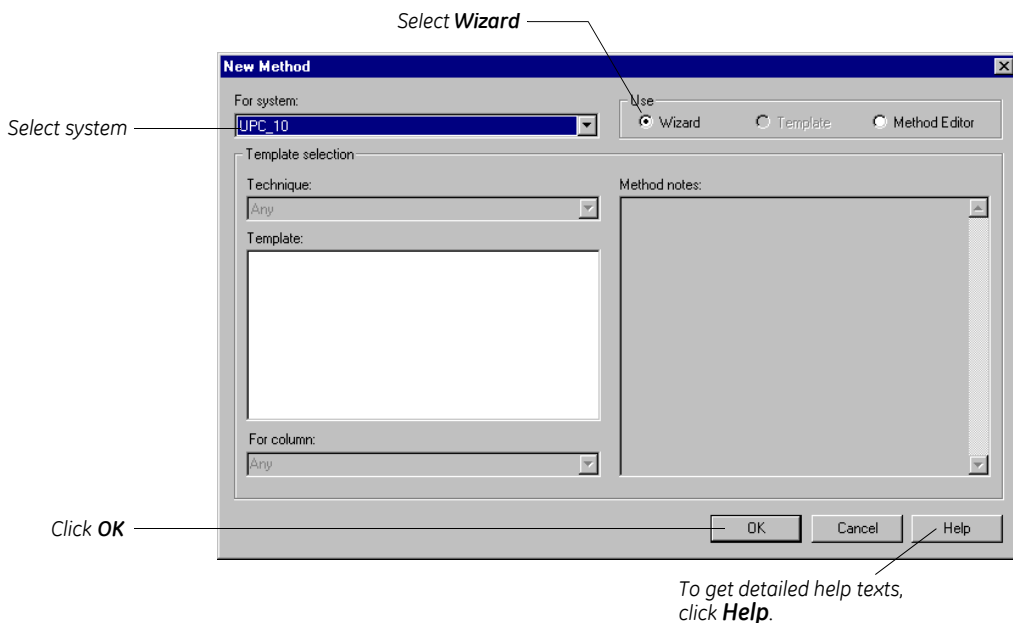
Note: ÄKTApurifier UPC screen shots are shown in the example.

To create a method:

- 1 Click the  icon in the **Method Editor** module. The Method Wizard is started.

or

Select **File:New** in the **Method Editor** module. The **New Method** dialog appears.

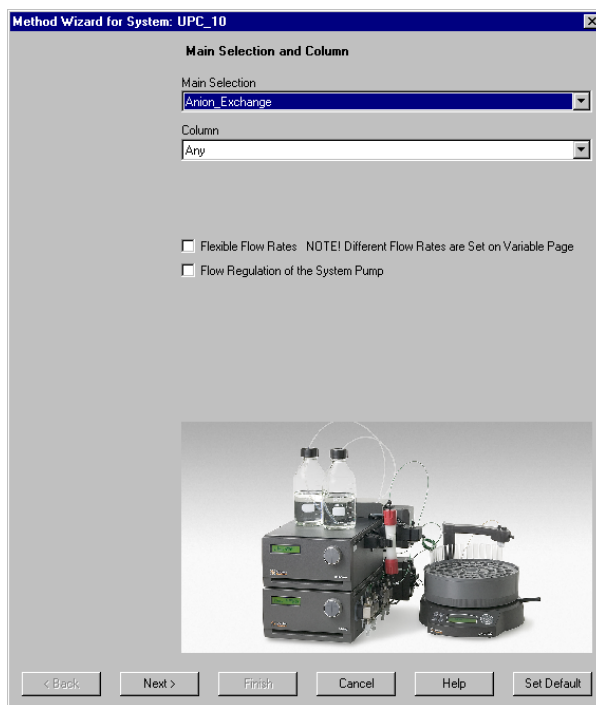


- Select **Wizard** from the **Use** options.
- Select which system you want to use.

4 Operation

4.2 Creating a method

- Click **OK**. The Method Wizard is started.
- 2 Select a chromatographic technique, for example **Anion_Exchange** from the **Main Selection** list.



- 3 Select the column you intend to use. The correct column volume, the recommended flow rate, and the correct pressure limit for that column will then be automatically implemented in the method.

Note: If you cannot find your column in the list, you can add one. Refer to the UNICORN User Manual and to section 8.4.3 "Selecting tubing kit for other columns".

Note: If you manually alter the default values of the column, and thereby exceed the recommended values for the selected column, you will get a warning when you save your method.

Note: If you want to perform a test run without a column, you should still select a column (a small one is recommended) to get suitable default parameters in the method. Then, when running the method, use a piece of tubing to replace the column.

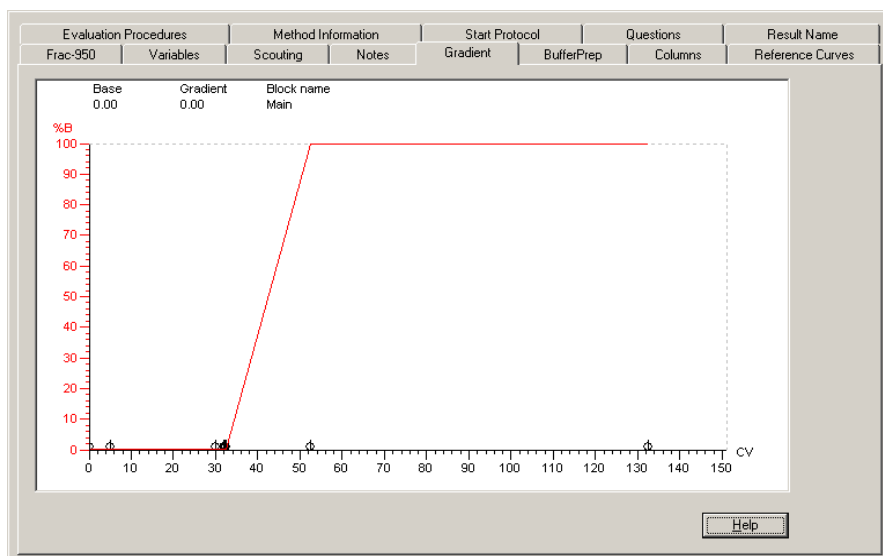
4 Operation

4.2 Creating a method

- Sample injection (see also section 4.4 "Sample application")
- Wash out unbound sample
- Fractionation (see also section 4.3 "Fraction collection")
- Gradient
- Clean after elution
- Re-equilibration

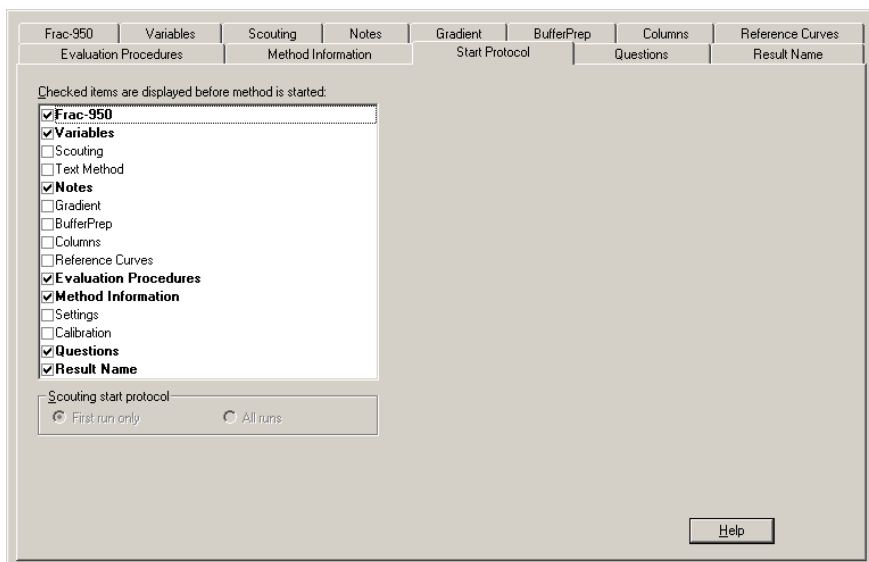
Some of the blocks contain a number of variables with suitable default values. The values can be changed to suit your application. Some of the variables are normally hidden but can be shown by checking the **Show details** box.

- 8 Click the **Gradient** tab to view the method graphically.



The length of each block is marked at the bottom of the graph. Click the x-axis to view the method in time, volume or column volumes.

- 9 Click the **Start Protocol** tab to decide which of the Run Setup pages is to be displayed at the start of a method run.



- 10 To save the method, select **File:Save**. In the **Save As** dialog, enter a name. Store the method in the directory of your choice by double-clicking on a directory. Click **OK**.

In **UNICORN Manager** module, the method name appears in the **Methods** pane. The method name, followed by three consecutive numbers starting with 001 will then be used as default name for the result file of your method after runs.

Now you are ready to start a run. Go to Section 4.6 "Starting a run".

For more information on:

- fraction collection, see Section 4.3 "Fraction collection"
- using Scouting in a method, see Section 5.3 "Scouting"
- using BufferPrep in a method, see Section 5.2 "BufferPrep".

4.3 Fraction collection

The fractions can be collected before and/or during the elution by using a fraction collector. The software provides the following methods for collecting fractions:

- Flowthrough fractionation
- Fixed volume fractionation
- Peak fractionation

Refer to the respective user manual for more information on using your fraction collector.

Note: *The delay volume (UV cell to fraction collector outlet) is not set at system delivery because it depends on the length of your outlet tubing. To set the delay volume in UNICORN, see Section 8.1.6 "Setting the delay volume".*

4.3.1 Fraction collection methods in the Method Wizard

The Method Wizard will automatically contain the fraction collection methods when the fraction collector is selected in UNICORN. By default, a fraction collector is automatically selected when the correct strategies are used.

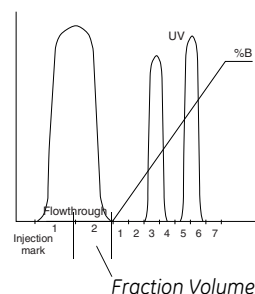
To check that the fraction collector is selected in UNICORN:

- 1 In **UNICORN Manager**, select **Administration: System Setup**.
- 2 In **System Setup** dialog, select the correct system symbol and click **Edit**.
- 3 In **Edit System** dialog, click **Component**.
- 4 Check that the fraction collector is selected.

Flowthrough fractionation

Flowthrough fractionation means that fixed volumes are collected before the elution starts. This fractionation method is available in all purification techniques, except for size exclusion.

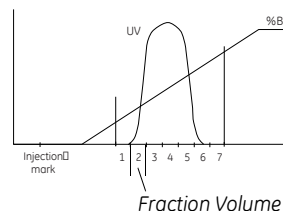
The parameters are found on the **Frac-920/950 Settings / Flowthrough Fractionation** page in the Method Wizard.



Fixed volume fractionation

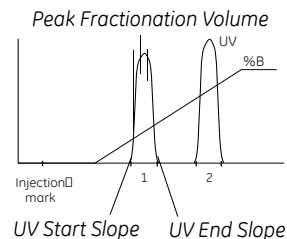
Fixed volume fractionation allows you to collect fixed volumes (Fraction Volume) during the elution.

The parameters are found on the **Elution Fractionation** page in the Method Wizard.

**Peak fractionation**

Peak fractionation means that only the peaks are collected during the elution. There are two ways to collect peaks:

- Each peak is collected in one fraction. The peak size is set to a volume (Peak Fractionation Volume) that is larger than the actual peak volume.
- Each peak is collected in several fractions. The peak size is set to a volume (Peak Fractionation Volume) that is smaller than the actual peak volume.



Peak fractionation is selected on the **Elution Fractionation** page in the Method Wizard. The parameters are found on the **Peak Fractionation** page.

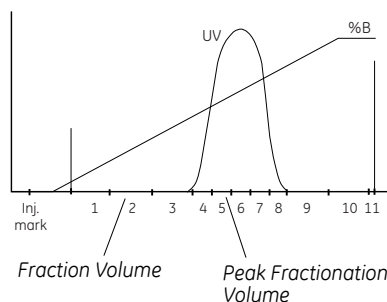
Examples on peak fractionation parameters are:

- start and end points of the fractions to be collected (the slope or level of the UV curve)
- minimum peak width to be collected
- peak fractionation volume

Refer to *UNICORN User manuals* for more information on the peak slopes and levels properties.

Fixed volume and peak fractionation

Fixed volume fractionation can also be combined with peak fractionation during the elution. Fixed volumes (Fraction Volume) will then be collected until a peak is detected. The peak is then collected in either one fraction volume or in several, using the Peak Fractionation Volume parameter. When the peak end is detected, the fixed volume fractionation continues.



4.3.2 Outlet valve (optional)

An outlet valve can be used for directing the liquid flow to either waste or to fractionation. To perform outlet fractionation, select **Outlet Valve** in the **Elution Fractionation** dialog in the wizard.

- Port 1 (valve default position) should always be connected to a waste flask of suitable size (W3).
- A fraction collector can be connected to the outlet valve with tubing G9/H9.
- Ports 2–8 of the outlet valve can also be used for collecting larger fractionations.

4.4 Sample application

With ÄKTApurifier, the sample can be applied in different ways to suit the application and the sample volume:

- Using sample loops, manually filled with a syringe, or a Superloop. As an option, the sample can also be applied using a sample pump, for example Pump P-960. More information is provided in the Pump P-960 package.
- (ÄKTApurifier) Directly onto the column using Pump P-960 or the system pump.
- (optional) Using an autosampler, A-900 or A-905.

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

Sample application technique	Volume to inject
Sample loop manual filling automated filling (optional)	0–2 ¹ ml 0.1–2 ¹
Directly onto the column using the system pump, or Pump P-960 (optional)	>1 ml
Superloop (optional)	1–150 ml
Autosampler ²	1 µl - 500 µl

¹ For partial filling of the sample loop the recommended volume is up to 1 ml.

² Depending on autosampler model and tubing selection. Refer to the A-900 or A-905 User Manual.

For scouting runs

If the sample volume is to be varied automatically in a series of scouting runs, one of the following techniques can be used:

- Automated partial filling of the sample loop using Pump P-960 (0.1–2 ml).
- Applying the sample directly onto the column with the Pump P-960 (> 1 ml).
- Using a Superloop (1–150 ml).

The subsequent Sections describe:

- applying small sample volumes using a sample loop
- applying large sample volumes using a Superloop

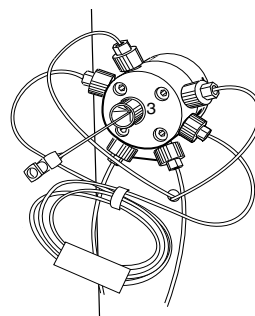
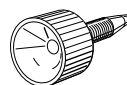
4.4.1 Applying small sample volumes using a sample loop

The sample loop should be manually filled before the method is started.

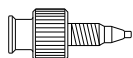
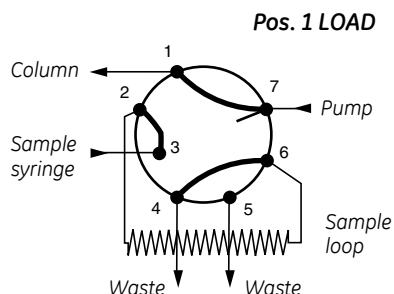
Preparation

Prepare the injection valve as follows:

- 1 Loosely thread the supplied injection fill port screw into valve port 3.
- 2 Insert the supplied injection needle (o.d. 0.7 mm) into the injection fill port.
- 3 Tighten the fill port until the nozzle has formed a seal around the needle's tip (i.e. when it feels as if you are penetrating a septum at the end of the injection fill port). The seal should allow easy insertion and removal of the needle.
- 4 Mount the syringe holder on the fill port.
- 5 Ensure that waste tubing is connected to port 4 of the injection valve.
- 6 Mount the sample loop between ports 2 and 6 of the injection valve.



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



A Union Luer female/1/16" male connector is supplied with ÄKTApurifier and is an alternative to the injection fill port. If used, the Union Luer connector replaces the injection fill port in port 3 of the injection valve.

Five sample loop sizes are available:

Sample loop	Catalogue no.
Loop 10 µl, 25 MPa	18-1120-39
Loop 100 µl, 25 MPa	18-1113-98
Loop 500 µl, 10 MPa	18-1113-99
Loop 1 ml, 10 MPa	18-1114-01
Loop 2 ml, 10 MPa	18-1114-02

When filling the loop with a sample volume equal to the loop volume, about 15% to 25% of the sample will be lost to waste because the fluid velocity in the sample loop tubing varies from a maximum at the tube axis to almost zero at the tubing wall. The exact amount of sample lost depends on the delivery flow rate.

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

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

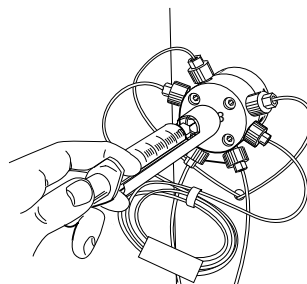
Partial filling

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

Partial filling is achieved as follows:

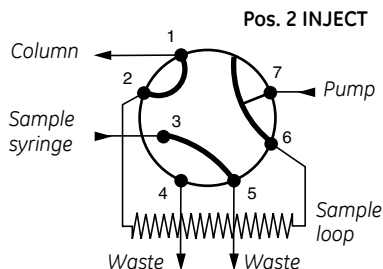
Note: The flow must be off before starting the filling procedure.

- 1 Set the injection valve to position LOAD.
- 2 Load the syringe with a large volume of buffer (5 times the loop volume).
- 3 Fill the sample loop carefully with buffer.



- 4 Set the injection valve to position INJECT before taking out the syringe.

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.

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

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

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

- 7 Gently load the syringe contents into the sample loop.
- 8 Leave the syringe in position. The sample will be injected onto the column when the valve is switched to INJECT in the method.

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

Complete filling

In this method, an excess of sample is used to ensure that the sample loop is filled completely. For analytical reproducibility, use a sample volume that is 5 times the volume of the sample loop. About 2 to 3 loop volumes of sample are required to achieve 95% of maximum loop volume. Five loop volumes ensure better precision.

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

Complete filling is achieved as follows:

- 1 Set the injection valve to position LOAD.
- 2 Load the syringe with sample (2–5 times the loop volume).
- 3 Gently load the syringe contents into the loop.
- 4 Leave the syringe in position. The sample will be injected onto the column when the valve is switched to INJECT in the method.

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

Emptying the sample loop

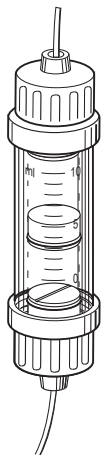
When emptying the sample loop, a buffer volume of at least 5 times the sample loop volume should be used to flush the loop and ensure that all sample is injected onto the column.

The volume to inject is set by the variable **Empty_loop_with** in the **Sample_Injection** block.

4.4.2 Applying large sample volumes using a Superloop



WARNING! Superloop 10 ml and Superloop 50 ml must not be used at pressures above 4 MPa (40 bar, 580 psi). Superloop 150 ml must not be used above 2 MPa (20 bar, 290 psi). At higher pressures, the glass tube might shatter.



Superloop permits the introduction of larger volumes of sample (1–150 ml) onto the column. Superloop must not be used with columns generating a column pressure higher than that stated in the table below. If the column pressure is higher, the flow must be reduced in order not to exceed the pressure limit. Set the pressure limit in the method with the variable **Pressure_limit**.

Superloop is an accessory available in three sizes:

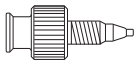
Volume	Max. allowed column pressure	Code no.
10 ml	4 MPa	18-1113-81
50 ml	4 MPa	18-1113-82
150 ml	2 MPa	18-1023-85

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.

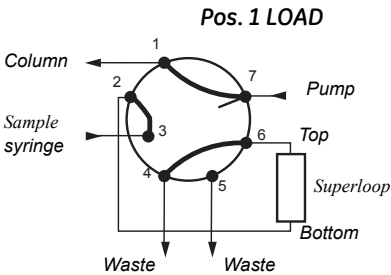
Superloop should be manually filled before the method is started.

Preparation

Prepare the injection valve and connect Superloop as follows:



- 1 Connect the supplied Union Luer female/1/16" male to port 3 of the injection valve.
- 2 Ensure that tubing for the waste is connected to port 4 of the injection valve.
- 3 Ensure that Superloop is filled with liquid (see separate Superloop Instruction).
- 4 Mount Superloop in a column holder as close to the injection valve as possible.
- 5 Connect the bottom tubing to injection valve port 2.

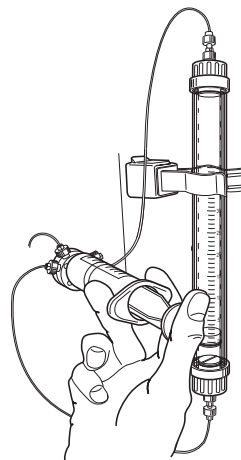


- 6 Connect the top tubing to injection valve port 6.
- 7 Make sure all connections are fingertight.

Filling Superloop

Filling Superloop is achieved as follows:

- 1 Set the injection valve to position INJECT.
- 2 Start Pump P-900 and let it run until the movable seal has reached the bottom of Superloop.
- 3 Stop Pump P-900 and set the injection valve to position LOAD.
- 4 Load a large volume syringe with sample.
- 5 Gently load the syringe contents into 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.
- 7 The volume to inject is set by the variable **Empty_loop_with** in the **Sample_Injection** block.
- 8 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.



WARNING! Make sure that the upper pressure limit of the system is lower than the maximum allowed pressure of the Superloop.

4.4.3 Mixing gradients

There are two different techniques available for mixing gradients. The standard technique using two separate buffers, one to each pump module, and the BufferPrep technique using four solutions, two to each pump module, generating the buffer on-line with a switch valve before each pump module. The minimum flow rate for BufferPrep is 1.0 ml/min. The outputs of the pump modules are routed to a mixer.

The BufferPrep method is described in section 5.2 "BufferPrep".

Note: To be able to use the BufferPrep function, the BufferPrep kit including two switch valves must be installed on the pump (see Section 5.2.2 "Installing the BufferPrep kit").

4 Operation

4.5 Preparing the system for a run

4.5 Preparing the system for a run

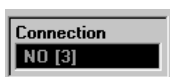
4.5.1 System connection

- 1 Click the **1. System Control** button in the Task bar at the bottom of the monitor.

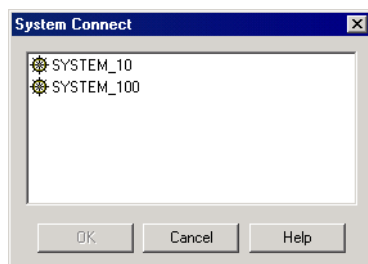


- 2 If **YES** is displayed in the **Connection** panel in the **Run Data** pane, UNICORN is connected to a system. Check that it is the correct system. The system name is displayed in the title bar of the window.

If it is the correct system, go to 4.5.2 "Preparing the flow path components".



- 3 If wrong system is connected or if the **Connection** panel says **NO**, select **System:Disconnect** and then click **OK**.
- 4 Select **System:Connect**. The System Connect dialog window appears:



- 5 Select your system from the list. If you are not connected to a network, only one system will be shown. Click **OK**.
- 6 When connected, **YES** is displayed in the **Connection** panel in the **Run Data** pane. You only have to connect once. If you do not select **System:Disconnect**, you will be automatically connected to the system the next time you login to UNICORN.

4.5.2 Preparing the flow path components

Preparing the tubing

- 1 Use the correct tubing kit for the column you intend to use. See Section 8.4 "Recommended tubing and columns". For most columns the i.d. 0.50 tubing kit can be used in ÄKTApurifier 10 and the i.d. 0.75 tubing kit in ÄKTApurifier 100.

Note: If using Frac-920 with ÄKTApurifier 10, we recommend a maximum flow rate of 3.5 ml/min with the i.d. 0.25 mm tubing kit. At higher flow rates, the drops will turn to a continuous liquid stream.

Note: If tubing with too large inner diameter is used, the peaks will become broader than necessary. If tubing with too small inner diameter is used, the backpressure from the tubing might become higher than the maximum pressure for the column and the run will stop immediately after it is started.

- 2 Immerse inlet tubing A in buffer A and inlet tubing B in buffer B (A2 and B2) respectively, if you changed this in the method).
- 3 Make sure that the waste tubing from the flow restrictor is put into an empty waste bottle.
- 4 If there is air in the inlet tubing or if you suspect air in the pump, purge the pump with a syringe as described in 2.4.1 "Preparing ÄKTApurifier" or in Chapter 2 in *Pump P-900 User Manual*.
- 5 If pH measurement is desired calibrate the pH monitor. Refer to the UNICORN User Manuals or the *Monitor pH/C-900 User Manual*. Mount the pH electrode in the flow cell.

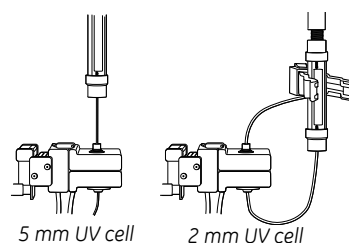
Checking the monitors

- 1 Make sure that the correct UV cell (2 or 5 mm) and filter is installed, and the correct wavelength set in the optical unit. See Chapter 2 in *Monitor UPC-900 User Manual*.
- 2 Make sure that pressure sensor, conductivity cell and pH electrode (optional) are calibrated according to the intervals listed in Section 6.4 "Calibration procedures".

Connecting the column

Monitor UV-900: Connect the column between the injection valve, port 1, and the inlet port of the UV cell.

Monitor UPC-900: The inlet port of the 5 mm UV cell is on top of the optical unit. The inlet port of the 2 mm UV cell is underneath the unit (view illustration).



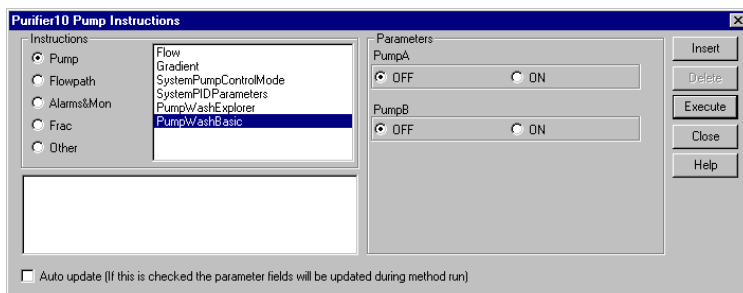
4 Operation

4.5 Preparing the system for a run

Filling the inlet tubing

- 1 Click the **System Control** button in the Task bar at the bottom of the monitor.
- 2 Fill the inlet tubing with the correct solutions by selecting **Manual:Pump**.

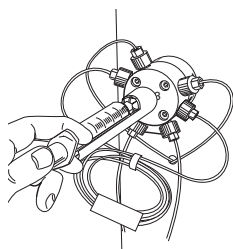
Select instruction **PumpWash** and set **PumpA** and **PumpB** to **ON**.



- 3 Click **Execute** to fill the inlet tubing. The injection valve will automatically switch to waste during the pump wash.
- 4 When the pump wash is finished, click **End** in the System Control toolbar.
- 5 **Close** the dialog.

Filling the sample loop or Superloop

- 1 Make sure that the correct loop or Superloop is mounted between port 2 and 6 on the injection valve.
- 2 Connect an injection fill port or a union luer female/1/16" male to port 3 on the injection valve.
- 3 Apply the sample manually with a syringe. More information on filling the loops is found in Section 4.4 "Sample application".



When using the fraction collector

- 1 Check that the tubing from the flow restrictor is connected to the fraction collector.
- 2 Put the waste tubing from the fraction collector into a waste bottle.
- 3 Insert a sufficient number of tubes into the fraction collector.

Note: The delay volume (UV cell to fraction collector outlet) is not set at system delivery because it depends on the length of your outlet tubing. To set the delay volume in UNICORN, see Section 8.1.6 "Setting the delay volume".

4.6 Starting a run

4.6.1 Final checks

Before starting any method, we recommend a number of checks to make sure that no problems occur once the run has been started.

- 1 Check that the inlet tubings are immersed in the correct bottles for the method selected.
- 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 with a syringe as described in 2.4.1 "*Preparing ÄKTApurifier*" or in Chapter 2 in *Pump P-900 User Manual*.
- 5 Check that the correct column has been fitted and equilibrated (if not included in the method).
- 6 Check that the correct max pressure is set for the column selected.
- 7 Check that the correct mixer chamber and tubing are installed for the method selected.
- 8 Check that the fraction collector (optional) has sufficient tubes fitted and is connected to the flow restrictor.

4.6.2 Setting up and starting the method

Note: ÄKTApurifier UPC screen shots are displayed in the example below.

- 1 Click the **System Control** icon.
- 2 Select **File:Run....** Select the method to start. Click **OK** (the method will not start yet).

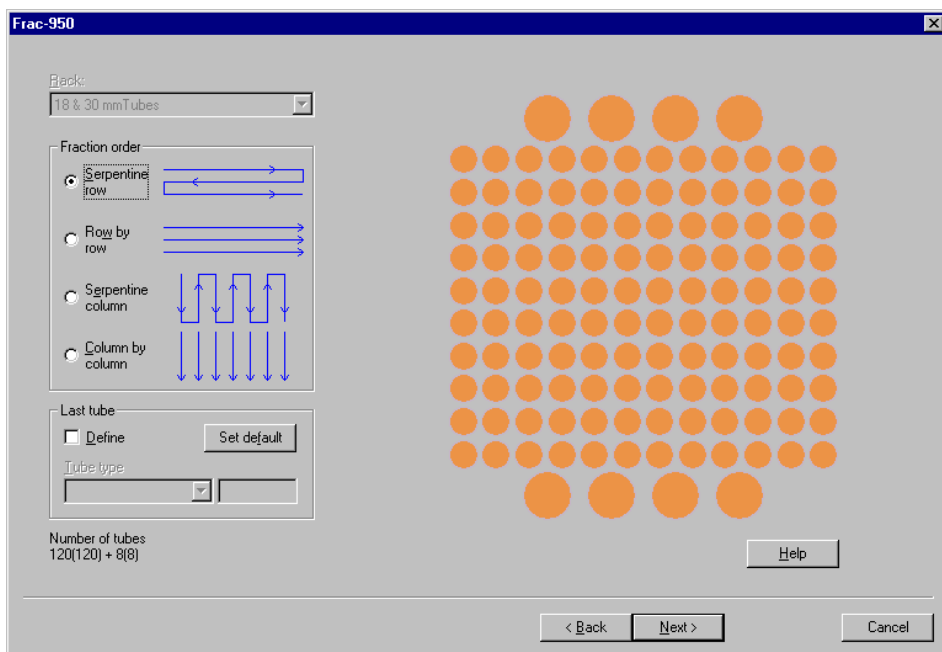
The method run is initiated in a series of pages in Run Setup in System Control.

Note: Only the pages set in the Start Protocol for the method will appear during the initialization. Some of the pages are briefly described in the steps below.

When a page is done, click **Next** to proceed.

- 3 If using Frac-950, the **Frac-950** page will appear if it has been selected in the Start Protocol.

On the Frac-950 page you can define the order of fractionation and set up the last tube used. The system will be paused when the last tube is reached and the fractionation will stop.



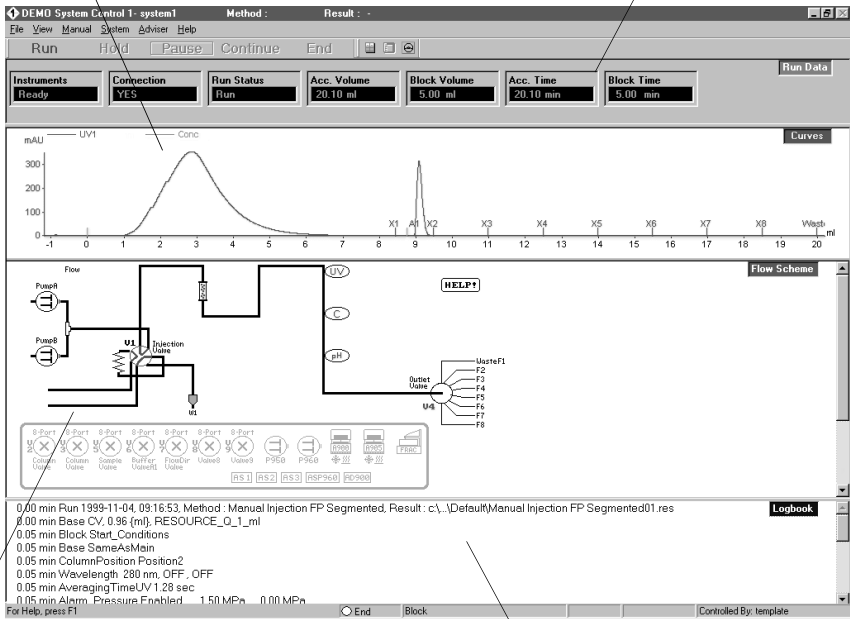
4 Operation

4.6 Starting a run

- 8 Click **START**. The run will start. You can view the run in the **System Control** module.

The Curves pane shows curves during the run.

The Run Data pane shows current values for running parameters



The Flow scheme is a graphical representation of the system flow path.

The Logbook pane shows when the instructions in the method are executed during the run.

4.7 During a run

4.7.1 Monitoring the run

Viewing progress

The progress of the method being used can be viewed in detail on UNICORN and the status of certain parameters of the instrument modules can be viewed directly on their front panel displays.

The **System Control** module in UNICORN displays the current status of ÄKTApurifier and can display up to four panes for monitoring different aspects of the run.



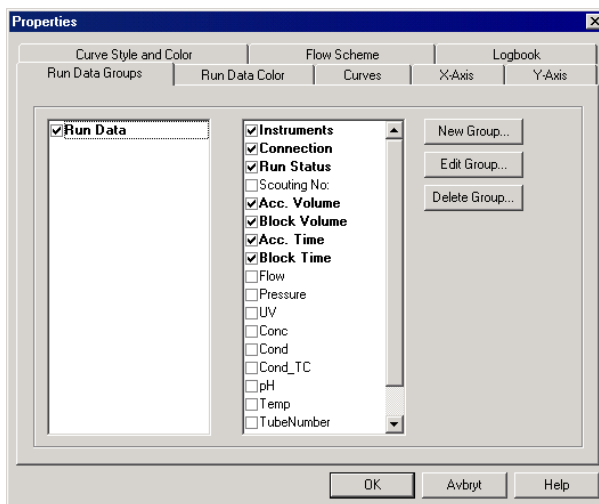
Click the **Customize panes** toolbar button or choose **View:Panels** from the menu to select which panes to display.

Run Data

The Run Data pane displays the current values for selected run parameters.

To customize the **Run Data** pane:

- 1 Right-click in the **Run Data** pane and select **Properties**, or select **View:Properties** from the **System Control** menu.
- 2 Select the run data items to be displayed and click **OK**.

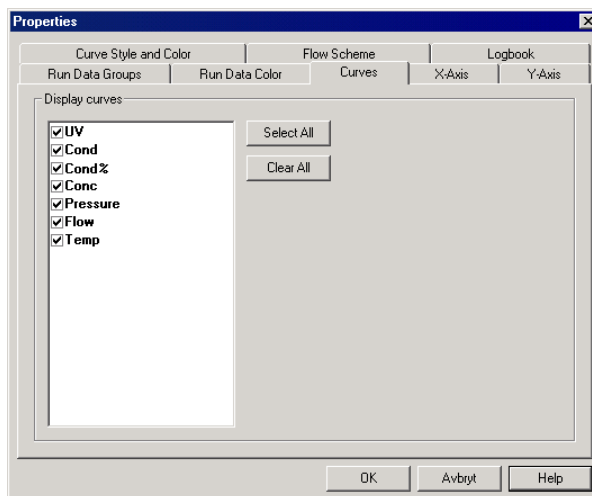


Curves

The curves pane displays the monitor signal values graphically.

To customize the **Curves** pane:

- 1 Right-click in the **Curves** pane and select **Properties...**, or select **View:Properties** from the **System Control** menu to select the curves to be displayed. All curves are always stored in the result file.
- 2 Click the different tabs in the **Curve Properties** pane to set the properties for the different curves. Normally the curves are scaled with auto scaling, i.e. the scale is adjusted continually to the highest and lowest values for each curve.



- 3 To fix the Y-axis scale for a curve, mark the curve, click **Y-axis**, click **Fixed**, and enter the max. and min. values. You can repeat this for other curves. Click **OK**.
- 4 To maximize the **Curve Data** pane, position the cursor in the **Curve Data** pane. Click the right mouse button and select **Maximize**. Go back to normal size by clicking **Restore**.
- 5 To shift to a scale for another curve click the Y-axis scale, or click the curve name at the top of the **Curve Data** pane. The color of a curve, its Y-scale, and its name are always the same. Click the X-axis to shift between time and volume.

Flow scheme

The flow scheme is a graphical representation of the flow path in the chromatography system. During a run, the flow scheme shows open flow paths and monitor signals with numerical displays.

Logbook

All actions and unexpected conditions such as warnings are logged for every run, with date, time and current username. The logbook provides a complete history of any given run. The log is saved in the result file.

Front panel display

The front panel displays of Monitor UV-900, Monitor UPC-900 and Pump P-900 can be set to show their current status. In each case, the main operating menu display shows the most important parameters.

Run	13.40 ml/min
2.00MPa	45.5%B

The main operating menu of Pump P-900 shows the current flow rate together with a mode indication, pressure and % B, if used.

The available modes are:

Run	The pump is running 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 gradient is held at the value displayed and the pump continues to run. The method is held in its current status.

λ 1 [215]	1.123	AU
λ 1 [254]	0.02345	AU
λ 1 [280]	0.1234	AU

ÄKTapurifier: The main operating menu of the Monitor UV-900 shows the absorbance values with 4 digits for up to 3 active wavelengths. The display for the third wavelength is reached by turning the dial clockwise. It is also possible to view all three wavelengths simultaneously by turning the dial one step further (only three digits).

AU	Cond%	Tc	pH
0.00002	015.0		12.50

ÄKTapurifier UPC: The main operating menu 1 of Monitor UPC-900 shows the absorbance value with 6 digits for the selected wavelength, the conductivity as a percentage of full scale and the pH value (optional).

pH12.50	Tc	22.4°C
735.8mS/cm	Tc	78.8%

By turning the dial one click, an alternative display of the conductivity is shown (main menu 2). This display shows pH, temperature and the actual conductivity value in mS/cm or µS/cm, together with the percentage value.

4.7.2 Changing parameters

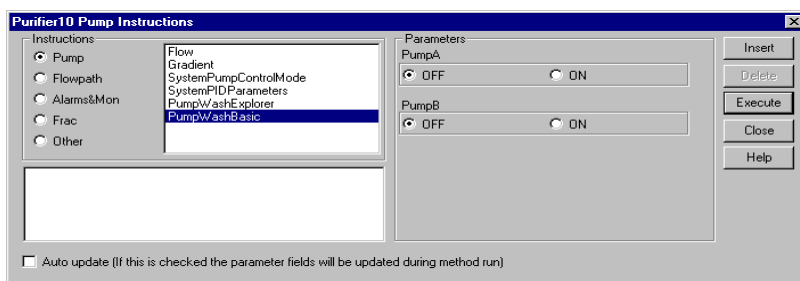
From UNICORN

ÄKTApurifier can be controlled with manual instructions issued from the **Manual** menu in **System Control** in UNICORN. These instructions can be used during a run to alter system conditions in response to the results observed.

The **Manual** menu in **System Control** opens a dialog box similar to the text instruction box in the Method editor.

For example, to change the flow rate:

- 1 Select **Manual:Pump**. The **Instructions** dialog is opened.



- 2 Select **Pump** and then **Flow**.
- 3 Type a new flow rate under **Parameters** and then click **Execute**. The new flow rate will be used until the end of the run or until a new flow rate instruction is reached in the method.
- 4 Close the box by clicking **Close**. All manual interactions are recorded in the **Logbook**.

Some instructions, for example gradient or fraction instructions, might take time to complete.

To stop the run before it is finished, click the **End** button at the top.



From the modules

Manual changes can also be performed on the pump and UV monitors using the selection dial.

Manual changes in UNICORN or on the modules are equivalent. Manual changes are normally recorded in the log book. The selection dial on the modules can be set in one of three different access modes:

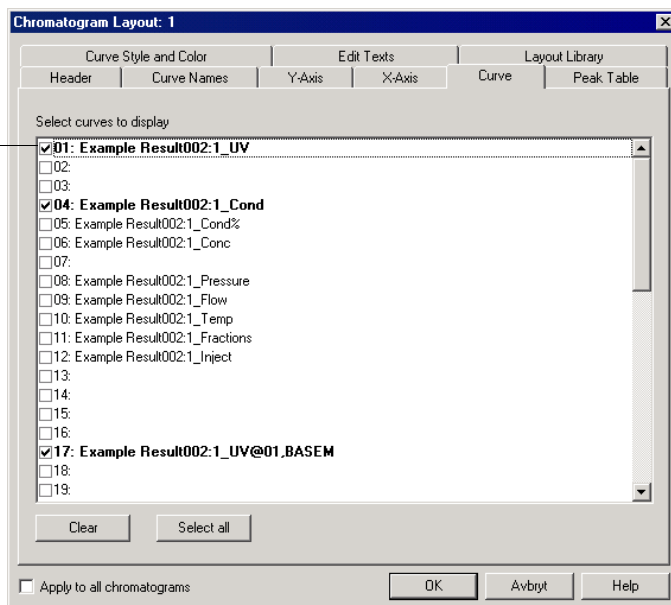
- ***Open*** – the dial on the module can be used for manual changes.
- ***KeyLocked*** – the dial on the module can be used to select different menus, but cannot be used to change any parameters.
- ***KeyAndDialLocked*** – Neither menu selection nor parameter changes can be performed.

To select access mode:

- 1 Select ***System settings*** in ***System Control*** and then ***Specials:Keyboard***.
- 2 Select ***Open***, ***KeyLocked*** or ***KeyAndDialLocked***.

- 3 Maximize the **Chromatogram** window by clicking the square in the upper right corner.
- 4 All changes regarding the presentation of the curves are done in the **Chromatogram Layout** window. Position the cursor in the **Chromatogram** window. Right-click and select **Properties.....**, or select **Edit: Chromatogram layout...** to activate this window.

Highlight curves to view



- 5 Select the curves to view under **Curves**.
The curves are named as **Result001:1_"curve"** where a curve can be, for example, UV_wavelength, pressure...etc. Click **OK** at the bottom of the **Chromatogram Layout** window.
- 6 To zoom in on the curves, click-and-drag in the chromatogram with the left mouse button. A rectangle appears on the screen. When you release the mouse button, the part within the rectangle will be enlarged. You can zoom further on the enlarged part.

To return to the complete chromatogram, right-click and select **Undo** or **Reset zoom**.
- 7 To change to a scale for another curve, click on the **Y-axis** scale. The style and color of a curve, its Y-scale and its X-scale can all be changed.

4 Operation

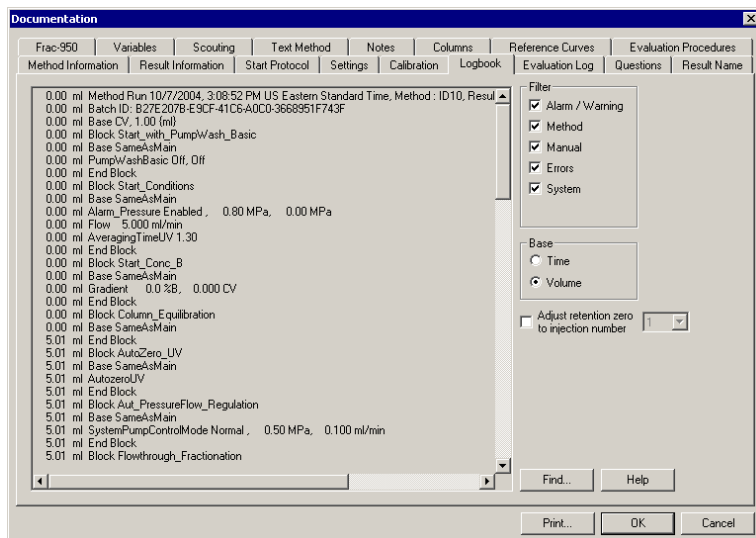
4.8 Viewing and printing the result

- 8 Open the **Chromatogram Layout** window again. Click on the **Y-axis** and **X-axis** tabs to set the scale for the different curves. Normally, the curves are scaled with auto scaling (i.e. the highest and lowest values for each curve set the scale).
- 9 To fix the Y-axis scale, mark a curve, click **Fixed**, and enter the max. and min. values for that curve. You can repeat this for other curves.
- 10 To fix the X-axis scale, click **Fixed** in the X-axis field, and enter the min. and max. values for the X-axis. Click **OK**.
- 11 Click **OK** at the bottom of the **Chromatogram Layout** window to execute all the changes.
- 12 When you have made the necessary changes in the Chromatogram layout window, they can be saved as a new layout:
 - Click the **Layout Library** tab at the top of the **Chromatogram layout** window.
 - Click **Save current layout as** and type a name. Click **OK**.

LAYOUTS CAN BE SELECTED IN **Save current layout** AND ALL YOUR SAVED SELECTIONS WILL APPLY. SAVED LAYOUTS CAN BE APPLIED TO ANY RESULT FILE.
- 13 Minimize the chromatogram window by clicking on the smaller squares in the upper right corner.

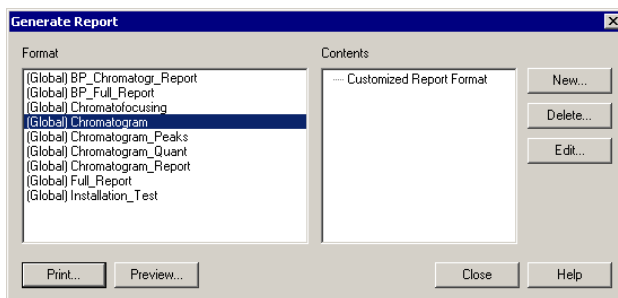


- 14 Click the **View Documentation** button. A number of pages appear as in the Run Setup in the Method Editor. All documentation about the run is stored here, e.g. the method, answers to questions, variables, logbook...etc. For example, click the **Notes** and **Logbook** pages to check the contents. Close the **Documentation** window by clicking the **X** in the upper right corner.



4.8.2 Printing and creating a report

- 1 To print the chromatogram, select **File:Report**. The **Generate Report** dialog opens.

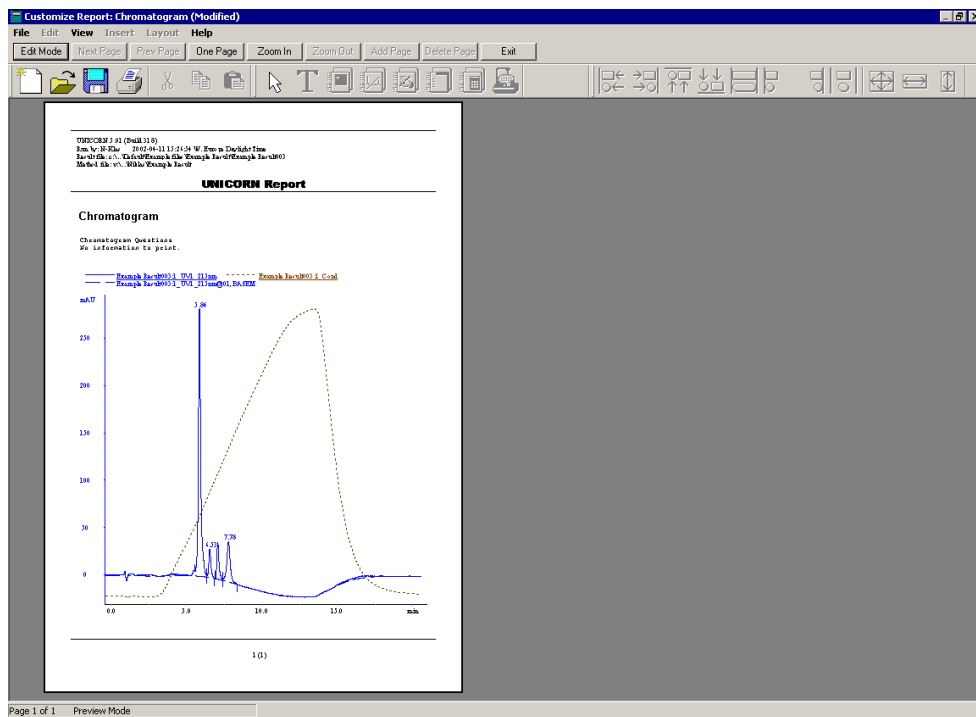


- 2 Select, for example, format **(Global) Chromatogram**. This will create a report containing the chromatogram and the questions on one page.

4 Operation

4.8 Viewing and printing the result

- 3 Click **Preview** to view the report on the screen.



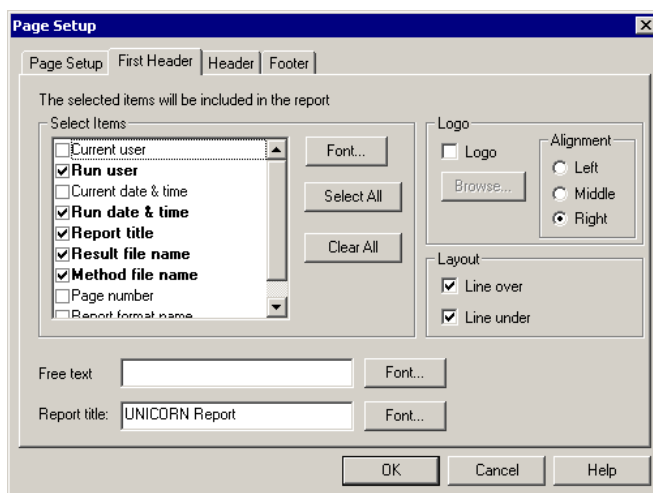
Adding information to the report

- 1 Click **Edit Mode** to enable changes in the report.
- 2 To add an empty page to the report, click **Add Page**.
- 3 Select from the **Insert** menu, the item to include. Items available are:
 - Free text
 - Picture
 - Text method
 - Chromatogram
 - Documentation
 - Evaluation log
 - Quantitate and molsize (optional)
 - Frac-950 (optional).

- 4 Move the mouse pointer into the page area of the window. You will notice that the mouse pointer has an additional symbol according to the item type you selected to insert.
- 5 Click-and-drag to create a box of the desired size. Release the mouse button. A dialog is displayed specific to the type of item inserted. Make the appropriate selections in the dialog and then click **OK** to view the inserted item.

Change page layout

- 1 If you want to change the page layout, select **Edit:Page Setup**. The **Page Setup** dialog opens and you can, for example select page size and items to be included in the header and in the footer. The information selected here will be printed in the report. Click **OK**.



- 2 Click **Print** to print the report.

4.8.3 Printing all method instructions with explanations

- 1 Select **Method Editor:File** and click **Print**. This opens a window containing instructions that are printable.
- 2 Make sure that the **Instruction Set** box is checked and clear any unwanted items.
- 3 Click **Print** to print the instructions.

4.9 Completion of a run and storage

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

4.9.1 Storage

Overnight: Let the system run isocratically at a low flow rate (approximately 10% of the used flow rate).

Weekend and long term storage: Flush the system with water and then fill it with 20% ethanol (not the pH electrode, if used, see separate instructions in *Optional Configurations ÄKTApurifier User manual*).

4.10 Cold room operation

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

4.10.1 Preparation

- 1 Place the separation unit in the cold room.
- 2 Place the computer outside the cold room. A 15 m UniNet cable is available as an accessory and should be used to connect the computer to the separation unit.
- 3 Allow the separation unit to stabilize at the new temperature for at least 12 hours.
- 4 Tighten all connections and pump water through the system to check for leaks.
- 5 Tighten any leaking connector.

4.10.2 Running in cold room

Before starting a run, check that the temperature of the buffers has reached the ambient temperature.

4.10.3 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 connectors.

5 Optional functions

This chapter describes how to use optional functions when operating ÄKTApurifier.

5.1 Mixing gradients

5.1.1 Gradients

There are two different techniques available for mixing gradients. The standard technique using two separate buffers, one to each pump module, and the BufferPrep technique using four solutions, two to each pump module, generating the buffer on-line with a switch valve before each pump module. The minimum flow rate for BufferPrep is 1.0 ml/min. The outputs of the pump modules are routed to a mixer.

The BufferPrep method is described in Section 5.2 "BufferPrep".

5.1.2 Mixer



WARNING! When using hazardous chemicals, flush the mixer chamber thoroughly with distilled water before removing it.

At delivery, the mixer is delivered with a 0.6 ml mixer chamber. It can be used at gradient volumes down to 5 ml (binary gradients).

The recommended minimum gradient volume for each mixing chamber is specified in the table below.

Mixing chamber volume	Recommended minimum gradient volume	
	Binary gradient	BufferPrep gradient
0.6 ml	5 ml	–
2 ml	16 ml	60 ml
5 ml	38 ml	80 ml
12 ml	90 ml	113 ml

When using eluents that are more difficult to mix, such as isopropanol and water, a larger mixer volume can be used to get optimum mixing.

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

Note: Always place the buffer bottles lower than the mixer when changing chambers, to prevent draining

5.2 BufferPrep

5.2.1 What is BufferPrep?

BufferPrep eliminates the time consuming manual buffer preparation and titration usually needed for every pH change in chromatography. For any pH and salt concentration entered, BufferPrep automatically calculates and prepares the composition of the buffer on-line, from four stock solutions. Linear and step salt gradients can be run and pH can be used as a variable scouting parameter.

Note: To be able to use the BufferPrep function, the BufferPrep kit including two switch valves must be installed on the pump (see Section 5.2.2 "Installing the BufferPrep kit" and Section 8.8 "Ordering information").

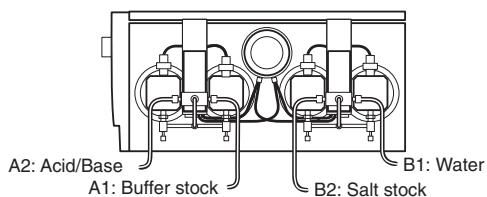
BufferPrep is optimized for use in ion exchange chromatography method development, but can also be used for size exclusion chromatography and for affinity chromatography.

The four stock solutions consist of:

- 1 A mix of buffering components. Up to five different buffering components with up to three pKa's each enabling a broad pH range to be covered.
- 2 An acid (HCl) or base (NaOH) for on-line titration of pH.
- 3 Distilled water.
- 4 An inert salt (e.g. NaCl or KCl) for elution gradient formation.

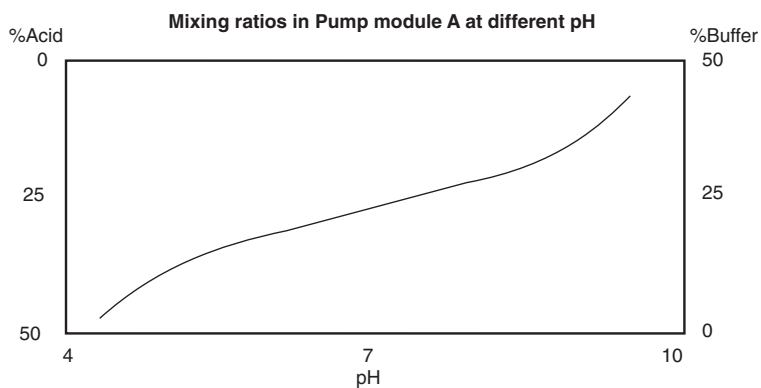
BufferPrep compensates for the pKa drift with changed ionic strength and temperature. A number of pre-defined recipes are available. New recipes can also be created, see Section 8.5.

The pH is generated from pump module A and the salt concentration from pump module B. The stock solutions containing the buffering component mix and the HCl or NaOH are connected to pump module A. The stock solutions containing salt and water are connected to pump module B. A switch valve on each pump module, together with the BufferPrep algorithms, generates the correct mixing ratios for the set pH. Each pump module delivers 50% of the set flow.



Pump module A

To achieve a set pH, the buffering stock solution and the acid/base stock solution are mixed via the switch valve in a ratio that depends on the buffering substance characteristics at the set pH. An example of the mixing ratios between the two stock solutions is shown below.

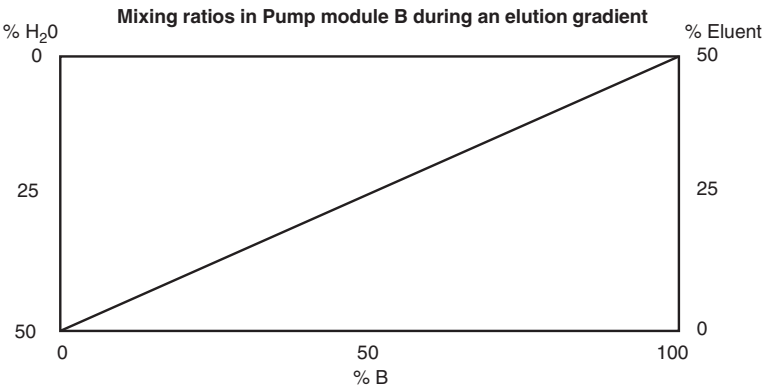


Note that the shape of the mixing ratio curve will differ for different buffering substances.

5 Optional functions
5.2 BufferPrep

Pump module B

To achieve the set eluent, the eluent stock solution and water are mixed via the switch valve in a ratio that depends on the programmed eluent concentration variation during elution.



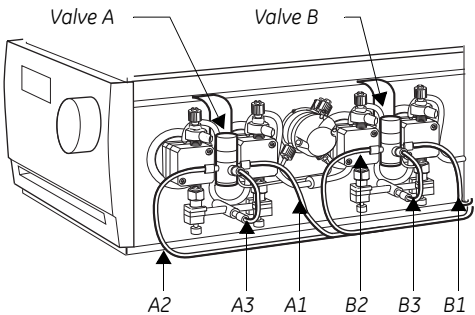
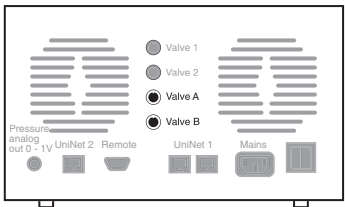
5.2.2 Installing the BufferPrep kit

The BufferPrep kit contains:

- two valves with valve holders
- tubing and connectors for ÄKTapurifier 10 (i.d. 1.6 mm, yellow ferrules)
- tubing and connectors for ÄKTapurifier 100 (i.d. 2.9 mm, blue ferrules).

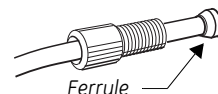
Installing the kit

- 1 Install the two switch valves between the respective pump heads by inserting the hooks into the channel in the pump.



- 2 Connect the valve A cable to socket **Valve A** at the rear of the pump and the valve B cable to **Valve B**.
- 3 Select the tubing and connectors intended for your system.

- 4 Connect the two short tubings in position A3 and B3 using the connectors and ferrules supplied (see the figure above). Note the direction of the ferrule!



- 5 Connect the four long tubings in position A1, A2, B1 and B2.

Setting up UNICORN for BufferPrep

To enable the BufferPrep function in UNICORN, the switch valves must be selected as components.

- 1 In **UNICORN Manager** select **Administration: System Setup**.
- 2 In the **System Setup** dialog select your system and click **Edit**.
- 3 In the **Edit System** dialog click **Component**.
- 4 In the **Component** dialog select **Switch valves**.
- 5 Click **OK** twice and finally **Close**.

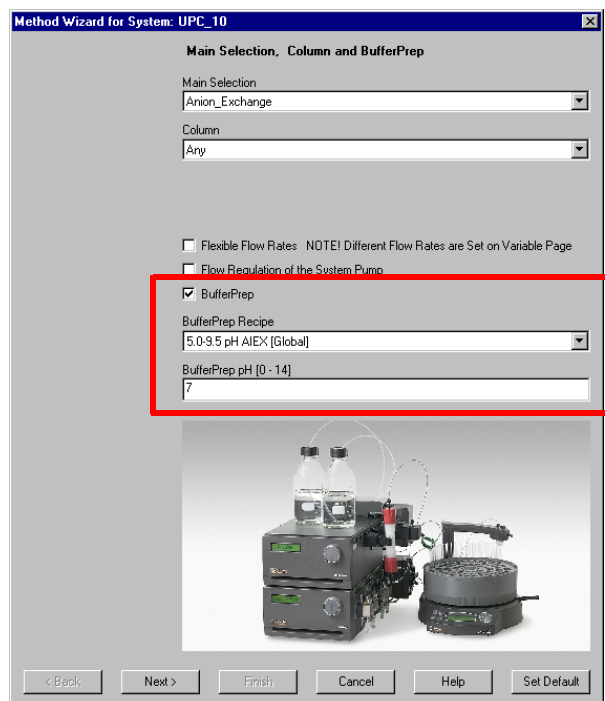
5.2.3 Strategy for using BufferPrep

BufferPrep should be used to quickly find the optimal pH for your protein purification procedure. Use it as follows:

- 1 Start with a recipe covering a broad pH range and scout for pH in steps of, for example, 0.5–1 pH units.
- 2 Select the pH that gives the best result.
- 3 Either continue with the broad pH range recipe or change to a single buffer recipe within the pH range that gave the best result.

5.2.4 Creating a method for pH scouting

- 1 Create a new method in the Method Wizard by selecting **BufferPrep** on the first page. Also select which recipe to use and the desired pH.



5.2.5 Preparing the system for a BufferPrep run

- 1 Calibrate the pH monitor. Refer to
ÄKTApurifier: Monitor pH/C-900 User Manual
ÄKTApurifier UPC: Monitor UPC-900 User Manual.
For high accuracy measurements, calibration should be performed with the pH electrode fitted in the flow cell at the flow rate to be used in the scouting run.
- 2 Prepare the required solutions. For details about the preparation and solutions, select **View:Run Setup** in **Method Editor** and click the **BufferPrep** tab.

Inlet A1: Buffer stock
Inlet A2: HCl or NaOH
Inlet B1: H₂O
Inlet B2: Eluent stock

- 3 Manually fill the inlet tubing with the stock solution by using the **PumpWash** instruction in UNICORN.
- 4 If equilibration is not programmed into the method, equilibrate the system manually without a column with the set pH before starting the run.
 - In **System Control** select **Manual:Other** and click **BufferPrep_Recipe**.
 - Select the same recipe as you have in your method. Click **Execute**.
 - Select **Pump** and select **BufferPrep_pH** and enter the same pH as set in the method. Click **Execute**.
 - Set a flow rate and click **Execute**.
- 5 Click the Variables page. Use the equilibration volumes (at minimum) listed in the table below to obtain a steady pH reading. The 0.6 ml mixer chamber is included in the systems at delivery, the other mixer chambers are optional.

System	Mixer chamber volume [ml]	Equilibration volume [ml]
ÄKTApurifier 10	0.6	9
	2	14
ÄKTApurifier 100	0.6	20
	2	30
	5	50
	12	100

- 6 When running pH scouting, the sample should, if possible, have a pH close to the highest pH in the scouting run for ALEX and close to the lowest pH for CIEX. The conductivity of the sample should be below 5 mS/cm.
- 7 Save the method.

5.2.6 Fine tuning

To obtain higher pH accuracy, the recipe can be fine tuned around a specific pH. When scouting over a broad pH range fine tuning is less beneficial. Run BufferPrep manually at 0 and 100%B as follows:

- 1 From the **System Control:Manual** menu select the recipe under **Other**, **BufferPrep_pH** and **Flow** under **Pump**. Start a run manually at 0% B. Make sure the switch valve is set correctly to the inlet A1.

5 Optional functions

5.2 BufferPrep

- 2 Check the pH reading when stable.
- 3 Change to 100%B by setting the **Gradient** instruction in **Manual:Pump** to 100%B for **Target** and 0 for **Length**. Click **Execute**.
- 4 Check the pH reading when stable at 100%B.
- 5 If the reading is acceptable at both 0 and 100%B, the run can be started.
- 6 If the pH reading is not acceptable, select **Correction Factors** in the **BufferPrep** page of the Method Editor. Enter the pH deviation at 0 and 100%B. (For example, if the pH has been set to 7 and the actual pH is 7.1 enter 0.1, or if the actual pH is 6.9, enter -0.1). If default correction factors exists, add the measured deviation to these factors.
- 7 Save the method.

Note: *The new correction factors will only apply while this method is used. When a new method is created and a recipe is selected, default correction factors will apply. To change default correction factors for a recipe see Section 8.5.4.*

5.2.7 Creating your own recipes

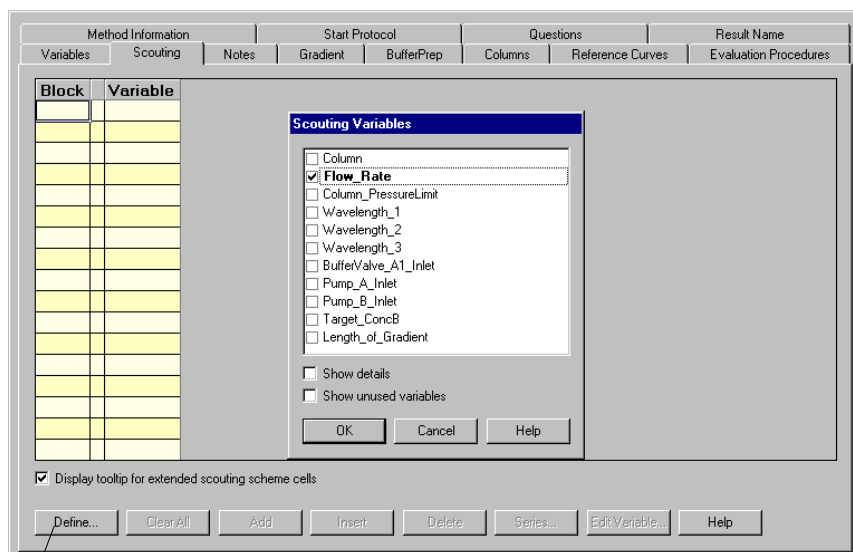
Refer to Section 8.5.1 "Creating your own recipes" for details on how to create recipes or edit existing recipes.

5.3 Scouting

Scouting allows any run parameters (e.g. flow rate) to be systematically varied automatically, in repeated runs.

Below is a description of how to perform a flow rate scouting.

- 1 Create a new method as described in Section 4.2 "Creating a method".
- 2 When the **Run Setup** window appears, click the **Scouting** tab.



Define other Scouting variables

- 3 A list of all the variables will appear. Select the variable **Flow_Rate** and any other variable you wish to alter (e.g. **Peak_Frac_Size**).
- 4 Click **OK**. The selected scouting variables will appear to the left with their default values inserted.
- 5 Values for variables selected for scouting are dimmed on the **Variables** page and cannot be changed there.
- 6 To change a variable value, position the cursor in the Run value field and double-click with the left mouse button. Type the new value.
- 7 To add a table column for the next run, click **Add**. A second column appears with the values from the previous run copied. Change the values as required.

Block	Variable	Run1
Flow_Rate	Flow_Rate (ml/min)	1.000

5 Optional functions

5.3 Scouting

- 8 If you want to insert a new run column after a specific column in the scouting scheme, position the cursor in the column and click **Insert**. A new column with identical values appears directly after the selected column.
- 9 Repeat step 6 until you have defined all the runs you require. If necessary, use the horizontal scroll bar to see more runs.
- 10 Click **Run1**, **Run2**, etc. at the top of the scheme with the right mouse button to toggle between **Run** and **Excluded** for the different runs. Those marked **Excluded** will not be run. A scouting scheme is now defined.
- 11 To save the scouting method, select **File:Save**.
- 12 When the method is started all the runs in the scheme will be performed automatically and the set flow for each run will be prepared automatically. Each run in the scouting scheme will generate a separate result file which are all stored in a special scouting directory.

5.4 Extra system pressure measurement

For low pressure columns, such as HiTrap and HiLoad, it is necessary to compensate for the pre-column pressure by measuring the pressure in the absence of the column. This is achieved by using the following method:

- 1 Set the injection valve (INV-907) in position **WASTE**.
- 2 Run the pump at the mandatory or intended flow rate.
- 3 Make a note of the back-pressure on the pump display or in the **Run Data** pane in UNICORN.
- 4 Add this value to the pressure limit value for the column (e.g. 0.5 MPa for HiLoad or 0.3 MPa for HiTrap).

The new total unit pressure value (measured pressure + max. column pressure) has to be introduced into the UNICORN column list and be defined as a personal column:

- 1 In the **Method Editor**, select **Edit:Column list** to open the **Column List** dialog. Clicking a column in the list will display its parameters in the field to the right of the box.
- 2 Click **Edit** to display the **Edit Column** dialog. In the **Parameter** column, enter in the field for **Max pressure** the new unit pressure limit, 0.5 MPa + the measured value.
- 3 Click **Save as** and enter a new name of your column. You can choose to save the column globally (i.e. available to all users) by checking the **Save as global** box. However, we recommend to clear the **Save as global** box in this situation. Click **OK**.

For further information, refer to *UNICORN User Manuals*.

5.5 Flow regulation

Flow regulation of the system pump is used for:

- Maintaining the requested pump flow rate.
- Making sure that the maximum pressure limit is not exceeded.

Flow regulation is useful in applications where high back-pressure can be expected, or when the back-pressure fluctuates, for example when using samples with high viscosity.

5.5.1 Regulation principle

There are two regulators involved in tuning the system pump. The first one is active as long as the pressure is below the set point. This regulator tunes the actual pump flow rate to the set flow rate. If the pressure exceeds the set point, another regulator decreases the flow rate in order to reduce the pressure. When the pressure falls below the set point, the first regulator again tunes the actual flow rate to the set flow rate, and so on.

The regulators use so-called PID flow regulation, where P, I and D are parameters that determine the tuning characteristics. The default PID values in UNICORN provide a robust flow regulation that suits most purposes. However, the parameters can be further optimized to suit a specific application (see 5.5.4 "Optimizing the PID parameters").

5.5.2 Including flow regulation in a method

Method Wizard

By default, flow regulation is not included when creating a method using the Method Wizard.

To include flow regulation:

- 1 Select **Flow Regulation of the System Pump** on the first page in the Method Wizard.
- 2 Enter suitable pressure and flow rate limits (see Section below).

Note: *Turning on the flow regulation means that the feedback control in UNICORN is activated. It will only be activated during sample application and wash-out of unbound sample.*

Turning on flow regulation in an existing method

- 1 Select **View: Run Setup** in **Method Editor**.
- 2 Click the **Variables** tab and select **Show details**.

- 3 In the **Aut_PressureFlow_Regulation** block, select **PressFlowControl** mode for the **System_Pump** variable.
- 4 Enter the pressure level in **System_PressLevel** (choose a value slightly below the column pressure limit) and the minimum allowed flow in **System_MinFlow**.

These parameter values as well as the PID parameter values can later be changed separately by selecting **System:Settings** in **System Control**.

Note: *If the flow rate falls below the System_MinFlow value, an Alarm will be issued and the system set to Pause. Therefore, we recommend using a Watch instruction (WatchPar_Flow) that is activated above this value. A suitable action is to continue to the next block.*

To prevent pressure peaks when continuing, use a lower flow rate in the block after the Watch instruction than used before the Watch instructions.

5.5.3 Manual flow regulation instructions

Flow regulation can also be used when running the system pump manually. The instructions are found in the **System Control** module by selecting **Manual:Pump** and are explained in Table 5-1.

Instruction	Parameter description
SystemPumpControlMode	To activate flow regulation, select PressFlowControl . PressLevel is the maximum allowed pressure. MinFlow is the minimum allowed flow rate
SystemPIDParameters	Flow_P , Flow_I and Flow_D are the parameters for tuning the actual flow rate to the set flow rate. Active below the PressLevel value. Press_P , Press_I and Press_D are the parameters for reducing the flow rate and thereby decreasing the pressure to below the pressure set point. Active above the PressLevel value.

Table 5-1. Flow regulation instructions

5.5.4 Optimizing the PID parameters

The two regulators for the system pump have separate PID parameters. The default PID parameters in UNICORN provide a robust flow regulation that is suitable for most purposes.

Note: *The PID parameters should only be changed by experienced users.*

The table below describes the three PID parameters.

Parameter	Description
P	The P parameter reduces the effect of an error but does not completely eliminate it. A simple P-regulator results in a stable stationary error between actual and requested flow.
I	The I parameter eliminates the stationary error, but results in a slight instability that might lead to oscillations in the actual flow. The I parameter can have values between 0 and infinity. Smaller values have a greater effect and a value of infinity has no effect. <i>Note:</i> The value infinity is set as 9999 in UNICORN.
D	In certain cases, the D parameter can reduce the oscillations introduced by a PI-regulator. D can have values between 0 and infinity, where larger values have a greater effect and a value of 0 has no effect. <i>Note:</i> Most often, a simple PI-regulator is preferable for control of flow rate, and ÅKTApurifier is therefore configured by default with the D parameter set to zero.

Table 5-2. PID parameters

Rules of thumb for optimizing the PID parameters:

- Use the default PID parameter values as start values.
To set the default values, select **System:Settings** in the **System Control** module and then **Specials**. Select the PID parameter to be restored and click **Set Selected Parameter To Strategy Default Value**.
- Keep the D parameter set to zero (i.e. use a simple PI-regulator).
- Start the pump before activating the regulator.
- Increasing P makes the regulator faster.
- Increasing I reduces oscillations.

6 Maintenance

6.1 Periodic maintenance

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

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

For details on how to perform the actions and for replacing components, please refer to the individual User Manuals and Instructions.

6.1.1 General precautions



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



WARNING! If there is a risk that large volumes of spilt liquid may penetrate the casing of the instruments and come into contact with the electrical components, immediately disconnect the mains cable and contact an authorized service technician.



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



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

CAUTION! When servicing and performing maintenance on the system, always place the buffer bottles on the laboratory bench to prevent draining.



6.1.2 Recycling and disposal

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

6.1.3 Maintenance schedule

Interval	Action
Every day	
System	- Inspect the complete system for eluent leakage. - The system can be left filled with buffer overnight. If you are not using the separation unit for a few days, wash the flow path with distilled water. Remove the column and the pH electrode (optional). Replace the column by a bypass capillary and fit the pH dummy electrode (if applicable). Then wash the system with 20% ethanol and store it in 20% ethanol. Make sure that all tubing and all flow paths used are rinsed.
Pump P-900	- Check for leakage. If there are signs of liquid leaking between the pump head and the housing side panel or increased or decreased volume of rinsing solution, replace the piston seals. Refer to Chapter 4 in <i>Pump P-900 User Manual</i>. - When changing eluent, it is important to remove trapped air. Purge the pump according to Chapter 2 in the <i>Pump P-900 User Manual</i>. If there is still air in the inlet tubing, stop and purge the pump according to Chapter 5 in the <i>Pump P-900 User Manual</i>. Note: If air is allowed to enter the columns, their performance can be adversely affected or destroyed.
pH electrode (optional)	Calibrate the pH electrode (optional). Refer to Section 6.4 in <i>"Calibration procedures"</i>.
Every week	
Inlet filters	Check the inlet filters visually and replace them if necessary.
Pump rinsing solution	- Change rinsing solution. Always use 20% ethanol as rinsing solution. - If the volume of rinsing solution in the storage bottle has increased, it can be an indication of internal pump leakage. Replace the piston seals according to Chapter 4 in <i>Pump P-900 User Manual</i>. - If the volume of rinsing solution in the storage bottle has decreased significantly, check if the rinsing system connectors are mounted properly. - If the rinsing system connectors are not leaking, the rinsing membranes or piston seals may be leaking. Replace the membranes and piston seals according to Chapter 4 in <i>Pump P-900 User Manual</i>.
Cleaning	Clean the system, see 6.2 <i>Cleaning the system surface</i>.
Fans	Check that all fans are running.

Interval	Action
Every month	
Flow restrictor	- Check that the flow restrictor generates the following back-pressures: FR-904..... 0.4 ± 0.05 MPa FR-902..... 0.2 ± 0.05 MPa Checking the back-pressure: 1 Disconnect the flow restrictor. 2 Connect a piece of tubing (approx. 1 m, i.d. 1.0 mm) to a free port in injection valve V2. Set the valve manually to this port. Put the open end in a waste container. 3 Run the pump at 10 ml/min with water. <i>Note the back-pressure (Bp1) on the pump display, or in the Run Data pane.</i> 4 Connect flow restrictor to the open end of the tubing (observe the IN marking). Put the flow restrictor in the waste container. 5 Run the pump at 10 ml/min with water. <i>Note the back-pressure (Bp2) on the pump display, or in the Run Data pane.</i> 6 Calculate the back pressure (Bp2-Bp1) of the flow restrictor. If is not within the limit, replace or contact GE Healthcare.
Every 3 months	
Monitor UV-900	Check the cell and optical fibre connectors according to Chapter 4 in <i>Monitor UV-900 User Manual</i>.
Monitor UPC-900	Check the instrument according to Chapter 4 in <i>Monitor UPC-900 User Manual</i>.
Every 6 months	
Monitor UV-900	Check the instrument according to Chapter 4 in <i>Monitor UV-900 User Manual</i>.
Monitor UPC-900	Clean the flow cell according to Chapter 4 in <i>Monitor UPC-900 User Manual</i>.
Mixer M-925	Check that the mixer chamber is clean and without damage. Check the tubing connectors. Replace if required.
Yearly	
Valve INV-907	Check for external and/or internal leakage. Replace the distribution plate yearly or when required. Refer to Chapter 4 in the valve's <i>Instructions</i>.

6 Maintenance

6.2 Cleaning the system surface

Interval	Action
Every 2 years	
Mixer M-925	• Replace the complete mixer chamber.
When required	
Pump P-900	• Replace piston seals. Refer to Chapter 4 in the <i>Pump P-900 User Manual</i>. • Replace piston. Refer to Chapter 4 in the <i>Pump P-900 User Manual</i>. • Clean or replace the inlet our outlet check valves. Refer to the Chapter 4 in <i>Pump P-900 User Manual</i>. • Calibrate the pressure sensor. Refer to Section 6.4 in "<i>Calibration procedures</i>".
Monitor UPC-900	• Clean the flow cell according to Chapter 4 in <i>Monitor UPC-900 User Manual</i>. • Calibrate the temperature sensor when changing conductivity cell. Refer to Section 6.4 in "<i>Calibration procedures</i>". • Enter a new cell constant when changing conductivity cell. Refer to Section 6.4 in "<i>Calibration procedures</i>". • Calibrate the cell constant if specific conductivity with high accuracy is measured. Refer to Section 6.4 in "<i>Calibration procedures</i>".
On-line filter	• Replace the on-line filter.

6.2 Cleaning the system surface

The system surface should be kept dry and clean to maintain proper safety and function of the system. Chemical stains and dust should be removed.

To clean the system surface:

- 1 Wipe the surface regularly with a damp cloth.
- 2 To remove stains, spray the system with 20% ethanol and wipe off the excess.

Note: Be careful not to bend any tubing.

- 3 Let the system dry completely before using it.

6.3 Cleaning the system flow path

The procedures below describe how to clean the system flow path.

To bypass the column, use a capillary tubing supplied with your system. If the column is to be left in the flow path, make sure that the maximum allowed flow and pressure for the column are not exceeded.

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

6.3.1 At the end of the day

If the system will be used with the same buffers the next day, let the system run isocratic at a low flow rate (approximately 0.02 ml/min).

If the system will be used with other buffers the next day, rinse the pump and the system with distilled water using the PumpWash instruction as follows:

- 1 Submerge the inlet tubings in distilled water.
- 2 Run the instruction

ÄKTApurifier	ÄKTApurifier UPC
<i>PumpWashPurifier</i>	<i>PumpWashBasic</i>

6.3.2 Leaving the system for a few days

Perform a *PumpWashPurifier/PumpWashBasic* with distilled water. Repeat with a bacteriostatic solution, i.e. 20% ethanol.

6.3.3 Monthly cleaning

Clean the system every month, or when problems such as ghost peaks occur. Also use this procedure to clean the flow path before service or maintenance.

- 1 Disconnect the column and replace it with bypass tubing.
- 2 If using a pH electrode, replace it with a dummy electrode.
- 3 Place all the inlet tubings in 1 M NaOH.
- 4 Manually perform *PumpWashPurifier/PumpWashBasic* for both inlet tubings.
- 5 Flush the whole system with 1 M NaOH for 20 minutes (1 ml/min).
- 6 Immediately repeat steps 3 and 4 with distilled water to rinse the system of NaOH.

6.3.4 Cleaning-in-place

After repeated separation cycles, contaminating material might progressively build up in the system and on the column. This material may not have been removed by the cleaning step described previously. The nature and degree of contamination depends on the sample and the chromatographic conditions employed. These should be considered when designing a cleaning process.

6.4 Calibration procedures

The table below lists the type and frequency of calibrations that can be done on ÄKTApurifier. Refer to the *UNICORN User Manuals* and to the individual component User Manuals and Instructions for descriptions of how to perform these calibrations.

The calibration is performed in UNICORN by selecting **System:Calibrate** in **System Control**.

Component	How often
Conductivity flow cell	Only necessary if specific conductivity with high accuracy is measured (Cond_Calib). Must be done when changing the conductivity cell (Temp). Must be done when changing the conductivity cell (Cond_Cell).
Cell constant	
Temperature	
Entering a new cell constant	Must be done when changing the conductivity cell (Cond_Cell).
Pressure reading	Once a year or when required.
pH electrode (optional)	Every day.

6.5 Moving the system

Two persons are required to lift the system

CAUTION! Never lift the system by the valves.

Before moving the system, disconnect all cables and tubing connected to peripheral components and liquid containers. Remove all items from the top of the system.

Grasp the system firmly by placing the fingers in the gap between the swivel platform and the base of the main unit and lift.

7 Troubleshooting

7.1 *Faults and actions*

This Section lists faults observed with specific monitor curves and specific modules. The faults and actions are listed as follows:

Type	Page
UV curve	108
Pressure curve	109
Conductivity curve	109
Pump P-900	111
Monitor UV-900	112
Monitor UPC-900	112
Mixer M-925	112
Valve INV-907	113
BufferPrep	113

If the suggested actions do not correct the fault, contact the local GE Healthcare service representative.

7.2 UV curve

Error symptom	Possible cause/Action
Ghost peaks	• Check that there is no air in the eluent. Degas if necessary. • Clean the system in accordance with Chapter 4. • Clean the column in accordance with the column instructions. • Check that the mixer is functioning properly and that the correct chamber volume is being used.
Noisy UV-signal, signal drift or instability	• The buffer may be impure. Check if the signal is still noisy with water. • There may be air in the flow cell. Check that the flow restrictor generates the following back-pressure: FR-904 0.4 ± 0.05 MPa FR-902 0.2 ± 0.05 MPa Check the back-pressure as follows: 1 Disconnect the flow restrictor. 2 Connect a piece of tubing (approx. 1 m, i.d. 1.0 mm) to a free port in injection valve V2. Set the valve manually to this port. Put the open end in the waste container. 3 Run the pump manually at 10 ml/min with water. Note the back-pressure (Bp1) on the pump display or in the Run Data pane. 4 Connect the flow restrictor to the open end of the capillary (note the IN marking). Put the flow restrictor in the waste container. 5 Run the pump at 10 ml/min with water. Note the back-pressure (Bp2) on the pump display or in the Run Data pane. 6 Calculate the back-pressure (Bp2-Bp1) of the flow restrictor. If it is not within limit, replace or contact GE Healthcare. • Degas the buffer before use. • Clean the UV flow cell, see Chapter 4 of <i>Monitor UPC-900 User Manual</i> or <i>UV-900 User Manual</i>.

7.3 Pressure curve

Error symptom	Possible cause/Action
Erratic flow, noisy baseline signal, irregular pressure trace Possible causes are:	
Air bubbles passing through or trapped in the pump	• Check that there is sufficient eluent present in the reservoirs. • Check all connections for leaks. • Follow the instructions in Chapter 5 of <i>Pump P-900 User Manual</i>.
Inlet or outlet check valves not functioning correctly	• Clean the valves according to Chapter 4 of <i>Pump P-900 User Manual</i>.
Piston seal leaking	• Replace the piston seal according to the instructions in Chapter 4 of <i>Pump P-900 User Manual</i>.
Blockage or partial blockage of flow path	• Flush through to clear the blockage. • If necessary, replace tubing. • Check the inlet tubing filter. It can become clogged if unfiltered buffers or samples are applied. • See instructions for flushing through at the end of the run in Chapter 3 of <i>Pump P-900 User Manual</i>.

7.4 Conductivity curve

Error symptom	Possible cause/Action
Conductivity measurement with the same buffer appears to change over time	• Clean the flow cell according to <i>Monitor UPC-900 User Manual</i> or <i>Monitor pH/C-900 User Manual</i>. • The ambient temperature might have decreased. Use a temperature compensation factor, see Reference information in <i>Monitor UPC-900 User Manual</i> or <i>Monitor pH/C-900 User Manual</i>.
Ghost peaks appear in the gradient profile	• A charged sample has been detected (e.g. protein). • Air bubbles are passing through the flow cell. Check for loose tubing connections. Use a flow restrictor.

Error symptom	Possible cause/Action
Baseline drift or noisy signal	• There may be air in the flow cell. • Check for leaking tubing connections. • Check that the column is equilibrated. If necessary, clean the column. • Check the operation of the mixer and the system pump. The mixer function is checked by placing a stirrer bar on top of the mixer housing. The stirrer bar should rotate when the system is in Run mode. The pump and mixer functions can also be checked by running the installation test. • Clean the flow cell according to <i>Monitor UPC-900 User Manual</i>.
Waves on the gradient	• Check that the pump and the valves are operating properly and are programmed correctly. • Change to a larger mixing volume if necessary. • Check the operation of the mixer. The mixer function is checked by placing a stirrer bar on top of the mixer housing. The stirrer bar should rotate when the system is in Run mode. The mixer function can also be checked by running the installation test.
Non-linear gradients or slow response to %B changes	• Check that the tubing has been washed properly and that the pump is operating. • If a larger mixer than the standard 0.6 ml chamber is used, try changing to a smaller mixer volume
Incorrect or unstable reading	• Check that the conductivity flow cell cable is connected properly to the rear of the monitor. • Check that the pump operates correctly. • If temperature compensation is used, check that the temperature sensor is calibrated and that the correct temperature compensation factor is used. • Check that the column is equilibrated. If necessary, clean the column. • Check the operation of the mixer. The mixer function is checked by placing a stirrer bar on top of the mixer housing. The stirrer bar should rotate when the system is in RUN mode. The mixer function can also be checked by running the installation test.

7.5 Pump P-900

Error symptom	Possible cause/Action
No text on the front display	Check that the mains cable is connected and the power switch is in ON-position I.
Liquid leaking between the pump head and the side panel	- Piston seal or rinsing membrane incorrectly fitted or worn. - Replace or re-install the seal or membrane. - Run-in carefully, see Chapter 4 of <i>Pump P-900 User Manual</i>.
Low eluent flow and noise as the piston moves	- Disassemble pump cylinder and examine the piston spring according to Chapter 4 of <i>Pump P-900 User Manual</i>. Replace if necessary. - If the spring is corroded, check piston seal and rinse membrane. Make sure that piston rinsing system is always used when working with aqueous buffers with high salt concentration. - Check the piston for damage. If damaged, replace the piston according to Chapter 4 of <i>Pump P-900 User Manual</i>. - Remember to replace the piston seal with new parts.
Leaking connection and/or crystallized material around a connector	- Unscrew the connector and check if it is worn or incorrectly fitted. If so, replace the connector. - Tighten the connector properly with your fingers.
Irregular pump pressure	To check the pump function, record the pressure or check it in UNICORN. By observing the piston status indicator in the Check menu together with the pressure trace, the pump cylinder that is functioning abnormally can be identified, see Reference information in the <i>Pump P-900 User Manual</i>. There can be several causes of an abnormal pressure recording, for example: - air trapped in the pump cylinders - partially blocked solvent filters - leaking connections - piston seal leakage - pump valve malfunction - piston damaged. Some examples of normal and abnormal pressure traces together with comments are shown in Chapter 5 of <i>Pump P-900 User Manual</i>.

7.6 Monitor UV-900

Error symptom	Possible cause/Action
No text on the front display	Check that the mains cable is connected and the power switch is in ON-position I.
Unstable UV baseline	Try using a larger mixer chamber instead of the standard 0.6 ml mixer chamber.

7.7 Monitor UPC-900

Error symptom	Possible cause/Action
No text on the front display	Check that the mains cable is connected and the power switch is in ON-position I.
Unstable UV baseline	Try using a larger mixer chamber instead of the standard 0.6 ml mixer chamber.
Absolute conductivity value wrong	- Turn the flow cell so that the end with the screws is facing the flow restrictor FR-902. - Recalibrate the conductivity cell. - Calibration solution, 1.00 M NaCl, not prepared correctly. Prepare a new calibration solution and recalibrate the conductivity cell.
Unstable conductivity curve	Try using a larger mixer chamber instead of the standard 0.6 ml mixer chamber.

7.8 Mixer M-925

Error symptom	Possible cause/Action
Leakage	- Check the tubing connections. Retighten or replace if necessary. - Check the mixer chamber. Replace if liquid has penetrated the mixer chamber walls and sealings.
Function test	- Test the mixer function by placing a stirrer bar on top of the mixer housing. The stirrer bar should rotate when the system is in Run mode. - The mixer function can also be checked by running the installation test.

7.9 Valve INV-907

Error symptom	Possible cause/Action
The valve is not switching	- Check the connection to the pump. The valve should be connected to the UniNet-2 socket. - Check the ID-switch on the valve. The ID number should correspond to the number set in UNICORN. - Check the UniNet cable and replace if required.
The valve is switching to wrong position	- The valve parts might have been incorrectly reassembled after replacement. - Check that the distribution plate marking 3 is horizontal. Refer to the relevant valve Instructions.
External leakage	Check the tubing connections. Tighten or replace if required.
Internal leakage	- Internal leakage can be detected at the small hole on the underside of the valve body. - Internal parts may be worn. Change channel plate and distribution plate. Refer to the relevant valve Instructions.
High back-pressure	- Perform cleaning-in-place according. Refer to the relevant valve Instructions. - Change channel plate and distribution plate. Refer to the relevant valve Instructions.

7.10 BufferPrep

Error symptom	Possible cause/Action
Unstable pH	- Check the system by running the method without a column or sample. - If still unstable: - Check that the correct mixer volume is used. - Check that the pump is operating correctly. - Check for a dirty or broken pH electrode. - Check that the valves are operating correctly. - Check that the pH is not set too far from the pKa of the buffer components. - If the pH is stable when running without column or sample, check if there is too low concentration of buffer components causing low buffering capacity.

Error symptom	Possible cause/Action
Incorrect pH	- Collect some eluent. Check the pH electrode calibration by measuring the pH of the collected eluent on a separate pH monitor. - When using standard recipes, use the following mixture to measure the pH separately. Mix, using a 25 ml pipette, 25 ml of the buffer stock solution, 25 ml of the acid and 50 ml of water. Check the pH with a known good quality pH electrode. The reading should be close to: 5.0 - 9.5 pH AIEX pH 6.5 6.0 - 9.0 pH AIEX pH 6.1 3.0 - 7.5 pH CIEX pH 3.7 If not, prepare new buffers. - If mixture pH is correct: Check for a dirty or broken pH electrode. Check the valves are operating correctly. - If a non-standard recipe is used: Check that the pH is not set too far from the pKa of the buffer components. Check if there is too low concentration of buffer components causing low buffering capacity. - Check that the correct pKa values are used and that all pKa values have been entered.
Drifting pH	Set the injection valve to position WASTE. Pump through at least 30 ml of buffer at the set pH to stabilize the pH. Switch the valve back to position LOAD. Equilibrate the column.

8 Reference information

8.1 System description

Note: Two versions are available; ÄKTApurifier with monitor UV-900 for multiple wavelengths detection and ÄKTApurifier UPC with monitor UPC-900 for combined measurement of UV absorption, pH and conductivity. When instructions or specifications are similar for both the models only ÄKTApurifier is mentioned.

8.1.1 The systems

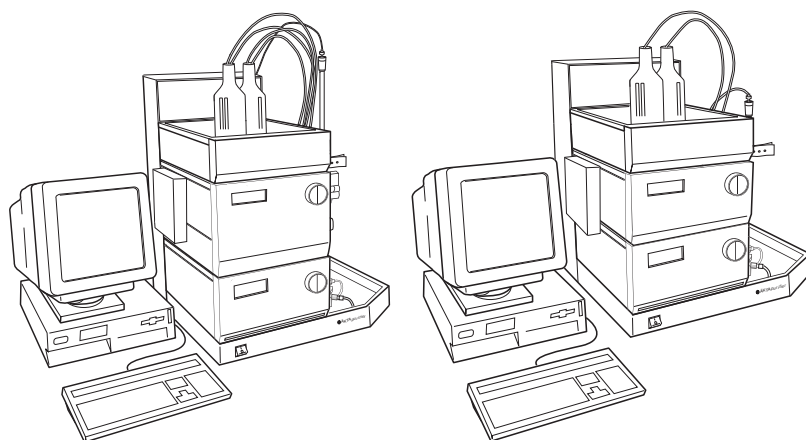


Fig 8-1. ÄKTApurifier to the left and ÄKTApurifier UPC to the right

ÄKTApurifier consists of a compact separation unit and a personal computer running UNICORN software version 5.10 or higher.

The individual modules and components are briefly described in this chapter. Detailed information on the components can be found in their respective User Manuals and Instructions. UNICORN is described in the separate UNICORN user documentation supplied.

Communication between the computer and the various components of ÄKTApurifier is achieved via high speed data network connections (UniNet-1 and UniNet-2).

All of the fluid handling components of ÄKTApurifier are mounted on the same side of the main components. This allows easy access to all components, tubing and other fluid components located on Pump P-900 and Monitor UV-900/Monitor UPC-900.

8.1.2 Component locations

The illustration below shows the location of the components of the separation unit

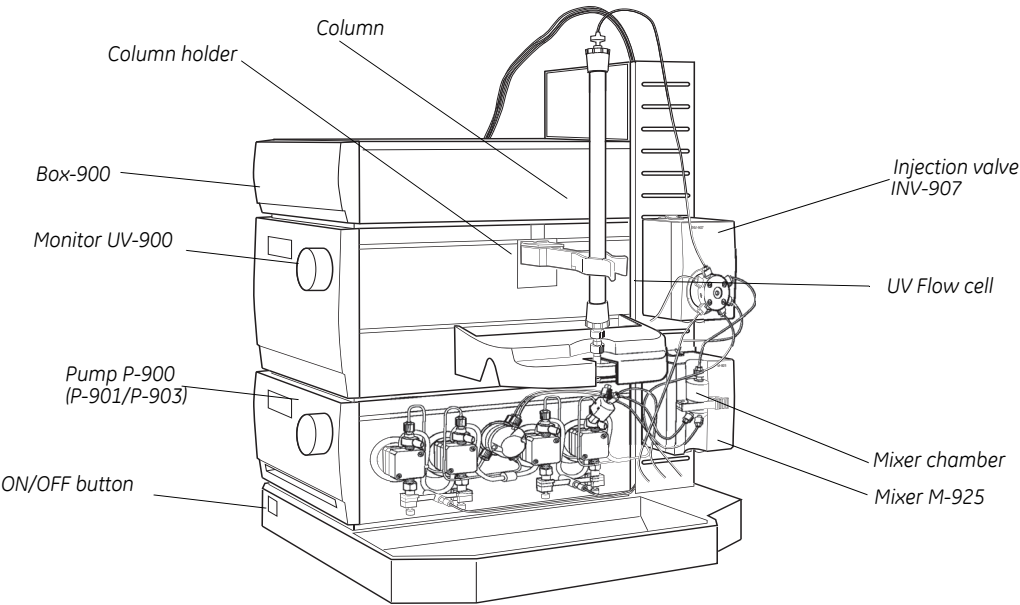


Fig 8-2. ÄKTApurifier component locations

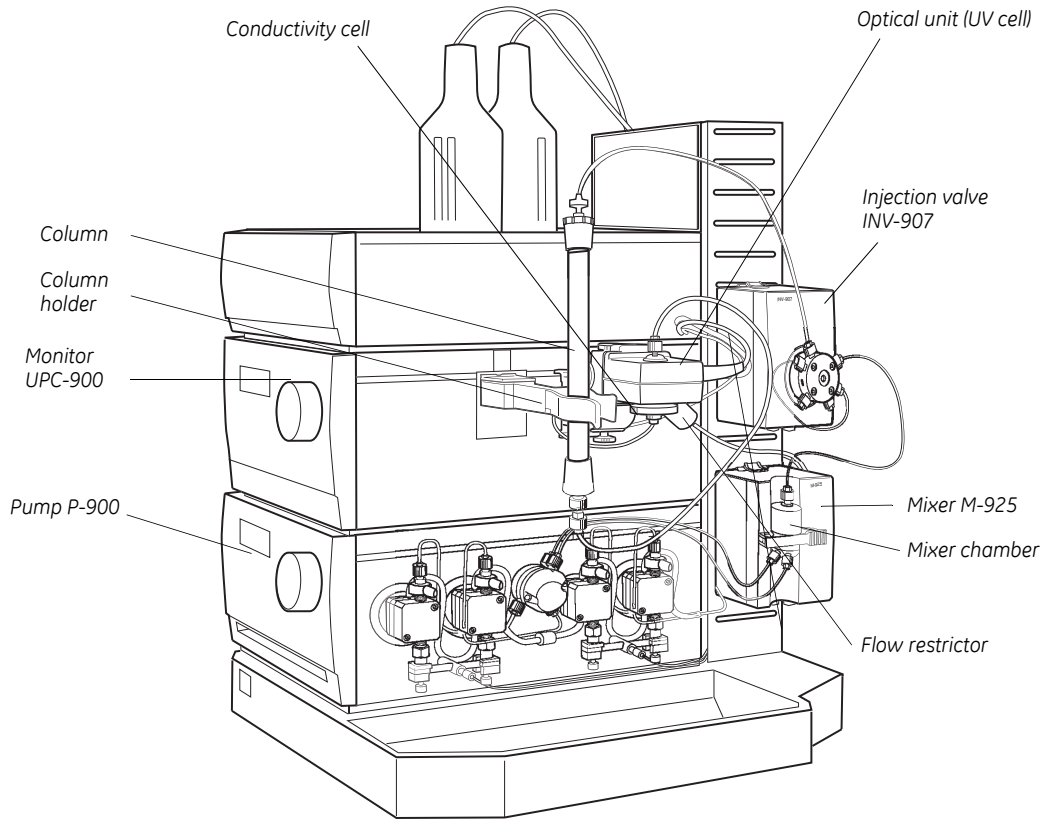
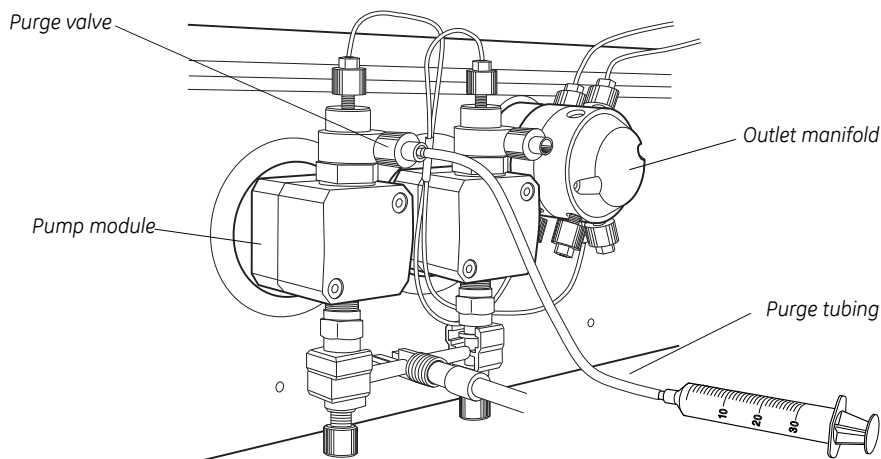


Fig 8-3. ÄKTApurifier UPC component locations

8.1.3 Purging parts

The figure below shows the purging parts for Pump P-900.



8.1.4 Electrical connections



WARNING! Never attempt to remove the mains fuse while mains voltage is applied to the system. For continued protection against risk of fire, replace only with a fuse of the specified type and rating. Refer to the "Technical specifications" for fuse data.

All electrical connections for ÄKTApurifier are located at the rear of the system. Only one mains input is required for the complete system. The supply voltage for the components in the system and the fraction collector is distributed from the rear of the system.

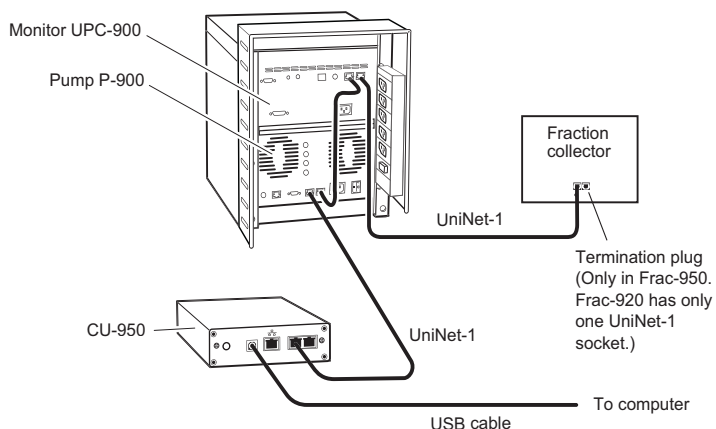
UniNet-1

Fig 8-4. UniNet-1 connections on ÄKTApurifier UPC. The connections on ÄKTApurifier is similar.

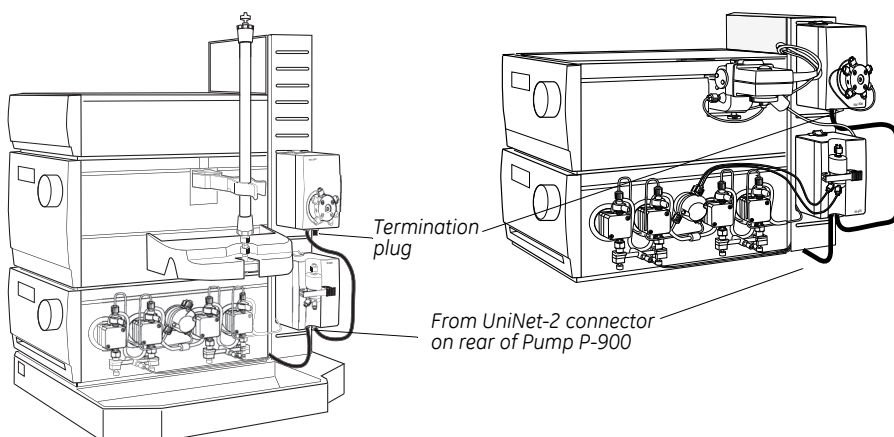
The computer communicates with the CU-950 controller unit through a USB cable.

The UniNet-1 data communication cable is routed from the CU-950 to the rear of Pump P-900. UniNet-1 is terminated at Monitor UV-900/Monitor UPC-900 with a termination plug, or if used, at the fraction collector.

UniNet-2

ÄKTApurifier

ÄKTApurifier UPC



The UniNet-2 data communication cable which supplies the valve and mixer with control signals and power is routed from the rear of Pump P-900, and links components on the right side of the system. The cable is terminated at the injection valve, V1, with a termination plug.

Mains cables

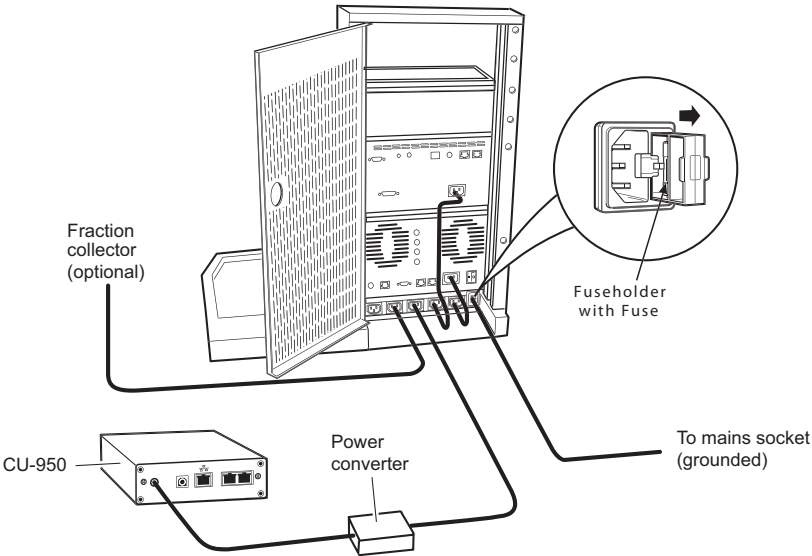


Fig 8-5. Mains cables in ÄKTApurifier system. Mains cables in ÄKTApurifier UPC is similar.



WARNING! Never attempt to remove the mains fuse while mains voltage is applied to the system. For continued protection against risk of fire, replace only with fuse of the specified type and rating.

Please, refer to the Technical specifications for fuse data.

Only one mains inlet is required. The modules, CU-950 and fraction collector (optional) are connected to the mains supply socket at the rear of the system.



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

The mains input fuse is located to the right of the mains input. To open the fuse holder, after first removing the power to the system, use a small bladed screwdriver to lever the holder outwards.

8.1.5 Fluid handling path

The following illustration shows the positions of the components and tubing in ÄKTApurifier. Refer to the flow diagram for their positions in the fluid handling path.

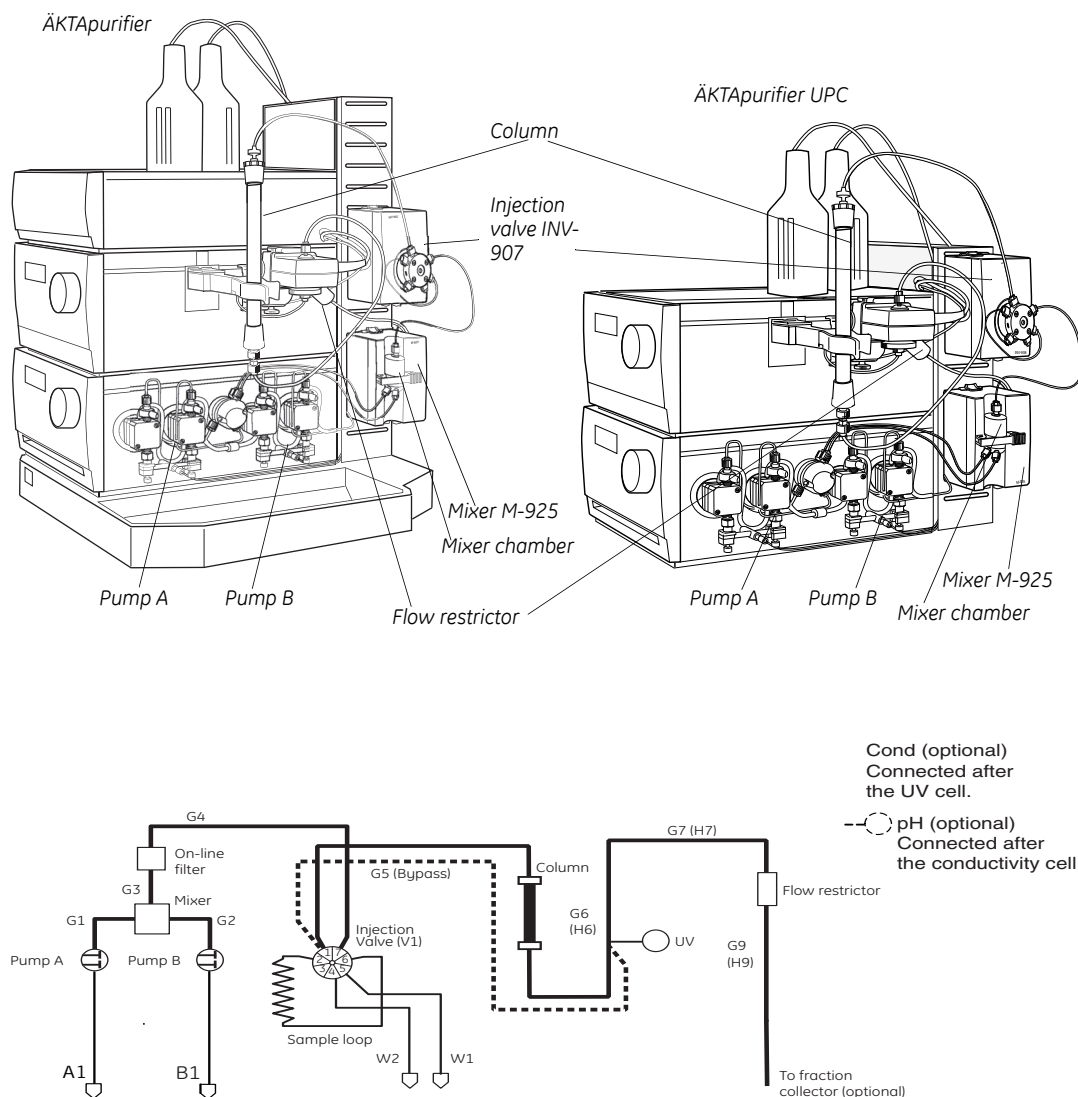


Fig 8-6. ÄKTApurifier flow diagram

8 Reference information

8.1 System description

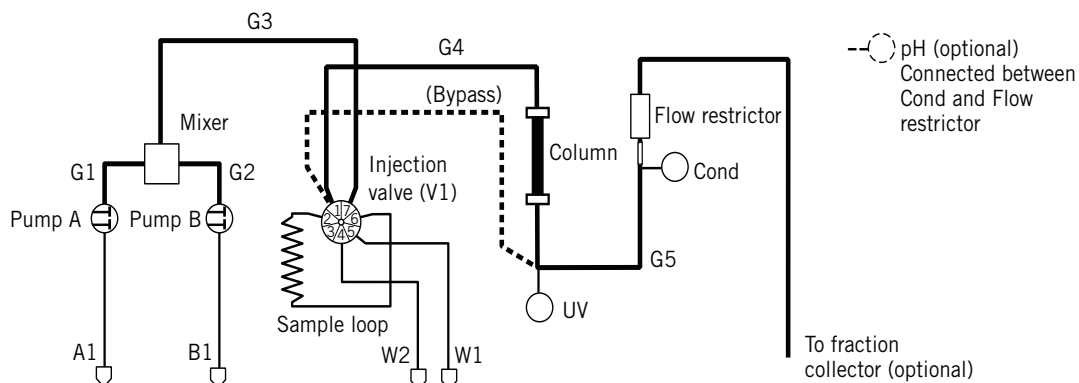


Fig 8-7. ÄKTApurifier UPC flow diagram

The G5 capillary is mounted from the factory, replacing the column. It is used as a bypass when the column is removed from the separation unit, e.g. at system wash.

Please note that there are two flow restrictors available for the system

- **FR-902**
Mounted from the factory, and pre-adjusted to give a back pressure of 0.2 MPa over the nominal flow range. This flow restrictor is used when a pH cell (optional) is fitted in the system, or when a low pressure column is used.
- **FR-904**
Pre-adjusted to give a back pressure of 0.4 MPa over the nominal flow range. This flow restrictor should be used when working with organic solvents, such as in RPC applications, to avoid air bubble formation in the flow cell.

The table below shows the tubing dimensions installed at the factory and their location in the system.

ÄKTApurifier 10

The table below shows the tubing available for ÄKTApurifier 10, and where they are located in the system. At delivery i.d. 0.5 mm PEEK tubing is installed from the pump to the UV-cell, and i.d. 0.25 mm Tefzel™ tubing from the outlet of the UV-cell to the fraction collector (if applicable). The column is installed either by using the tubing supplied with the column or using pieces of 0.25 mm PEEK tubing cut by the user to suitable length (0.25 mm PEEK tubing, blue, is supplied with the ÄKTApurifier 10 system).

Tubing design	Length	Tubing i.d.	Tubing o.d.	Material	Color	Volume of 10 cm
INV-907-column		0.25 mm	1/16"	PEEK	Blue	4.9 µl
G5	40 cm	0.50 mm	1/16"	PEEK	Orange	19.6 µl
UV-Frac		0.25 mm	1/16"	PEEK	Blue	4.9 µl
G1	33 cm	0.50 mm	1/16"	PEEK	Orange	19.6 µl
G2	33 cm	0.50 mm	1/16"	PEEK	Orange	19.6 µl
G3	20 cm	0.50 mm	1/16"	PEEK	Orange	19.6 µl
G4	18 cm	0.50 mm	1/16"	PEEK	Orange	19.6 µl
H6	16 cm	0.50 mm	1/16"	Tefzel	Clear	19.6 µl
H7	8 cm	0.50 mm	1/16"	Tefzel	Clear	19.6 µl
H8	14 cm	0.50 mm	1/16"	Tefzel	Clear	19.6 µl
H9	60 cm	0.50 mm	1/16"	Tefzel	Clear	19.6 µl
W1-W3	100 cm	0.75 mm	1/16"	Tefzel	Clear	44.2 µl
A1, B2	170 cm	1.6 mm	1/8"	Teflon	Clear	201.1 µl
A2	190 cm	1.6 mm	1/8"	Teflon	Clear	201.1 µl
B1	150 cm	1.6 mm	1/8"	Teflon	Clear	201.1 µl
A3, B3	15 cm	1.6 mm	1/8"	Teflon	Clear	201.1 µl

8 Reference information

8.1 System description

ÄKTApurifier 100

In ÄKTApurifier 100 i.d. 0.75 mm PEEK tubing (green) is mounted from the factory, from pump P-901 to the outlet. The table below shows the tubing available for ÄKTApurifier 100, and where it is located in the system.

Tubing design	Length	Tubing i.d.	Tubing o.d.	Material	Color	Volume of 10 cm
G1, G2	33 cm	0.75 mm	1/16"	PEEK	Green	44.2 µl
G3	9 cm	0.75 mm	1/16"	PEEK	Green	44.2 µl
G4	16 cm	0.75 mm	1/16"	PEEK	Green	44.2 µl
G5	30 cm	0.75 mm	1/16"	PEEK	Green	44.2 µl
G6 (H6)	16 cm	0.75 mm	1/16"	PEEK	Green	44.2 µl
G7 (H7)	8 cm	0.75 mm	1/16"	PEEK	Green	44.2 µl
G8 (H8)	14 cm	0.75 mm	1/16"	PEEK	Green	44.2 µl
G9 (H9)	60 cm	0.75 mm	1/16"	PEEK	Green	44.2 µl
W1-W3	100 cm	0.75 mm	1/16"	Tefzel	Clear	44.2 µl
W1-W3	100 cm	1.00mm	1/16"	Tefzel	Clear	78.5 µl
A1, B2	170 cm	2.9 mm	3/16"	Teflon	Clear	660 µl
A2	190 cm	2.9 mm	3/16"	Teflon	Clear	660 µl
B1	150 cm	2.9 mm	3/16"	Teflon	Clear	660 µl
A3, B3	15 cm	2.9 mm	3/16"	Teflon	Clear	660 µl

Changing tubing kits – ÄKTApurifier 10

Two different tubing kits, with different internal diameters, are available for use from the injection valve to the outlet valve (or the fraction collector if applicable) in ÄKTApurifier 10:

- Tubing kit 0.25 (G6–G9). PEEK tubing, blue, marked G. Installed from factory at delivery. Used for most columns.
- Tubing kit 0.50 (H6–H9). Tefzel tubing, clear, marked H. For low-pressure columns, at high flow rates.

Tubing kit 0.25 and Tubing kit 0.50

The system is delivered with Tubing kit 0.25 installed. Tubing kit 0.50 should be fitted when columns with a low max pressure are used at high flow rates.

When changing from/to Tubing kit 0.25 to/from Tubing kit 0.50, change the tubings designated G6–G9 with H6–H9. Refer to the flow diagram in section 8.1.5 for their location in the fluid handling path

Tubing¹	Length (mm)	i.d. (mm)	Location
G1	330	0.50	Pump P-900A (inner) to mixer
G2	330	0.50	Pump P-900B (outer) to mixer
G3	200	0.50	Mixer to on-line filter
G4	180	0.50	On-line filter to injection valve pos. 7
G5	400	0.50	Bypass: injection valve (pos.1) to UV cell
<i>Tubing kit 0.25 mm</i>			
G6	160	0.25	UV cell to conductivity cell
G7	80	0.25	Conductivity cell to flow restrictor
G8	140	0.25	Flow restrictor to outlet valve (centre port)
G9	600	0.25	Outlet valve (port 2) to fraction collector (optional)
F3	1000	0.50	Outlet valve (port 3)
A3	150	1.6	SV-903A (IN) to pump P-900A
B3	150	1.6	SV-903B (IN) to pump P-900B
A1	1700	1.6	Buffer vessel A1 to SV-903A (NO)
A2	1900	1.6	Buffer vessel A2 to SV-903A (NC)
B1	1500	1.6	Buffer vessel B1 to SV-903B (NO)
B2	1700	1.6	Buffer vessel B2 to SV-903B (NC)
W1	1000	0.75	Injection valve (port 4) to waste
W2	1000	0.75	Injection valve (port 5) to waste
W3	1000	0.75	Outlet valve (port 1) to waste
<i>Tubing kit 0.50 mm</i>			
H6	160	0.50	UV cell to conductivity cell
H7	80	0.50	Conductivity cell to flow restrictor
H8	140	0.50	Flow restrictor to outlet valve (centre port)
H9	600	0.50	Outlet valve (port 2) to fraction collector (optional)

1. G = General tubing, H = High flow tubing, W = Waste tubing, A = Inlet tubing A, B = Inlet tubing B, F = Fraction tubing.

8 Reference information

8.1 System description

ÄKTApurifier UPC 10

The column is installed either using the tubing supplied with the column, or with PEEK tubing cut by the user to suitable lengths (i.d. 0.5 mm orange PEEK tubing is supplied with ÄKTApurifier UPC 10). A male/male union connects the conductivity cell to the flow restrictor.

Tubing design	Length	Tubing i.d.	Tubing o.d.	Material	Color	Volume of 10 cm
A1, B1	170 cm	1.6 mm	1/8"	Teflon	Clear	201.1 µl
G1, G2	33 cm	0.50 mm	1/16"	PEEK	Orange	19.6 µl
G3	25 cm	0.50 mm	1/16"	PEEK	Orange	19.6 µl
G4	30 cm	0.25 mm	1/16"	PEEK	Blue	4.9 µl
G5	17 cm	0.25 mm	1/16"	PEEK	Blue	4.9 µl
W1–W3	100 cm	0.75 mm	1/16"	Tefzel	Clear	44.2 µl

ÄKTApurifier UPC 100

The column is installed either using the tubing supplied with the column, or with PEEK tubing cut by the user to suitable lengths (i.d. 0.75 mm green PEEK tubing is supplied with ÄKTApurifier UPC 100). A male/male union connects the conductivity cell to the flow restrictor.

Tubing design.	Length	Tubing i.d.	Tubing o.d.	Material	Color	Volume of 10 cm
A1, B1	170 cm	2.9 mm	1/16"	Teflon	Clear	660 µl
G1, G2	33 cm	0.75 mm	1/16"	PEEK	Green	44.2 µl
G3	25 cm	0.75 mm	1/16"	PEEK	Green	44.2 µl
G4	30 cm	0.75 mm	1/16"	PEEK	Green	44.2 µl
G5	17 cm	0.75 mm	1/16"	PEEK	Green	44.2 µl
W1–W3	100 cm	0.75 mm	1/16"	Tefzel	Clear	44.2 µl

8.1.6 Setting the delay volume

The delay volume is the total volume of the tubing and components between the UV cell and the outlet of the fraction collector. This volume is used in UNICORN for synchronizing the UV peak detection with the fraction collection.

Note: *The delay volume is not set at system delivery because it depends on the length of the outlet tubing. When the installation is finished, the delay volume should be calculated and then set in UNICORN.*

When the system configuration after the UV cell has been changed, for example by changing a component or tubing, the delay volume might change as well. The new delay volume must then be calculated and set in UNICORN.

Calculating the delay volume

Calculate the contribution from each component and tubing from the UV cell to the fraction collector outlet.

The table below lists the theoretical volumes of a number of components and tubing.

Component/capillary	Volume (µl)
Valve PV-908	7
Valve IV-908	27
Valve FV-903	113
Conductivity cell	24
pH flow cell	100
Accumulator (Frac-950)	40
Union 1/16" male/ 1/16" male (0.25 mm i.d.)	1.8
Union 1/16" male/ 1/16" male (0.5 mm i.d.)	7
Flow restrictor FR-902	5
Capillary i.d. 0.25 x 100 mm	4.9
Capillary i.d. 0.50 x 100 mm	19.6
Capillary i.d. 0.75 x 100 mm	44.2
Capillary i.d. 1.0 x 100 mm	78.5

8 Reference information

8.1 System description

Example:

This example shows how to calculate the delay volume in ÄKTApurifier UPC 10, including fraction collector Frac-920, based on the tubing lengths on the previous page and the values in the table above. The tubing length from the flow restrictor to the outlet on the delivery arm is assumed to be 1000 mm.

The tubing volume is calculated according to the following example: A 300 mm long i.d. 0.25 mm tubing has the volume $3 \times 4.9 = 14.7 \mu\text{l}$.

Calculation of the delay volume:

Component/Tubing	Length [mm]	Volume (μl)
Tubing, UV cell–conductivity cell (i.d. 0.25 mm)	170	$1.7 \times 4.9 = 8.3$
Conductivity cell	–	24.0
Union 1/16" male/ 1/16" male (i.d. 0.25 mm)	–	1.8
Flow restrictor FR-902	–	5.0
Tubing, FR-902–flow diversion valve on Frac-920 (i.d. 0.25 mm)	700	$7 \times 4.9 = 41.0$
Flow diversion valve FV-903	–	113
Tubing, FV-903–delivery arm on Frac-920 (i.d. 0.25 mm)	300	$3 \times 4.9 = 14.7$
Delay volume		207.8

Setting the delay volume in UNICORN

To change the delay volume in UNICORN:

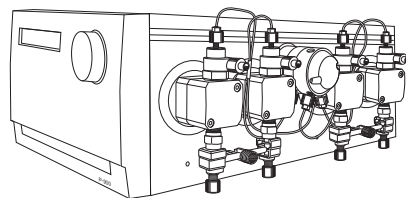
- 1 Select **System:Settings** in **System Control**.
- 2 Select **Specials** and then **FracParameters**.
- 3 Enter the delay volume and click **OK**.

8.2 Component description

8.2.1 Pump P-900

Pump P-900 is the collective name for a pump family.

P-900 is a high performance laboratory pump for use where accurately controlled liquid flow is required. It is a low pulsation pump equipped with two pump modules; A and B. This allows for binary gradients with high pressure mixing. A pressure sensor is connected to pump module A (left hand pair of pump heads).

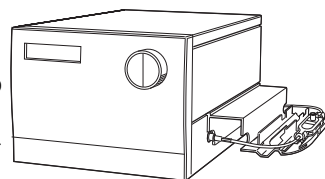


The model installed in **ÄKTApurifier 100 has 100 ml pump heads** and is referred to as Pump P-901 in the accessories list. P-901 has an operating flow rate range of 0.01–100 ml/min in both isocratic and gradient mode, and a **pressure range of 0–10 MPa** (100 bar, 1450 psi).

The model installed in **ÄKTApurifier 10 has 10 ml pump heads** and is referred to as Pump P-903 in the accessories list. P-903 has an operating flow rate range of 0.001–10 ml/min in both isocratic and gradient mode, and a **pressure range of 0–25 MPa** (250 bar, 3625 psi).

8.2.2 Monitor UV-900

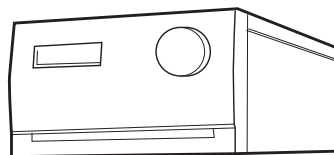
Monitor UV-900 is a multi-wavelength UV-Vis monitor that uses advanced fiber optic technology to monitor with high sensitivity at up to three wavelengths simultaneously in the wavelength range 190–700 nm. The use of fiber optics together with a unique flow cell design ensures a high signal-to-noise ratio with a minimal drift and refractive index effects.



Monitor UV-900 consists of a main unit, optical fibers and a choice of two flow cells.

8.2.3 Monitor UPC-900

Monitor UPC-900 is a high precision on-line combined monitor for measurement of UV absorption, pH and conductivity in liquid chromatography. The UPC-900 offers fixed wavelengths of 214 (Zn lamp), 254 or 280 nm (Hg lamp), fast response, high accuracy and reproducibility and flow cells with low dead volumes. Additional wavelengths are obtainable using optical filters (accessories).

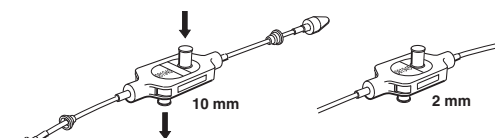


Monitor UPC-900 consists of a control unit, an optical unit with lamp assembly and a choice of two flow cells (optical path length 2 mm or 5 mm), a conductivity flow cell with integrated temperature sensor and a pH flow cell with pH electrode (optional).

8.2.4 UV and conductivity flow cells

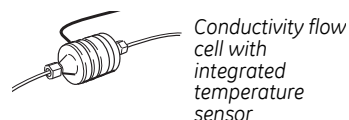
The type of UV flow cell used depends on the sample amount applied and the size of the column.

ÄKTApurifier 10 is delivered with the 10 mm cell fitted. A 2 mm cell (optical pathlength 2 mm, internal volume 2 μ l) is available as an accessory. If a lower detection sensitivity is desired, due to output signal limitation, the 2 mm flow cell should be used.



ÄKTApurifier 100 is delivered with the 2 mm cell fitted. A 10 mm cell (optical pathlength 10 mm, internal volume 8 μ l) is available as an accessory. For higher detection sensitivity, the 10 mm flow cell should be used.

ÄKTApurifier UPC is delivered with the 2 mm cell fitted. A 5 mm cell is also included in the package. If a lower detection sensitivity is desired due to output signal limitation, the 2 mm cell should be used.



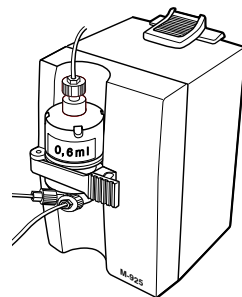
Conductivity flow cell with integrated temperature sensor



UV flow cells with 2 and 5 mm path lengths

8.2.5 Mixer M-925

Mixer M-925 is a dynamic, single chamber mixer powered and controlled from Pump P-900. All eluents commonly used in ion exchange, hydrophobic interaction, affinity and reversed phase chromatography can be mixed with a high degree of accuracy and reproducibility. The mixer is positioned directly after the Pump P-900.



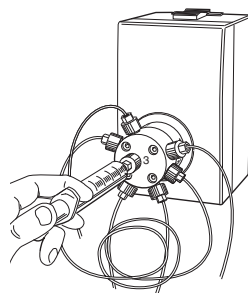
Mixer M-925 is supplied with a 0.6 ml mixing chamber, which can be exchanged for larger chambers. 2, 5 and 12 ml mixer chambers are available as options to obtain optimal mixing over the entire flow rate range of ÄKTApurifier.

8.2.6 Valve INV-907

A seven port motorized valve is used as a sample injection valve.

Three different operating positions to:

- Load a sample loop without disturbing column equilibration.
- Wash the sample loop while the column is in operation.
- Wash the pump for quick eluent exchange without disturbing the column.



Sample volumes up to 150 ml can be applied via loops connected to the injection valve:

- Using a range of fixed volume loops for applying samples from 10 µl to 2 ml with accuracy and precision.
- Using Superloop 10 ml, Superloop 50 ml, or Superloop 150 ml for applying samples in the range 1–10 ml, 1–50 ml, and 1–150ml respectively. All three are loaded by a syringe.

8.2.7 Flow restrictor FR-902/904



The flow restrictor generates a steady back-pressure to prevent air bubbles being formed in the flow cells.

FR-902 is set at the factory to 0.2 MPa, and is used when a pH cell (optional) is fitted in the system, or when using low pressure columns. FR-902 is mounted in the system at delivery.

FR-904 is set at the factory to 0.4 MPa. This flow restrictor should be used when working with organic solvents, such as in RPC applications, to avoid air bubble formation in the flow cell.

8.2.8 On-line filter



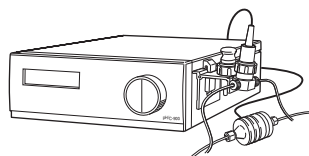
The on-line filter is fitted between the output of Mixer M-925 and position 7 of the injection valve. Arrows on the on-line filter indicates the flow direction. It generates a back-pressure of 0.5 MPa maximum.

The filter is a depth type filter and has a pore size of 2 μm . The filter used in ÄKTApurifier 10 systems is made of titanium. In ÄKTApurifier 100 systems, it is made of polypropene.

The filter should be replaced every week. When changing the filter, use a tool if necessary, to unscrew the filter body. When assembling the on-line filter, tighten the filter body by hand only. Never use a tool.

8.2.9 Monitor pH/C-900 (optional)

Monitor pH/C-900 is an option for ÄKTApurifier. It is a combined monitor for accurate, on-line monitoring of pH, conductivity and temperature in a wide range of liquid chromatography applications. Its accurate response coupled with high precision over a wide measuring range makes it ideal for use in all chromatography techniques, from reversed phase with very low conductivity eluents to hydrophobic interaction chromatography in high salt solutions.



8.2.10 Fraction collector (optional)

A fraction collector, Frac-920 or Frac-950, can be used for both small scale and preparative scale purifications with ÄKTApurifier. A number of racks for different tubes sizes are supplied with the fraction collector.

In ÄKTApurifier, the fraction collector allows fixed volume fractionation, or automatic peak fractionation. The latter function is based on UV peak detection using slopes or level sensing. Fraction marks and fraction numbers make it easy to identify fractions and peaks.

8.3 *Optional configurations*

ÄKTApurifier standard system configuration can easily be changed to optional configurations. This built-in flexibility in the standard ÄKTApurifier system allows the user to enhance already used purification methods and also to develop new, more complex methods.

Optional configurations are selected, installed and implemented by the user. An optional configuration consists of both hardware components and software instructions.

To support the process of implementing optional configurations, general guidelines regarding installation and operation are given in the separate manual ÄKTApurifier Optional Configurations User Manual included in ÄKTApurifier Manual Box.

Optional configurations are monitored and controlled via methods run by the UNICORN control system in the same way as the ÄKTApurifier standard configuration.

Optional configurations supported by ÄKTApurifier are:

- Connection of up to 9 motorised multi-port valves. These valves can be used to accomplish the following functions:
- Buffer selection.
- Sample selection.
- Column selection.
- Optional functions.
- On-line pH measurement.
- Connection of a dedicated sample pump.
- Sample application using a Superloop.
- Connection of a fraction collector.
- Connection of an monitor for pH and conductivity measurement (ÄKTApurifier).
- Connection of an autosampler.
- Connection of up to four air sensors.

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8.3 Optional configurations

- Connection of external equipment using digital input/output signals through the system pump P-901/903 REMOTE connector.
- Connection of an AD converter.

8.4 Recommended tubing and columns

The tables below shows which tubing kit should be used for each column. It is important that the recommendations in these tables are followed. The tubing to be changed is described in Section 8.1.2 in "Component locations".

8.4.1 ÄKTApurifier10

Note: In order to use low pressure columns, such as HiTrap, HiPrep™ and HiLoad, an extra system pressure measurement must be performed. This is described later in this Section.

X = recommended tubing kit

P = recommended for purity checks only

RF = can be used with indicated tubing if the optimal recommended flow rate in the column list is reduced in order not to exceed the column maximum pressure

Ion Exchange Columns

Code no	Column name	0.25	0.50
17-5177-01	Mini Q™ 4.6/50 PE	X, P	
17-5166-01	Mono Q™ 5/50 GL	X	
17-5167-01	Mono Q 10/100 GL	RF	X
17-0506-01	Mono Q HR 16/10	RF	X
17-5178-01	Mini S™ 4.6/50 PE	X, P	
17-5168-01	Mono S™ 5/50 GL	X	
17-5169-01	Mono S 10/100 GL	RF	X
17-0507-01	Mono S HR 16/10	RF	X
17-5181-01	SOURCE™ 15Q 4.6/100 PE	RF	X
17-5182-01	SOURCE 15S 4.6/100 PE	RF	X
17-1177-01	RESOURCE™ Q, 1 ml		X
17-1179-01	RESOURCE Q, 6 ml		X
17-1178-01	RESOURCE S, 1 ml		X
17-1180-01	RESOURCE S, 6 ml		X
11-0013-02	HiTrap Capto™ Q, 1 ml		X
11-0013-03	HiTrap Capto Q, 5 ml		RF
28-9165-37	HiTrap Capto DEAE, 1 ml		X
28-9165-40	HiTrap Capto DEAE, 5 ml		RF
17-5441-22	HiTrap Capto S, 1 ml		X
17-5441-23	HiTrap Capto S, 5 ml		RF
11-0032-73	HiTrap Capto MMC, 1 ml		X

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8.4 Recommended tubing and columns

Code no	Column name	0.25	0.50
11-0032-75	HiTrap Capto MMC, 5 ml		RF
28-4058-44	HiTrap Capto adhere, 1 ml		X
28-4058-46	HiTrap Capto adhere, 5 ml		RF
17-1153-01	HiTrap Q HP, 1 ml		X
17-1154-01	HiTrap Q HP, 5 ml		RF
17-1151-01	HiTrap SP HP, 1 ml		X
17-1152-01	HiTrap SP HP, 5 ml		RF
17-6002-33	HiTrap IEX Selection Kit, 7×1 ml		X
17-5053-01	HiTrap Q FF, 1 ml		X
17-5156-01	HiTrap Q FF, 5 ml		RF
17-5054-01	HiTrap SP FF, 1 ml		X
17-5157-01	HiTrap SP FF, 5 ml		RF
17-5055-01	HiTrap DEAE FF, 1 ml		X
17-5154-01	HiTrap DEAE FF, 5 ml		RF
17-5056-01	HiTrap CM FF, 1 ml		X
17-5155-01	HiTrap CM FF, 5 ml		RF
17-5162-01	HiTrap ANX FF (high sub), 1 ml		X
17-5163-01	HiTrap ANX FF (high sub), 5 ml		RF
17-5158-01	HiTrap Q XL, 1 ml		X
17-5159-01	HiTrap Q XL, 5 ml		RF
17-5160-01	HiTrap SP XL, 1 ml		X
17-5161-01	HiTrap SP XL, 5 ml		RF
17-5092-01	HiPrep 16/10 Q XL		RF
17-5093-01	HiPrep 16/10 SP XL		RF
17-5090-01	HiPrep 16/10 DEAE FF		RF
17-5091-01	HiPrep 16/10 CM FF		RF
17-5190-01	HiPrep 16/10 Q FF		RF
17-5191-01	HiPrep 16/10 ANX FF (high sub)		RF
17-5192-01	HiPrep 16/10 SP FF		RF
17-1064-01	HiLoad 16/10 Q Sepharose™ HP		X
17-1066-01	HiLoad 26/10 Q Sepharose HP		X
17-1137-01	HiLoad 16/10 SP Sepharose HP		X
17-1138-01	HiLoad 26/10 SP Sepharose HP		X

Reversed Phase Columns

Code no	Column name	0.25	0.50
17-5068-01	SOURCE 15RPC ST 4.6/100	X	
17-5116-01	SOURCE 5RPC ST 4.6/150	X	
17-1181-01	RESOURCE RPC 1 ml	RF	X
17-1182-01	RESOURCE RPC 3 ml	RF	X
17-0704-01	μRPC C2/C18 SC 2.1/10	X, P	
17-5057-01	μRPC C2/C18 ST 4.6/100	X, P	

Size Exclusion Columns

Code no	Column name	0.25	0.50
17-1458-01	Superdex™ Peptide PC 3.2/30	X, P	
17-5176-01	Superdex Peptide 10/300 GL	X	
17-0771-01	Superdex 75 PC 3.2/30	X, P	
28-9205-04	Superdex 75 5/50 GL	X, P	
17-5174-01	Superdex 75 10/300 GL	X	
17-1089-01	Superdex 200 PC 3.2/30	X, P	
17-5175-01	Superdex 200 10/300 GL	X	
28-9065-61	Superdex 200 5/150 GL	X, P	
17-5172-01	Superose™ 6 10/300 GL	X	
17-0673-01	Superose 6 PC 3.2/30	X, P	
17-5173-01	Superose 12 10/300 GL	X	
17-0674-01	Superose 12 PC 3.2/30	X, P	
17-1408-01	HiTrap Desalting, 5 ml		RF
17-5087-01	HiPrep 26/10 Desalting		RF
17-1139-01	HiLoad 16/60 Superdex 30 prep grade		X
17-1140-01	HiLoad 26/60 Superdex 30 prep grade		X
17-1068-01	HiLoad 16/60 Superdex 75 prep grade		X
17-1070-01	HiLoad 26/60 Superdex 75 prep grade		X
17-1069-01	HiLoad 16/60 Superdex 200 prep grade		X
17-1071-01	HiLoad 26/60 Superdex 200 prep grade		X
17-1165-01	HiPrep 16/60 Sephacryl™ S-100 HR		RF
17-1194-01	HiPrep 26/60 Sephacryl S-100 HR		RF
17-1166-01	HiPrep 16/60 Sephacryl S-200 HR		RF
17-1195-01	HiPrep 26/60 Sephacryl S-200 HR		RF

8 Reference information

8.4 Recommended tubing and columns

Code no	Column name	0.25	0.50
17-1167-01	HiPrep 16/60 Sephacryl S-300 HR		RF
17-1196-01	HiPrep 26/60 Sephacryl S-300 HR		RF

Hydrophobic Interaction Columns

Code no	Column name	0.25	0.50
17-5186-01	SOURCE 15PHE 4.6/100 PE		X
17-1184-01	RESOURCE ETH 1 ml		X
17-1185-01	RESOURCE ISO 1 ml		X
17-1186-01	RESOURCE PHE 1 ml		X
17-1187-01	RESOURCE HIC Test kit, 3×1 ml		X
28-4110-07	HiTrap HIC Selection Kit, 7×1 ml		X
17-1085-01	HiLoad 16/10 Phenyl Sepharose HP		X
17-1086-01	HiLoad 26/10 Phenyl Sepharose HP		X
17-5095-01	HiPrep 16/10 Phenyl FF (high sub)		RF
17-5094-01	HiPrep 16/10 Phenyl FF (low sub)		RF
17-5096-01	HiPrep 16/10 Butyl FF		RF
17-5097-01	HiPrep 16/10 Octyl FF		RF
17-1355-01	HiTrap Phenyl FF (high sub), 1 ml		X
17-5193-01	HiTrap Phenyl FF (high sub), 5 ml		RF
17-1353-01	HiTrap Phenyl FF (low sub), 1 ml		X
17-5194-01	HiTrap Phenyl FF (low sub) 5 ml		RF
17-1351-01	HiTrap Phenyl HP, 1 ml		X
17-5195-01	HiTrap Phenyl HP, 5 ml		RF
17-1359-01	HiTrap Octyl FF, 1 ml		X
17-5196-01	HiTrap Octyl FF, 5 ml		RF
28-4110-01	HiTrap Butyl HP, 1 ml		X
28-4110-05	HiTrap Butyl HP, 5 ml		RF
17-1357-01	HiTrap Butyl FF, 1 ml		X
17-5197-01	HiTrap Butyl FF, 5 ml		RF
17-0978-13	HiTrap Butyl-S FF, 1 ml		X
17-0978-14	HiTrap Butyl-S FF, 5 ml		RF

Affinity Columns

Code no	Column name	0.25	0.50
17-5247-01	HisTrap™ HP, 1 ml		X
17-5248-01	HisTrap HP, 5 ml		RF
17-5319-01	HisTrap FF, 1 ml		X
17-5255-01	HisTrap FF, 5 ml		RF
17-5256-01	HisPrep™ FF, 16/10		RF
11-0004-58	HisTrap FF crude, 1 ml		X
17-5286-01	HisTrap FF crude, 5 ml		RF
17-0920-03	HiTrap IMAC HP, 1 ml		X
17-0920-05	HiTrap IMAC HP, 5 ml		RF
17-0921-02	HiTrap IMAC FF, 1 ml		X
17-0921-04	HiTrap IMAC FF, 5 ml		RF
17-0921-06	HiPrep IMAC FF 16/10		RF
17-0408-01	HiTrap Chelating HP, 1 ml		X
17-0409-01	HiTrap Chelating HP, 5 ml		RF
17-0716-01	HiTrap NHS-activated HP, 1 ml		X
17-0717-01	HiTrap NHS-activated HP, 5 ml		RF
17-5281-01	GSTrap™ HP, 1 ml		X
17-5282-01	GSTrap HP, 5 ml		RF
17-5130-01	GSTrap FF, 1 ml (5 pcs)		X
17-5131-01	GSTrap FF, 5 ml		RF
17-5234-01	GSTPrep™ FF 16/10		RF
28-4017-45	GSTrap 4B, 1 ml		X
28-4017-46	GSTrap 4B, 5 ml		RF
28-9187-78	MBPTrap™ HP, 1 ml		X
28-9187-79	MBPTrap HP, 5 ml		RF
28-9075-46	StrepTrap™ HP, 1 ml		X
28-9075-47	StrepTrap HP, 5 ml		RF
28-4082-53	HiTrap MabSelect™, 1 ml		X
28-4082-55	HiTrap MabSelect, 5 ml		RF
11-0034-93	HiTrap MabSelect SuRe™, 1 ml		X
11-0034-94	HiTrap MabSelect SuRe, 5 ml		RF
28-4082-58	HiTrap MabSelect Xtra™, 1 ml		X
28-4082-60	HiTrap MabSelect Xtra, 5 ml		RF

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8.4 Recommended tubing and columns

Code no	Column name	0.25	0.50
17-5079-01	HiTrap rProtein A FF, 1 ml		X
17-5080-01	HiTrap rProtein A FF, 5 ml		RF
17-0402-01	HiTrap Protein A HP, 1 ml		X
17-0403-01	HiTrap Protein A HP, 5 ml		RF
17-0404-01	HiTrap Protein G HP, 1 ml		X
17-0405-01	HiTrap Protein G HP, 5 ml		RF
17-0406-01	HiTrap Heparin HP, 1 ml		X
17-0407-03	HiTrap Heparin HP, 5 ml		RF
17-5189-01	HiPrep 16/10 Heparin FF		RF
17-0412-01	HiTrap Blue HP, 1 ml		X
17-0413-01	HiTrap Blue HP, 5 ml		RF
17-5110-01	HiTrap IgM Purification HP, 1 ml		X
17-5111-01	HiTrap IgY Purification HP, 5 ml		RF
17-5112-01	HiTrap Streptavidin HP, 1 ml		X
17-5143-01	HiTrap Benzamidine FF (high sub), 1 ml		X
17-5144-01	HiTrap Benzamidine FF (high sub), 5 ml		RF

Chromatofocusing Columns

Code no	Column name	0.25	0.50
17-5171-01	Mono P™ 5/200 GL	X	
17-5170-01	Mono P 5/50 GL	X	

8.4.2 ÄKTApurifier 100

Note: When using low pressure columns, such as HiTrap and HiLoad, change to the Flow restrictor FR-902. If the pressure exceeds the pressure limit value given for these columns, an extra system pressure measurement must be performed. This is described later in this Section.

X = recommended tubing kit

(+) = may improve resolution

(x) = can be used if flow < 5 ml/min

(o) = should be used if flow > 30 ml/min

Ion Exchange Columns

Code no	Column name	0.50	0.75	1.0
17-5166-01	Mono Q 5/50 GL	(+)	X	
17-5167-01	Mono Q 10/100 GL		X	
17-5168-01	Mono S 5/50 GL	(+)	X	
17-5169-01	Mono S 10/100 GL		X	
17-1177-01	RESOURCE Q, 1 ml	(+)	X	
17-1179-01	RESOURCE Q, 6 ml		X	(o)
17-1178-01	RESOURCE S, 1 ml	(+)	X	
17-1180-01	RESOURCE S, 6 ml		X	(o)
11-0013-02	HiTrap Capto Q, 1 ml	(+)	X	
11-0013-03	HiTrap Capto Q, 5 ml		X	
11-0032-73	HiTrap Capto MMC, 1 ml	(+)	X	
11-0032-75	HiTrap Capto MMC, 5 ml		X	
17-1153-01	HiTrap Q HP, 1 ml	(+)	X	
17-1154-01	HiTrap Q HP, 5 ml		X	
28-9165-37	HiTrap Capto DEAE, 1 ml	(+)	X	
28-9165-40	HiTrap Capto DEAE, 5 ml		X	
17-5441-22	HiTrap Capto S, 1 ml	(+)	X	
17-5441-23	HiTrap Capto S, 5 ml		X	
17-1151-01	HiTrap SP HP, 1 ml	(+)	X	
17-1152-01	HiTrap SP HP, 5 ml		X	
28-4058-44	HiTrap Capto adhere, 1 ml	(+)	X	
28-4058-46	HiTrap Capto adhere, 5 ml		X	
17-6002-33	HiTrap IEX Selection Kit, 7 x 1 ml	(+)	X	
17-5053-01	HiTrap Q FF, 1 ml	(+)	X	

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8.4 Recommended tubing and columns

Code no	Column name	0.50	0.75	1.0
17-5156-01	HiTrap Q FF, 5 ml		X	
17-5054-01	HiTrap SP FF, 1 ml	(+)	X	
17-5157-01	HiTrap SP FF, 5 ml		X	
17-5055-01	HiTrap DEAE FF, 1 ml	(+)	X	
17-5154-01	HiTrap DEAE FF, 5 ml		X	
17-5056-01	HiTrap CM FF, 1 ml	(+)		X
17-5155-01	HiTrap CM FF, 5 ml		X	
17-5162-01	HiTrap ANX FF (high sub), 1 ml	(+)		X
17-5163-01	HiTrap ANX FF (high sub), 5 ml		X	
17-5158-01	HiTrap Q XL, 1 ml	(+)		X
17-5159-01	HiTrap Q XL, 5 ml		X	
17-5160-01	HiTrap SP XL, 1 ml	(+)		X
17-5161-01	HiTrap SP XL, 5 ml		X	
17-5092-01	HiPrep 16/10 Q XL		X	
17-5093-01	HiPrep 16/10 SP XL		X	
17-5090-01	HiPrep 16/10 DEAE FF		X	
17-5091-01	HiPrep 16/10 CM FF		X	
17-5190-01	HiPrep 16/10 Q FF		X	
17-5191-01	HiPrep 16/10 ANX FF (high sub)		X	
17-5192-01	HiPrep 16/10 SP FF		X	
17-5181-01	SOURCE 15Q 4.6/100 PE		X	
17-5182-01	SOURCE 15S 4.6/100 PE		X	
17-1064-01	HiLoad 16/10 Q Sepharose HP		X	
17-1066-01	HiLoad 26/10 Q Sepharose HP		X	
17-1137-01	HiLoad 16/10 SP Sepharose HP		X	
17-1138-01	HiLoad 26/10 SP Sepharose HP		X	

Size Exclusion Columns

Code no	Column name	0.50	0.75	1.0
17-5176-01	Superdex Peptide 10/300 GL	(+)	X	
17-5174-01	Superdex 75 HR 10/300 GL	(+)	X	
17-5175-01	Superdex 200 HR 10/300 GL	(+)	X	
17-5172-01	Superose 6 10/300 GL	(+)	X	
17-5173-01	Superose 12 10/300 GL	(+)	X	
17-1408-01	HiTrap Desalting, 5 ml		X	
17-5087-01	HiPrep 26/10 Desalting		X	
17-1139-01	HiLoad 16/60 Superdex 30 prep grade		X	
17-1140-01	HiLoad 26/60 Superdex 30 prep grade		X	
17-1068-01	HiLoad 16/60 Superdex 75 prep grade		X	
17-1070-01	HiLoad 26/60 Superdex 75 prep grade		X	
17-1069-01	HiLoad 16/60 Superdex 200 prep grade		X	
17-1071-01	HiLoad 26/60 Superdex 200 prep grade		X	
17-1165-01	HiPrep 16/60 Sephacryl S-100 HR		X	
17-1194-01	HiPrep 26/60 Sephacryl S-100 HR		X	
17-1166-01	HiPrep 16/60 Sephacryl S-200 HR		X	
17-1195-01	HiPrep 26/60 Sephacryl S-200 HR		X	
17-1167-01	HiPrep 16/60 Sephacryl S-300 HR		X	
17-1196-01	HiPrep 26/60 Sephacryl S-300 HR		X	

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8.4 Recommended tubing and columns

Hydrophobic Interaction Columns

Code no	Column name	0.50	0.75	1.0
17-1184-01	RESOURCE ETH 1 ml		X	
17-1185-01	RESOURCE ISO 1 ml		X	
17-1186-01	RESOURCE PHE 1 ml		X	
17-1187-01	RESOURCE HIC Test kit, 3 x 1 ml	(+)	X	
28-4110-07	HiTrap HIC Selection Kit, 7 x 1 ml	(+)		X
17-1085-01	HiLoad 16/10 Phenyl Sepharose HP		(x)	X
17-1086-01	HiLoad 26/10 Phenyl Sepharose HP		(x)	X
17-5095-01	HiPrep 16/10 Phenyl FF (high sub)		X	
17-5094-01	HiPrep 16/10 Phenyl FF (low sub)		X	
17-5096-01	HiPrep 16/10 Butyl FF		X	
17-5097-01	HiPrep 16/10 Octyl FF		X	
17-1355-01	HiTrap Phenyl FF (high sub), 1 ml		X	
17-5193-01	HiTrap Phenyl FF (high sub), 5 ml		RF	
17-1353-01	HiTrap Phenyl FF (low sub), 1 ml		X	
17-5194-01	HiTrap Phenyl FF (low sub), 5 ml		RF	
17-1351-01	HiTrap Phenyl HP, 1 ml		X	
17-5195-01	HiTrap Phenyl HP, 5 ml		RF	
17-1359-01	HiTrap Octyl FF, 1 ml		X	
17-5196-01	HiTrap Octyl FF, 5 ml		RF	
17-1357-01	HiTrap Butyl FF, 1 ml		X	
17-5197-01	HiTrap Butyl FF, 5 ml		RF	
17-0978-13	HiTrap Butyl-S FF, 1 ml		X	
17-0978-14	HiTrap Butyl-S FF, 5 ml		RF	

Reversed Phase Columns

Code no	Column name	0.50	0.75	1.0
17-5116-01	SOURCE 5RPC ST 4.6/150	X		
17-5068-01	SOURCE 15RPC ST 4.6/100		X	
17-1181-01	RESOURCE RPC 1 ml	(+)	X	
17-1182-01	RESOURCE RPC 3 ml		X	
17-0704-01	μRPC C2/C18 SC 2.1/10	X, P		
17-5057-01	μRPC C2/C18 ST 4.6/100	X, P		

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8.4 Recommended tubing and columns

Affinity Columns

Code no	Column name	0.50	0.75	1.0
17-5247-01	HisTrap HP, 1 ml	(+)	X	
17-5248-01	HisTrap HP, 5 ml		X	
17-5319-01	HisTrap FF, 1 ml	(+)	X	
17-5255-01	HisTrap FF, 5 ml		X	
17-5256-01	HisPrep FF, 16/10		X	
11-0004-58	HisTrap FF crude, 1 ml	(+)	X	
17-5286-01	HisTrap FF crude, 5 ml		X	
17-0920-03	HiTrap IMAC HP, 1 ml	(+)	X	
17-0920-05	HiTrap IMAC HP, 5 ml		X	
17-0921-02	HiTrap IMAC FF, 1 ml	(+)	X	
17-0921-04	HiTrap IMAC FF, 5 ml		X	
17-0921-06	HiPrep IMAC FF 16/10		X	
17-0408-01	HiTrap Chelating HP, 1 ml	(+)	X	
17-0409-01	HiTrap Chelating HP, 5 ml		X	
17-0716-01	HiTrap NHS-activated HP, 1 ml	(+)	X	
17-0717-01	HiTrap NHS-activated HP, 5 ml		X	
17-5281-01	GSTrap HP, 1 ml	(+)	X	
17-5282-01	GSTrap HP, 5 ml		X	
17-5130-01	GSTrap FF, 1 ml	(+)	X	
17-5131-01	GSTrap FF, 5 ml		X	
17-5234-01	GSTPrep FF 16/10		X	
28-4082-53	HiTrap MabSelect, 1 ml	(+)	X	
28-4082-55	HiTrap MabSelect, 5 ml		X	
11-0034-93	HiTrap MabSelect SuRe, 1 ml	(+)	X	
11-0034-94	HiTrap MabSelect SuRe, 5 ml		X	
28-4082-58	HiTrap MabSelect Xtra, 1 ml	(+)	X	
28-4082-60	HiTrap MabSelect Xtra, 5 ml		X	
17-5079-01	HiTrap rProtein A FF, 1 ml	(+)	X	
17-5080-01	HiTrap rProtein A FF, 5 ml		X	
17-0402-01	HiTrap Protein A HP, 1 ml	(+)	X	
17-0403-01	HiTrap Protein A HP, 5 ml		X	
17-0404-01	HiTrap Protein G HP, 1 ml	(+)	X	
17-0405-01	HiTrap Protein G HP, 5 ml		X	

Code no	Column name	0.50	0.75	1.0
17-0406-01	HiTrap Heparin HP, 1 ml	(+)	X	
17-0407-01	HiTrap Heparin HP, 5 ml		RF	
17-5189-01	HiPrep 16/10 Heparin FF		X	
17-0412-01	HiTrap Blue HP, 1 ml	(+)	X	
17-0413-01	HiTrap Blue HP, 5 ml		RF	
17-5110-01	HiTrap IgM Purification HP, 1 ml	(+)	X	
17-5111-01	HiTrap IgY Purification HP, 5 ml		X	
17-5112-01	HiTrap Streptavidin HP, 1 ml	(+)	X	
17-5143-01	HiTrap Benzamidine FF (high sub), 1 ml	(+)	X	
17-5144-01	HiTrap Benzamidine FF (high sub), 5 ml		RF	

Chromatofocusing Columns

Code no	Column name	0.25	0.75	1.0
17-5171-01	Mono P 5/200 GL	(+)	X	
17-5170-01	Mono P 5/50 GL	(+)	X	

8.4.3 Selecting tubing kit for other columns

For the columns not available in the tables in 8.4 in "Recommended tubing and columns", select the tubing kit as described below.

Note: Before starting to perform the described method below, make sure that the column itself is clean and does not generate too high back-pressure.

- 1 Select **View:Run Setup** in the **Method Editor** for the method that is used.
- 2 Note the maximum specified back-pressure for the column at the variable **Column_PressureLimit** on the **Variables** page.

Note: The maximum allowed back-pressure on a self-packed column should never exceed 75% of the back-pressure used during the packing procedure.

- 3 Install the column and test to run at the flow rate and with the eluents to be used, with the i.d. 0.75 mm tubing kit.
- 4 Select the appropriate tubing kit as follows:
 - If the generated back-pressure at the flow to be used is within the set column pressure limit, use the tubing kit already installed.
 - If the generated back-pressure at the flow rate to be used is well below the column pressure limit and the predicted peak volume is less than 1 ml, it is preferable to change to a narrower tubing kit, i.d. 0.5 mm. If the demands on low band-broadening are less critical, use the tubing kit already installed.
 - If the generated back-pressure at the flow rate to be used exceeds the set column pressure limit, change to a wider tubing kit, i.d. 1.0 mm. If the set column pressure limit is still exceeded with the i.d. 1.0 mm tubing kit installed, change to Flow restrictor FR-902, and check the generated back-pressure according to the description below.

Note: The back-pressure might increase during e.g. sample injection and gradient formation due to viscosity variations. Make sure that these variations have been taken into consideration when selecting tubing i.d.

8.5 BufferPrep details

8.5.1 Creating your own recipes

- 1 Select **Edit:BufferPrep Recipes** in the **Method Editor**.
- 2 Click **New** to open the **New Recipe** dialog.
- 3 Select a buffer substance from the list. If the required buffer substance is not listed, a new buffer substance can be defined, see Section 8.5.2 in "Defining a new buffer substance" below. Up to 5 buffering components can be added.
- 4 Set the stock concentration of the buffer.

Buffer concentrations of 2–4 times higher compared to normal preparation should be used. When BufferPrep is used, the buffer will be diluted between 2–10 times, dependent on the amount of acid/base that has to be used to reach the desired pH.

We recommend that the total concentration for all buffer substances selected for the recipe should be between 0.03 M and 0.2 M (typically 0.1 M for ion exchange chromatography).

- 5 Select either **Acid** or **Base** (HCl or NaOH) from the pull-down list and set the inlet concentration (typically 0.1 M).
- 6 Select a salt from the pull-down list. If the required salt is not listed, a new salt can be defined, see Section 8.5.3 in "Defining a new salt" below.
- 7 Set the maximum outlet concentration of the salt to 100 %B (usually 1.0 M). The maximum outlet salt concentration is half the concentration of the inlet salt stock solution.
- 8 Set the pH range required for the buffer.

Note: To set a useful pH range the pKa must be known. Click on the Buffer substance button and select the buffer component. The pKa values are shown in the list. Typically, a pH range ± 0.5 units around the pKa is useful. For exact ranges, check the buffer tables.

- 9 To add notes about the recipe, click **Notes** and enter the required text in the box displayed. Click **OK** to close the **Recipe Notes** dialog.
- 10 When you have created your recipe, click **Save As**. Give the recipe a name. The recipe is checked and if the pH range selected is not feasible, a warning is displayed giving the error condition and making suggestions for its correction. Correct the recipe and click **Save As** again.

Note: *The check does not include whether the buffering capacity is large enough over the entire pH range.*

- 11 Click **Close** to close the **BufferPrep Recipes** dialog.

8.5.2 Defining a new buffer substance

Before defining a new buffer substance, make sure that all pKa values for the substance are available. The pKa values entered should be true pKa (i.e. the pKa value at indefinite dilution) as opposed to apparent pKa (i.e. measured at a non-zero concentration). Refer to Section 8.5.5 in "*Examples and tips*" for information on true and apparent pKa. The pKa values should be given at 25 °C.

- 1 Click **New** in the **BufferPrep Recipes** dialog to open the **New Recipe** dialog. Click the **Buffer substance** button and click **New** in the **Define Buffer Substance** dialog that opens.
- 2 In the **New Component** dialog, enter the name of the new buffer substance. Click **OK**.
- 3 In the **Define Buffer Substance** table, enter the pKa **Value**. Up to 3 values (**pKa1- pKa3**) can be entered for each buffering component. The pKa values must be entered in order of increasing value, starting with the lowest pKa value. When the buffering component has less than three pKa, the other pKa values should be set to zero.
- 4 Enter the dpKa/dT **Value** (the change of pKa dependent on temperature) for each pKa (**dpKa1/dT- dpKa3/dT**). Zero means that the pKa does not change with temperature.
- 5 Enter the **Number of acidic protons** for the buffer substance in the form that is actually weighed in. (Example: For NaH₂PO₄ enter 2, for Na₂HPO₄ enter 1, for Tris enter 0).
- 6 Enter the **Charge** of the completely **deprotonated ion**. This will be a negative value for an acid, 0 for a base. (Example: For NaH₂PO₄ enter -3, for Tris enter 0).
- 7 Click **OK** to add the new buffer substance to the list of available buffers.

8.5.3 Defining a new salt

When defining a new salt make sure that the new salt is inert, i.e. a salt with no buffering properties.

- 1 In the **New Recipe** dialog, click **Salt** and click **New** in the **Define Salt** dialog.
- 2 In the **New Component** dialog, enter the name of the new salt. Click **OK**.

- 3 Enter the **Charge of the Anion** (Example: for Cl^- enter -1, for SO_4^{2-} enter -2).
- 4 Enter the **Charge of the Cation** (Example: For Na^+ enter 1, for Mg^{2+} enter 2).
- 5 Click **OK** to add the new salt to the list of available salts.

8.5.4 Correction factors

To obtain higher pH accuracy, the recipe can be fine tuned around a specific pH at the flow rate to be used. When scouting over a broad pH range, fine tuning is less beneficial. Run BufferPrep manually at 0 and 100 %B as follows:

- 1 From the **System Control:Manual** menu select **Other**.
In the **Other instructions** dialog select **BufferPrep Recipe**.
Select the recipe in the **Recipe Name** list.
Click **Execute**.
Select **Pump** to switch to the **Pump instructions** dialog.
Select **Flow**.
Select **FlowRate**.
Click **Execute**.
 - 2 Start a run manually at 0 %B. Make sure the buffer valve (optional) is set to the required inlet (A11–A18).
 - 3 Check the pH reading when stable.
 - 4 Change to 100 %B by setting the **Gradient** instruction in **Manual:Pump** to 100 %B for **Target** and 0 for **Length**. Click **Execute**.
 - 5 Check the pH reading when stable at 100 %B.
 - 6 If the reading is acceptable at both 0 and 100 %B, the correction factors do not need to be changed.
 - 7 If the pH reading is not acceptable, it is possible to correct the factors for the recipe. Select **Edit:BufferPrep Recipes** in the Method Editor.
Click **New**.
In the **New Recipe** dialog click **Correction factors**. Enter the pH deviation at 0 and 100% B. (For example, if the pH set is 7.0 and the actual pH is 7.1, enter 0.1, or if the actual pH is 6.9, enter -0.1).
- Note:** Some of the pre-programmed recipes have default correction factors. Add your deviation to these to obtain the correct value.
Example: If your pre-set correction factor is -0.2 and your reading at pH 7 is 7.1, enter $-0.2+0.1 = -0.1$.
- 8 Click **Save As** to save the recipe.

8.5.5 Examples and tips

Buffering capacity

If the buffer capacity of the broad range BufferPrep recipes are too low, there are two alternatives:

- Switch to a recipe with one buffer component with a pKa close to the required pH.
- Increase the concentration of all buffers in the broad range recipe. Increase the acid concentration by the same factor as the buffer concentration. Note that a new recipe has to be created. The ionic strength of the start eluent will also be increased.

Ionic strength

Buffer components with several pKa values will give a higher ionic strength at the start when the pH is set above the second pKa and even higher above the third pKa for acidic buffer components. This may cause problems if you have peaks that elute early in the gradient. To reduce this, use low concentrations or if possible change to buffer components that only have one pKa.

Example:

A recipe with 0.1 M Citrate (pKa 3.13, 4.76, and 6.40) will have an ionic strength of approximately 0.22 at pH 6, but only 0.05 M at pH 4. Use a 0.03 M solution instead or use 0.1 M MES at pH 6.

Choice of eluent

To avoid local disturbances in pH caused by buffering ions participating in the ion exchange process, select an eluent with buffering ions of the same charge as the substituting groups on the ion exchanger.

Choose the start buffer pH so that substances to be bound to the ion exchanger are charged, e.g. at least 1 pH unit below the isoelectric point for cation exchangers.

Example:

A recipe with Na₂HPO₄ (pKa 2.15, 7.20 and 12.33) will not give accurate pH above approximately pH 7.6 (when titrated with HCl).

Conversion of apparent pKa's to thermodynamic pKa's

Only pKa values at thermodynamic conditions (pKa at infinite dilution) must be entered into BufferPrep. When entering a new buffer substance with only apparent pKa known, a conversion to thermodynamic pKa has to be performed.

The apparent pKa is measured at a non-zero concentration. Find the thermodynamic pKa by taking the apparent pKa and add the value, found in the table below, for the buffer concentration at which the apparent pKa has been measured.

Conc [M]	Base pKa1	Base pKa2	Acid pKa1	Acid pKa2
0.02	-0.05	-0.26	+0.05	+0.26
0.05	-0.07	-0.37	+0.07	+0.37
0.1	-0.09	-0.47	+0.09	+0.47
0.2	-0.12	-0.60	+0.12	+0.60

Example:

Bis-Tris has a listed pKa of 6.46 at a concentration of 0.1 M. This is a base with only one pKa. Using the table above we find that 6.37 should give more accurate results ($6.46 - 0.09 = 6.37$).

Zwitter ions

Using zwitter ions in a BufferPrep recipe can be difficult. All zwitter ions have at least two pKa values. Since BufferPrep has to know all pKa values for the buffer component, do not use a zwitter ionic buffer component if the pKa values are not known. The second important issue is to know in which form the component is, i.e. if the molecule contains acidic protons.

Example:

These examples can be used as a template for zwitter ions:

Bicine	pKa1 = 1.84 pKa2 = 8.33 number of acidic protons = 1 charge of deprotonated ion = -1	dpKa1/dT = value unknown, insert 0 dpKa2/dT = -0.017
HEPES	pKa1 ~ 3 pKa2 = 7.39 number of acidic protons = 1 charge of deprotonated ion = -1	dpKa1/dT = value unknown, insert 0 dpKa2/dT = -0.014

Cold room

If the dpK_a/dT values are correct, there is no problem using BufferPrep in a cold room. To fine tune the ALEX and CLEX recipes, use the following correction factors:

ALEX at 5 °C	0% B = +0.10	100% B = 0.00
CLEX at 5 °C	0% B = 0.00	100% B = -0.40

8.5.6 Recipe overview

Data for pre-programmed AIEX recipes

Gradient 0.0–1.0 M NaCl

Buffer	Titrate with	~ pH range	Default correction at 0%B 100% B		Start conductivity low - high pH
5.0–9.5 pH AIEX mixture 0.05 M 1-methyl-piperazine 0.05 M Bis-Tris 0.025 M Tris	0.1 M HCl	5.0–9.5	0.0	0.0	3.2 - 0.8 mS/cm
6.0–9.0 pH AIEX mixture 0.07 M Bis-Tris 0.05 M Tris	0.1 M HCl	6.0–9.0	0.0	0.0	2.5 - 0.4 mS/cm
0.1 M Bis-Tris	0.1 M HCl	6.0–7.7	+0.2	0.0	2.2 - 0.4 mS/cm
0.1 M 1-methyl-piperazine	0.1 M HCl	5.0–5.5 8.6–10.3	0.0 -0.1	-0.1 -0.2	2.4 - 0.6 mS/cm 3.3 - 2.8 mS/cm
0.1 M Piperazine	0.1 M HCl	6.0–6.7 9.4–10.5	-0.1 -0.2	-0.3 -0.4	2.4 - 1.1 mS/cm 3.4 - 2.9 mS/cm
0.1 M Tris	0.1 M HCl	7.5–8.5	0.0	-0.2	2.4 - 1.3 mS/cm

	2	3	4	5	6	7	8	9	10	11	12
5.0–9.5 pH AIEX				5.0	-----				9.5		
6.0–9.0 pH CIEX					6.0	-----		9			
1-methyl-piperazine				5.0	—	5.5					
Bis-Tris					6.0	-----	7.7				
Piperazine					6	—	6.7				
Tris						7.5	-----	8.5			
1-methyl-piperazine							8.6	-----	10.3		
Piperazine								9.4	-----	10.5	
	2	3	4	5	6	7	8	9	10	11	12

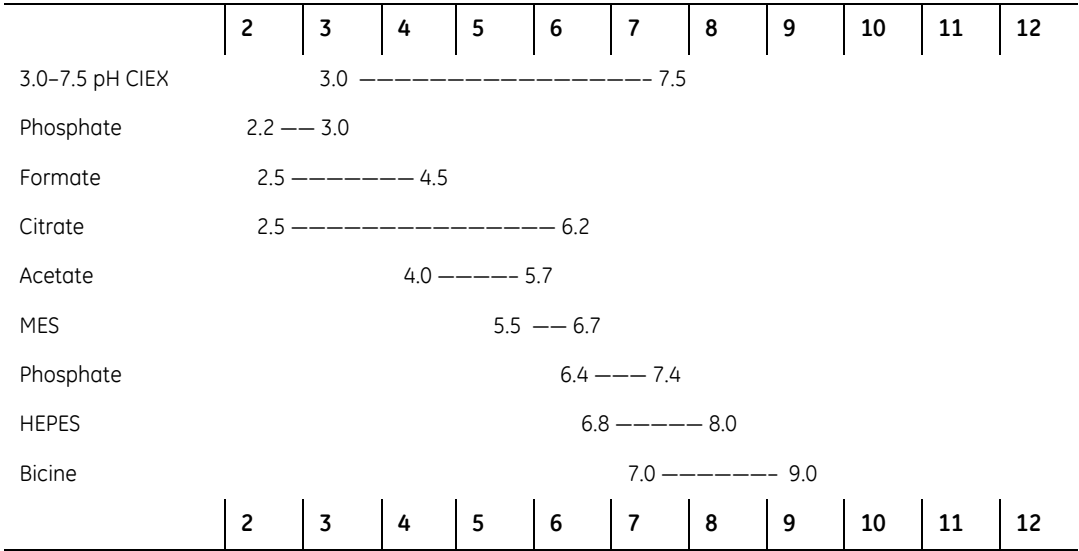
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8.5 BufferPrep details

Data for pre-programmed CIEX recipes

Gradient 0.0–1.0 M NaCl

Buffer	Titrate with	~ pH range	Default correction at 0%B	100% B	Start conductivity low - high pH
3.0–7.5 pH CIEX 0.03 M Phosphate Na ₂ HPO ₄ 0.03 M Formate Na 0.06 M Acetate Na	0.1 M HCl	3.0–7.5	0.0	-0.2	4.2 - 5.7 mS/cm
0.1 M Acetate Na	0.1 M HCl	4.0–5.7	0.0	-0.1	3.2 - 3.8mS/cm
0.1 M Bicine	0.1 M NaOH	7.0–9.0	+0.1	0.0	0.4 - 1.7 mS/cm
0.03 M Citrate Na3	0.1 M HCl	2.5–6.2	0.0	-0.2	4.2 - 3.0mS/cm
0.1 M Formate Na	0.1 M HCl	2.5–4.5	0.0	-0.2	4.4 - 3.5 mS/cm
0.1 M HEPES	0.1 M NaOH	6.8–8.0	+0.2	+0.3	0.6 - 1.6 mS/cm
0.1 M MES	0.1 M NaOH	5.5–6.7	+0.1	+0.1	0.7 - 1.6 mS/cm
0.03 M Phosphate Na ₂ HPO ₄	0.1 M HCl	2.2–3.0 6.4–7.4	0.0 0.0	-0.3 -0.3	2.4 - 2.6 mS/cm 5.0 - 2.6 mS/cm



8.6 Technical specifications

For the complete specifications for each component, refer to the individual User Manuals and Instructions. The relevant system specifications are listed below.

8.6.1 Operating data

Pump P-901	
Flow rate range	0.01–100 ml/min
Flow rate increments	0.01 ml/min
Pressure range	0–10 MPa (100 bar, 1450 psi)
pH stability range	1–13 (1–14 < 1 day exposure)
Viscosity	Max. 5 cP
Flow rate accuracy	± 2% or 20 µl/min whichever is greater
Flow rate reproducibility	rsd < 0.5% at flow rate 0.5 ml/min
Gradient composition Accuracy Reproducibility	± 1% at 0.5–100 ml/min rsd < 0.25% at flow rate <0.5–100 ml/min
Internal volume Stroke volume Total volume	286 µl 1850 µl
Pump P-903	
Flow rate range Flow rate increments	0.001–10 ml/min 0.001 ml/min
Pressure range	0–25 MPa (250 bar, 3625 psi)
pH stability range	1–13 (1–14 < 1 day exposure)
Viscosity	Max. 5 cP
Flow rate accuracy	± 2% or 2 µl/min whichever is greater (with compression compensation activated)
Flow rate reproducibility	rsd < 0.5% at flow rate 0.05 ml/min
Gradient composition Accuracy Reproducibility	± 1% at 0.05–10 ml/min rsd < 0.25% at flow rate <0.05–10 ml/min
Internal volume Stroke volume Total volume	35.6 µl 556 µl

8 Reference information

8.6 Technical specifications

Monitor UV-900	
Wavelength range	190-700 nm in step of 1 nm, 3 wavelengths simultaneously
Bandwidth	4 nm
Wavelength accuracy	± 2 nm
Wavelength reproducibility	± 0.01 nm
Wavelength switch time	< 500 ms (one cycle from 214 nm to 254 nm and back to 214)
Linearity ¹	< 2% deviation up to 2 AU at 260 nm with Uracil at pH 2
Noise ^{1, 2} (at 230 nm)	< 6×10 ⁻⁵ AU, with 10 mm cell, H ₂ O at 1 ml/min
Drift ¹ (at 254 nm)	< 2×10 ⁻⁴ AU/h
Flow cell Max. flow rate Max. pressure	100 ml/min 2 MPa (20 bar, 290 psi)
1. Specified for 10 mm flow cell 2. Typical values at room temperature after warm up	
Monitor UPC-900 UV measurement	
Absorbance range	0.001–5.0 AU (full scale)
Autozero range	-0.2–2.0 AU
Baseline adjust	Adjustable 0–100% of full scale
Wavelengths Hg lamp, fixed by changing filter Zn lamp	254 and 280 nm 313, 365, 405, 436 and 546 nm 214 nm
UV flow cell, 2 mm	
Flow rate	0–100 ml/min
Max. pressure	4.0 MPa (40 bar, 580 psi)
Max. back-pressure	0.05 MPa at 100 ml/min
Liquid temperature range	+4 to +60 °C
Optical path length	2 mm

Cell volume	2 µl (30 µl detector volume)
UV flow cell, 5 mm	
Flow rate	0–20 ml/min
Max. pressure	4.0 MPa (40 bar, 580 psi)
Max. back-pressure	0.02 MPa at 20 ml/min
Optical path length	5 mm
Cell volume	6 µl (10 µl detector volume)
Conductivity unit	
Conductivity range	1 µS/cm–999.9 mS/cm
Deviation from theoretical conductivity	Max. ± 2% of full scale calibration range or ± 10 µS/cm whichever is greater in the range 1 µS/cm–300 mS/cm
Reproducibility	Max. ± 1% maximum or ± 5 µS/cm whichever is greater in the range 1 µS/cm– 300 mS/cm
Noise	Max. ± 0.5% of full scale calibrated range
Conductivity flow cell	
Flow rate	0–100 ml/min
Max. pressure	5 MPa (50 bar, 725 psi)
Max. back-pressure	0.01 MPa at 100 ml/min
pH measurement (optional)	
pH range	0 to 14

8.6.2 Physical data

Degree of protection	IP 20
Power requirement	100–240 V ~ 50–60 Hz
Power consumption	600 VA
Fuse specification	ÄKTApurifier: T 6.3 AL/ 250 VAC
Dimensions, W × H × D	450 × 610 × 490mm
Weight	ÄKTApurifier: 41 kg ÄKTApurifier UPC: 38 kg
Environment	+4 to +40 °C, 10–95% relative humidity (non-condensing), 84–106 kPa (840–1060 mbar atmospheric pressure).

8.6.3 Compliance with safety standards

The CE symbol and declaration of conformity	The CE symbol and corresponding declaration of conformity is valid for the instrument only if it is: • used for laboratory purposes, not for clinical or <i>in vitro</i> purposes • used as a stand-alone unit • used in the same state as it was delivered from GE Healthcare except for alterations described in this User Manual • connected to other CE labelled GE Healthcare modules or other products as recommended.
Safety standards	This product meets the requirement of the Low Voltage Directive (LVD) 73/23/EEC through the following harmonized standards: • EN 61010-1 • IEC 61010-1 • CAN/CSA-C22.2 No. 61010-1 • UL 61010-1

EMC standards	This device meets the requirements of the EMC Directive 89/336/EEC through the following harmonized standards: • EN 61326 (emission and immunity) • EN 55011, GR 1, Class A (emission) This is a Class A product. In a domestic environment, it may cause radio interference, in which case the user may be required to take suitable measures. This device complies with part 15 of the FCC rules (emission). Operation is subject to the following two conditions: • This device may not cause harmful interference. • This device must accept any interference received, including interference that may cause undesired operation.
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8.6.4 Hardware requirements

Refer to Section *System requirements* in the *UNICORN User Manuals* supplied.

8.6.5 Software requirements

Refer to Section *System requirements* in the *UNICORN User Manuals* supplied.

8.6.6 Network requirements

Refer to Section *System requirements* in the *UNICORN User Manuals* supplied.

8.6.7 ÄKTApurifier component materials

The wetted materials of ÄKTApurifier are listed below:

	FFKM	Silicone	PEEK	PTFE	ETFE	CTFE	Kalrez	PP	PVDF	PE	Titan. alloy	Quartz	Gold	Alum. oxide	Stainl. st.	Ruby/ sapphire
Pump P-900			X	X			X		X	X	X			X	X	X
Monitor UV-900			X	X							X	X				
Monitor UPC-900	X		X	X	X	X					X	X				
Flow restrictor			X	X	X								X			
Mixer M-925	X		X	X												
INV-907			X													
Unions/ Connectors			X		X											
Online filter			X					X								
Inlet filters								X			X					

FFKM = perfluororubber

PEEK™ = polyetheretherketone

PTFE = polytetrafluoroethylene

ETFE = ethylenetetrafluoroethylene

CTFE=chlorotrifluoroethylene

ECTFE = ethylenechlorotrifluoroethylene

PP = polypropylene

PVDF = polyvinylidene fluoride

PE = polyethylene

8.7 Chemical resistance guide and chemical compatibility

The chemical resistance of ÄKTApurifier to some of the most commonly used chemicals in liquid chromatography is indicated in the table below.

The ratings are based on the following assumptions:

- 1 The synergistic effects of the chemical mixtures have not been taken into account.
- 2 Room temperature and limited over-pressure is assumed.

Note: Chemical influences are time and pressure dependent. Unless otherwise stated, all concentrations are 100%.

Chemical	Exposure		Comments
	< 1 day	up to 2 months	
Acetaldehyde	OK	OK	
Acetic acid, < 5%	OK	OK	
Acetic acid, 70%	OK	OK	
Acetonitrile	OK	OK	FFKM, PP and PE swell. PEEK is affected by long term use
Acetone, 10%	OK	Avoid	PVDF is affected by long term use
Ammonia, 30%	OK	OK	Silicone is affected by long term use
Ammonium chloride	OK	OK	
Ammonium bicarbonate	OK	OK	
Ammonium nitrate	OK	OK	
Ammonium sulphate	OK	OK	
1-Butanol	Ok	OK	
2-Butanol	OK	OK	
Citric acid	OK	OK	
Chloroform	OK	Avoid	ECTFE, PP and PE are affected by long term use
Cyclohexane	OK	OK	
Detergents	OK	OK	
Dimethyl sulphoxide	Avoid	Avoid	PVDF is affected by long term use
1, 4-Dioxane	Avoid	Avoid	ETFE, PP, PE and PVDF are affected by long term use
Ethanol	OK	OK	

8 Reference information

8.7 Chemical resistance guide and chemical compatibility

Chemical	Exposure		Comments
	< 1 day	up to 2 months	
Ethyl acetate	OK	Avoid	Silicone not resistant. Pressure limit for PEEK decreases.
Ethylene glycol	OK	OK	
Formic acid	OK	OK	Silicone not resistant
Glycerol	OK	OK	
Guanidinium hydrochloride	OK	OK	
Hexane	OK	Avoid	Silicone not resistant. Pressure limit for PEEK decreases.
Hydrochloric acid, 0.1 M	OK	OK	Silicone not resistant
Hydrochloric acid, > 0.1 M	OK	Avoid	Silicone not resistant. Titanium is affected by long term use
isopropanol	OK	OK	
Methanol	OK	OK	
Nitric acid, diluted	OK	Avoid	Silicone not resistant
Nitric acid, 30%	Avoid	Avoid	Elgiloy is affected by long term use
Phosphoric acid, 10%	OK	Avoid	Titanium and aluminium oxide are affected by long term use
Potassium carbonate	OK	OK	
Potassium chloride	OK	OK	
Pyridine	Avoid	Avoid	ETFE, PP and PE not resistant
Sodium acetate	OK	OK	
Sodium bicarbonate	OK	OK	
Sodium bisulphate	OK	OK	
Sodium borate	OK	OK	
Sodium carbonate	OK	OK	
Sodium chloride	OK	OK	
Sodium hydroxide, 1-2 M	OK	Avoid	Titanium, PVDF and borosilicate glass are affected by long term use
Sodium sulphate	OK	OK	
Sulphuric acid, diluted	OK	Avoid	PEEK and titanium are affected by long term use
Sulphuric acid, medium concentration	Avoid	Avoid	
Tetrachloroethylene	Avoid	Avoid	Silicone, PP and PE are not resistant

Chemical	Exposure		Comments
	< 1 day	up to 2 months	
Tetrahydrofuran	Avoid	Avoid	Silicone, ETFE, CTFE, PP and PE are not resistant
Toluene	OK	Avoid	Pressure limit for PEEK decreases
Trichloroacetic acid, 1%	OK	OK	
Trifluoroacetic acid, 1%	OK	OK	
Urea	OK	OK	
o-Xylene p-Xylene	OK	Avoid	Silicone, PP and PE are affected by long term use

8.8 Ordering information

Item	Quant. /pack	Code no.
ÄKTApurifier 10 system	1	28-4062-64
ÄKTApurifier 100 system	1	28-4062-66
ÄKTApurifier UPC 10 system	1	28-4062-68
ÄKTApurifier UPC 100 system	1	28-4062-71
Monitor pH/C -900	1	18-1107-76
Pump P-901		
Pump P-901	1	18-1114-00
Sealing kit, 100 ml, including 4 piston seals and 4 rinse membranes	1	18-1113-12
Piston kit, 100 ml	1	18-1112-13
Inlet tubing kit, 100 ml	1	18-1114-40
Pump head capillaries 100 ml (two capillaries for one pump module)	1	18-1117-52
Rinsing/draining housing 100 ml	1	18-1112-03
Pump head, 100 ml complete	1	18-1128-48
Pump P-903		
Pump P-903	1	18-3000-00
Sealing kit, 10 ml, including 2 piston seals, 2 membranes and 2 O-rings	1	18-1120-77
Piston kit, 10 ml	1	18-1120-75
Inlet tubing kit, 10 ml	1	18-1119-26
Pump head capillaries 10 ml (two capillaries for one pump module)	1	18-1120-81
Rinsing/draining housing 10 ml	1	18-1120-76
Pump head, 10 ml complete	1	18-1128-47
Pumps P-901 and P-903		
Tubing kit for rinsing system	1	18-1113-32
Purge kit	2	18-1124-53
Purge valve	1	18-1128-87
O-ring for purge valve	10	19-0036-01
Check valve kit including: 1 Inlet check valve 1 Outlet check valve	1	18-1128-66

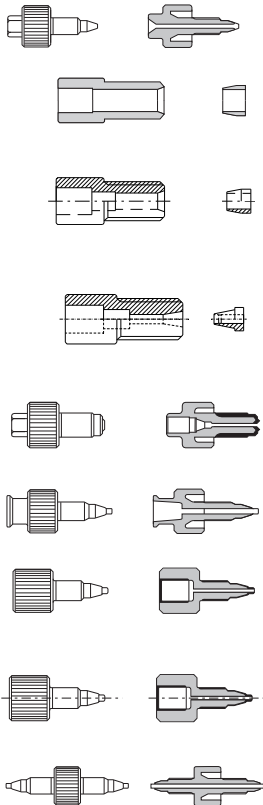
Item	Quant. /pack	Code no.
Monitor UPC-900		
Monitor UPC-900	1	18-1128-95
HG lamp & housing complete	1	18-1128-22
Zn lamp & housing complete	1	18-1128-23
UV flow cell 5 mm	1	18-1128-24
UV flow cell 2 mm	1	18-1128-25
Filter 214 nm	1	18-0622-01
Filter 254 nm	1	18-0620-01
Filter 280 nm	1	18-0621-01
Filter 313 nm	1	18-0623-01
Filter 365 nm	1	18-0624-01
Filter 405 nm	1	18-0625-01
Filter 436 nm	1	18-0626-01
Filter 546 nm	1	18-0627-01
Filter wheel complete	1	18-0647-01
Monitor UV-900		
Monitor UV-900 complete but without flow cells	1	18-1108-35
Flow cell UV-900, 2 mm	1	18-1111-10
Flow cell UV-900, 10 mm	1	18-1111-11
Fibre detachment tool	1	18-1111-16
pH and conductivity monitoring		
pH electrode with cell and holder, round tip	1	18-1134-84
pH electrode, round tip	1	18-1111-26
Dummy electrode, round tip	1	18-1111-03
Conductivity cell	1	18-1111-05
Mixer M-925		
Mixer M-925 including one UniNet cable	1	18-1118-89
Mixing chambers		
0.6 ml	1	18-1118-90
2 ml	1	18-1118-91
5ml	1	18-1118-92
12 ml	1	18-1118-93

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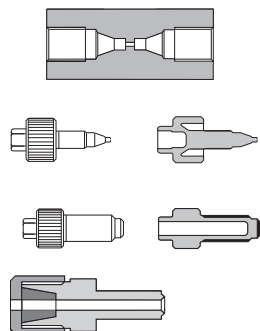
Item	Quant. /pack	Code no.
Valve INV-907		
Valve INV-907 including one UniNet cable (fill port, needle and syringe holder are not included)	1	18-1108-40
Injection kit INV-907 including: fill port 0.7 mm, needle and syringe holder	1	18-1110-89
Valve kit INV-907 including channel plate and distribution plate	1	18-1109-05
Mounting bracket	1	18-1109-11
Sample loops:		
10 µl	1	18-1120-39
100 µl	1	18-1113-98
500 µl	1	18-1113-99
1 ml	1	18-1114-01
2 ml	1	18-1114-02
Cables		
UniNet, 0.18 m	1	18-1109-72
UniNet, 0.3 m	1	18-1109-73
UniNet, 0.7 m	1	18-1109-74
UniNet, 1.5 m	1	18-1117-75
UniNet, 3.0 m	1	18-1109-75
UniNet, 15.0 m	1	18-1117-74
Mains cable, US standard	1	19-2447-01
Mains cable, EU standard	1	19-2448-01
Signal Cable, 6 pin mini DIN-open	1	18-1110-64
Inlet filter		
Inlet filter set	5	11-0004-14
Filter holder kit	1	11-0004-07
Screw lid GL45 kit	1	11-0004-10
Inlet filter kit, 1 filter, 1 net, 1 lid (GL45)	1	18-1113-15
Tubing		
PEEK tubing, i.d. 0.25 mm, o.d. 1/16"	2 m	18-1120-95
PEEK tubing, i.d. 0.50 mm, o.d. 1/16"	2 m	18-1113-68
PEEK tubing, i.d. 0.75 mm, o.d. 1/16"	2 m	18-1112-53
PEEK tubing, i.d. 1.0 mm, o.d. 1/16"	2 m	18-1115-83

Item	Quant. /pack	Code no.
Tubing		
Tefzel tubing, i.d. 0.25 mm, o.d. 1/16"	2 m	18-1121-36
Tefzel tubing, i.d. 0.50 mm, o.d. 1/16"	2 m	18-1120-96
Tefzel tubing, i.d. 0.75 mm, o.d. 1/16"	2 m	18-1119-74
Teflon tubing, i.d. 0.75 mm, o.d. 1/16"	2 m	18-1112-54
Teflon tubing, i.d. 1.6 mm, o.d. 1/8"	3 m	18-1121-16
Teflon tubing, i.d. 2.9 mm, o.d. 3/16"	3 m	18-1112-47
Tubing kit: ÄKTApurifier 10, i.d. 0.25 mm	1	18-1123-34
Tubing kit: ÄKTApurifier 10, i.d. 0.5 mm	1	18-1123-33
Connectors and unions		
Fingertight connector, 1/16" for 1/16" o.d. tubing, PEEK	10	18-1112-55
Tubing connector, for 3/16" o.d. tubing, PEEK	10	18-1112-49
Ferrules, for 3/16" o.d. tubing, PEEK	10	18-1112-48
Tubing connector for 1/8" o.d. tubing, PEEK	10	18-1121-17
Ferrules for 1/8" o.d. tubing, PEEK	10	18-1121-18
Tubing connector for 1/16" o.d. tubing, PEEK	10	18-1127-07
Ferrules for 1/16" o.d. tubing, PEEK	10	18-1127-06
Union, 1/16" female/M6 male, PEEK	6	18-1112-57
Union, luer female/1/16" male, PEEK	2	18-1112-51
Union, M6 female/1/16" male, PEEK	8	18-1112-58
Union 5/16"-32 female/HPLC male, PEEK	8	18-1142-08
Union, 1/16" male/1/16" male, for 1/16" o.d. tubing, PEEK	10	18-1120-92



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Item	Quant. /pack	Code no.
Connectors and unions		
Union, 1/16" female/1/16" female, for 1/16" o.d. tubing, titanium	1	18-3855-01
Stop plug, 1/16", PEEK	5	18-1112-52
Stop plug, 5/16", PEEK	5	18-1112-50
Tubing connector/M6 male	1	18-1017-98
Accessories		
ÄKTApurifier BufferPrep kit, including two switch valves and holders. Tubing and connectors for 10-systems and 100-systems.	1	28-4044-73
Flow restrictor, FR-902, 0.2 MPa	1	18-1121-35
Flow restrictor, FR-904, 0.4 MPa	1	18-1119-63
Short column holder	1	18-1113-17
Long column holder	1	18-1126-32
Small column clamp	1	18-1149-98
Lab rod holder	1	18-1113-19
Tubing cutter	1	18-1112-46
On-line filter, (10 ml systems)	1	18-1118-01
On-line filter kit, (10 ml systems) 2 filters	2	18-1120-94
On-line filter, (100 ml systems)	1	18-1112-44
On-line filter kit, (100 ml systems) 10 filters and 2 nets	10	18-1027-11
On-line filters, (100 ml systems) 25 filters	25	18-1130-23

Item	Quant. /pack	Code no.
User Manuals		
ÄKTApurifier Manual Box complete, containing all User Manuals and Instructions for all components in ÄKTApurifier 10/100	1	11-0014-51
ÄKTApurifier UPC Manual Box complete, containing all User Manuals and Instructions for all components in ÄKTApurifier UPC 10/100	1	11-0014-51
UNICORN 5.10 Manual kit including - Getting Started - User Reference Manual Chapter part 1 - User Reference Manual Chapter part 2 - Administration and Technical Manual	1	28-4010-25
ÄKTApurifier User Guide	1	28-4079-53
ÄKTApurifier Getting started	1	28-4079-54
Optional Configurations ÄKTApurifier User manual	1	28-4079-56
Pump P-900 User Manual	1	18-1120-04
Monitor UPC-900 User Manual	1	18-1125-55
Monitor UV-900 User Manual	1	18-1120-05

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