ÄKTApilot™

Instrument Handbook

Important user information

This document is a handbook with complementing information to the Operating Instructions. All users must read the safety instructions in the ÄKTApilot Operating Instructions to fully understand the safe use of the ÄKTApilot system, before installing, using, or maintaining the system.

Do not operate the ÄKTApilot system in any other way than described in the user documentation, or you may be exposed to hazards that can lead to personal injury, and damage the equipment.

WARNING!

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

Caution!

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

Note

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

CE Conformity

This product complies with the European directives listed in the table, by fulfilling the corresponding harmonized standards.

2006/42/EC Machinery Directive (MD)

2006/95/EC Low Voltage Directive (LVD)

2004/108/EC Electromagnetic Compatibility (EMC) Directive

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

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

- used as a stand-alone unit, or
- connected to other products recommended or described in this manual, and
- used in the same state as it was delivered from GE Healthcare except for alterations described in this manual.

Recycling

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

WARNING!

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

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1 Introduction

ÄKTApilot™ is a high performance, automated liquid chromatography system. The system is designed for process development, process scale-up and scale-down, and small scale production.

In process development, ÄKTApilot simplifies the transition from laboratory purification to full scale production. Scale-up to production is predictable and trouble-free. It can also be used in scale-down studies in process robustness, cleaning and virus validation, media selection, media lifetime studies, and method scouting.

In small-scale production, the system can be used to produce purified material for clinical testing programs, or for small-scale production of diagnostic or therapeutic products.

ÄKTApilot system is based on proven liquid chromatography techniques, such as ion exchange, gel filtration, affinity chromatography, and hydrophobic interaction. The system can be used with pre-packed columns as well as with standard laboratory and pilot-scale columns packed with media. ÄKTApilot can also be used for packing columns.

ÄKTApilot system is biocompatible, hygienic and sanitizable. It meets all GLP and cGMP demands for Phase I–III in drug development and final-scale production.

ÄKTApilot features:

- Liquid flow rates from 4 to 800 ml/min.
  (The gradient performance is limited from 400 to 800 ml/min. See also *ÄKTApilot Operating instructions*.)
- Operating pressure up to 20 bar.

1.1 The Instrument Handbook

This handbook provides safety instructions, technical information and basic operating instructions for ÄKTApilot system. In addition, maintenance schedules and instructions for troubleshooting and user maintenance are included.

1.2 ÄKTApilot system

ÄKTApilot system comprises ÄKTApilot separation unit and a PC. A flat-screen monitor is included in the system.

Fig 1-1. The ÄKTApilot system

1.2.1 System control

UNICORN™ control system controls and supervises the ÄKTApilot chromatography system. It runs on the PC under Windows® XP operating system, and includes hardware for interfacing the controlling PC to the chromatography liquid handling parts of ÄKTApilot.

UNICORN controls the run data acquisition from sensors and monitors. UNICORN also evaluates results and generates reports.

1.2.2 Separation unit components

The location of the components in the separation unit is shown in the following figure.

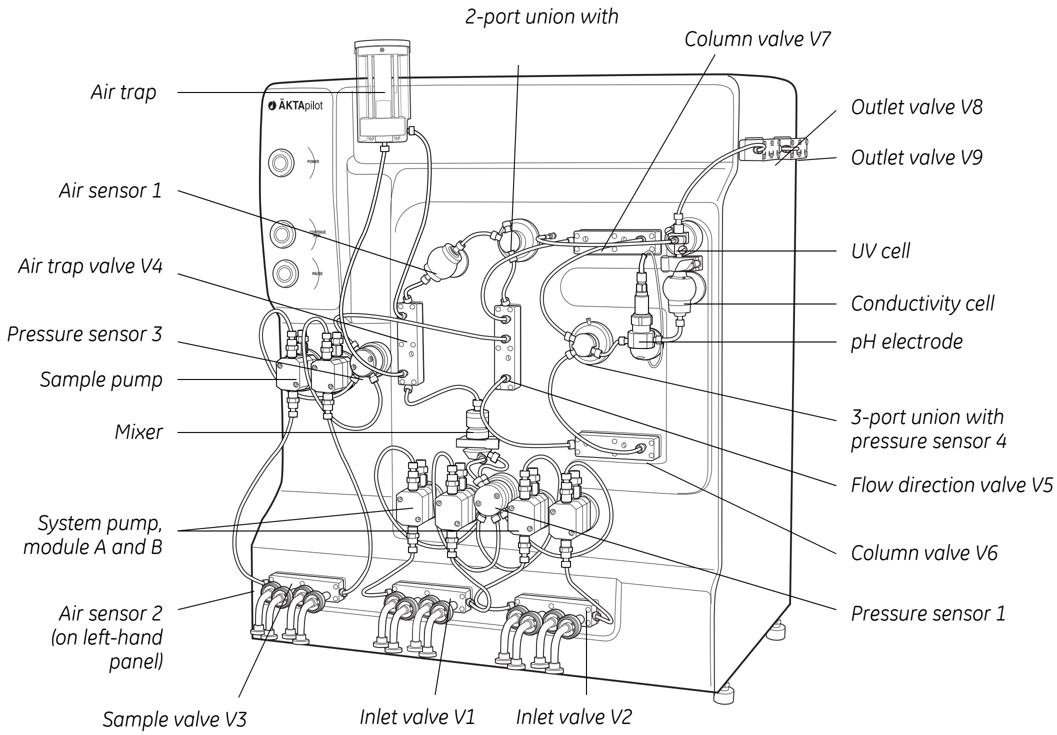


Fig 1-2. Location of components

1.2.3 Installation and localization of system

> **WARNING!** Installation, moving and service of ÄKTApilot must only be performed by authorized GE service personnel.

ÄKTApilot is designed for indoor use only. It requires a working area of about 200 × 80 cm (width × depth) and should be placed on a stable laboratory bench that can withstand a minimum weight of 200 kg.

> **CAUTION!** To maintain correct ventilation, the system requires a minimum clearance of at least 0.3 m on all sides. Do not block the ventilation inlets (underneath the system and next to the front panel) or outlets on the system.

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

WARNING! In a situation where there is a risk of injury, switch off the mains power switch (located on the left side of the system) to position **o**.

WARNING! Do not block the mains plug. In case of emergency the mains plug must always be easy to access.

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

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

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

See section 5.3.1 for detailed technical specifications.

1.2.4 Identification and rating labels

The identification and rating labels are located on the lower part of the rear panel of ÄKTApilot separation unit (see Fig. 1-3). These ratings determine the electrical hazards of the equipment connected to the supply voltage. There are, however, other hazards that might be more severe, see *ÄKTApilot Safety Handbook*.

Voltage	100–240V~
Frequency	50–60 Hz
Power, max	800 VA

CE

System No. 123456	Code No. 18-1164-95

Fig 1-3. Layout of identification and rating labels

1.2.5 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.

1.3 Operating principles

This section gives an introduction to the function of ÄKTApilot separation unit.

ÄKTApilot contains all the fluid handling components required to perform buffer delivery and mixing, sample application, and in-line monitoring. The individual control units for the fluid handling components are located inside ÄKTApilot separation unit.

1.3.1 Liquid flow path

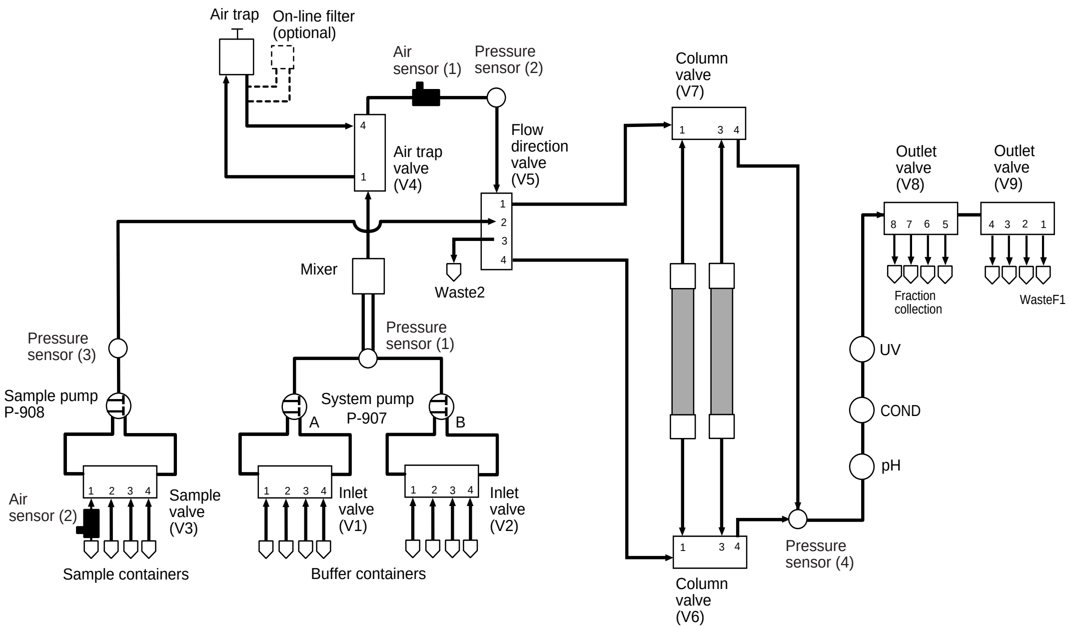


Fig 1-4. The liquid flow path of ÄKTApilot

1. The sample valve selects an appropriate sample inlet. The sample pump then delivers the sample solution to the column via a pressure sensor, the flow direction valve and the selected column valve. An air sensor can be used on the inlet tubing to detect when the sample container is empty.

2. The inlet valves select buffer. The system pump then delivers the buffer through a pressure sensor and a mixer to the air trap valve. The flow can also be directed through an air trap (used for column protection).

3 After the air trap valve, the buffer flow is directed through an air sensor and a pressure sensor to the flow direction valve. The air sensor is used to prevent air from entering the column.

4 The flow path continues through one of the column valves to the column. The flow passes through the packed column where the separation takes place.

5 The flow then passes a pressure sensor, the pH cell holder, the conductivity cell, and the UV cell.

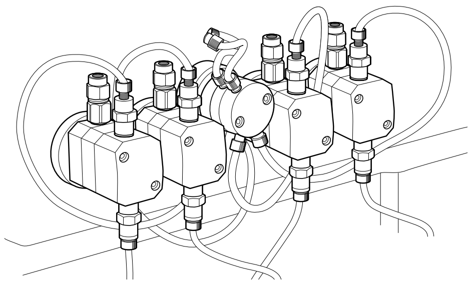
6 The flow path continues to the outlet valves, which are used to divert the flow to waste or fraction collection.

Pressure sensors continuously monitor the pressure in the flow path.

1.3.2 Liquid delivery

System pump

The system pump, P-907, is a high-performance laboratory pump producing an accurately controlled liquid flow. It is a low pulsation pump equipped with two pump modules; A and B. This allows for binary gradients with efficient mixing.



A pressure sensor (Press1) is connected to pump module A (the left-hand pump heads). The pressure sensor can be used in combination with an over-pressure alarm function, which is controlled from UNICORN.

The pump heads are equipped with check valves at the system flow inlet and outlet, and at the rinsing flow outlet.

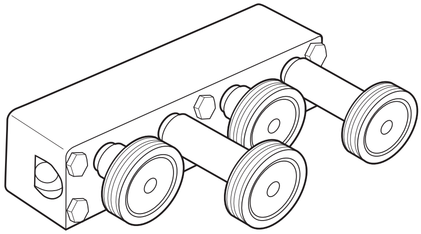
The system pump has an operating flow rate range up to 800 ml/min in isocratic mode (400 ml/min in gradient mode) and a maximum allowed pressure of 20 bar.

Sample pump

The sample pump, P-908, is identical with the system pump but has only one pump module. P-908 is also equipped with a pressure sensor (Press2).

Membrane valves

Stepper motor-actuated membrane valves control the liquid flow in the separation unit. The valves are located in nine valve blocks.



A valve block consists of a connection block containing the ports and the membranes, and a mechanical housing containing the stepper motors, cams and actuating pistons.

The valve blocks have different numbers of inlet and outlet ports depending on their position in the flow path.

There are six main types of valve block:

- Inlet valve V1 and V2 and sample valve V3
- Air trap valve V4
- Flow direction valve V5
- Column valve V6 and V7
- Outlet valve V8
- Outlet valve V9

The valve seats in the valves have two different sizes in order to withstand the operating pressure in the flow path where the valve is located.

1.3.3 Mixing

Buffers delivered by the system pump are dynamically mixed by an electrically operated mixer. The volume of the mixer chamber included in the system is 5 ml.

1.3.4 Sample application

The sample solution is applied directly onto the column by the sample pump or the system pump.

1.3.5 Columns

A wide range of columns for ion exchange, gel filtration, hydrophobic interaction and affinity chromatography is suitable for use with ÄKTApilot. It includes some pre-packed columns as well as standard laboratory and pilot-scale columns that can be packed with media using ÄKTApilot.

Contact your local GE representative for recommended columns.

WARNING! OVER-PRESSURE. Use columns that withstand expected pressures. If not, the columns might rupture, resulting in injury.

WARNING! OVER-PRESSURE. The flow rate may under no circumstances exceed the specified column maximum flow rate. The flow might affect the packed column, causing the pressure to exceed the specified column maximum pressure.

1.3.6 Detectors and monitors

ÄKTApilot is equipped with detectors for continuous in-line measurement of pH, conductivity and UV absorbance. The detectors provide accurate and reliable monitoring.

The flow cells are connected close together, which minimizes band broadening and time delay between the detectors. The flow cells are easily accessible from the front panel to facilitate maintenance.

pH measurement

The pH electrode is mounted in the pH cell holder after pressure sensor 4 in the flow path. The electrode is in continuous contact with the liquid. The pH monitor provides pH measurement in the range 1–14 (2-12 within specifications).

Conductivity measurement

The conductivity cell is placed after the pH electrode in the flow path. It is used to verify gradients, follow peak positions relative to ionic strength, and for temperature measurement using a built-in sensor. The measurement range is 1 µS/cm to 999.9 mS/cm.

UV absorbance measurement

The UV cell is placed after the conductivity cell in the flow path. It is used for measuring the UV absorbance of the liquid. The location of the UV cell near the outlet minimizes the delay volume during fractionation. Up to three wavelengths can be measured simultaneously in the range 190–700 nm.

1.3.7 Air trap and air sensors

The buffer flow can be directed through an air trap by the air trap valve V4. The air trap is used for removing air from the buffer in order to enhance system performance and to prevent air from damaging the column performance.

There are also two air sensors in the system for preventing air from entering and damaging the packed column. The air sensor at the sample inlet detects air in the sample inlet tubing, for example, when the sample container is empty. The other air sensor is located between the air trap valve V4 and the flow direction valve V5.

Note: *Make sure that the air sensors are filled with liquid before enabling. If not, an alarm will be raised immediately and the system set to Pause.*

1.3.8 Tubing and connectors

At delivery of ÄKTApilot system, all internal tubing is connected. The tubing has an inner diameter (i.d.) of 2.9 mm and an outer diameter (o.d.) of 3/16", and is made of ETFE. The connectors have rubber O-rings for proper sealing and sanitizability.

The system inlet and outlet tubing is packed separately at delivery. The inlet tubing has i.d. 6 mm and o.d. 13 mm and is equipped with TC connectors at both ends. The outlet tubing has i.d. 2.9 mm and o.d.
3/16" and the same type of connector as the internal tubing at one end.

Small-bore tubing

Tubing with i.d. 1.6 mm is available to enhance system performance at low flow rates. The tubing replaces the original tubing i.d. 2.9 mm tubing from the sample valve and the inlet valves to the outlet valves. Observe that when using this tubing, the system flow path might not be sanitizable.

1.3.9 Fraction collection

When the purified material has passed the monitor flow cells, it can be collected in fractions using the outlet valves. The outlet valves have 8 outlets in total. The minimum volume for a fraction is 40ml.

A separate fraction collector, e.g. Fraction Collector Frac-950, can be connected to the outlet valves for collecting multiple fractions. Observe that when using Frac-950, the maximum allowed flow rate during fractionation is 100 ml/min.

1.4 Sanitization of the flow path

The fluid handling components in ÄKTApilot have been designed to be compatible with recommended sanitization procedures. The Method Wizard includes a ready-made method for sanitizing the system. To facilitate compliance with GLP and cGMP requirements, all wetted parts in the flow path are replaceable.

Note: *The circlips are not resistant to alkali and acids.*

More information about sanitizing the system is found in the *ÄKTApilot Operating instructions*.

1.5 Associated documentation

The documentation listed below is also included with ÄKTApilot system.

The *ÄKTApilot Operating Instructions* contains operating instructions.

The *ÄKTApilot User Manual* contains operating instructions.

The *ÄKTApilot Safety Handbook* contains safety information.

UNICORN control system package includes three manuals:

- *Getting Started*
- *User Reference Manuals (2 pcs.)*
- *Administration and Technical Manual*

2 Basic operation

This chapter provides basic operating instructions for ÄKTApilot system. See *ÄKTApilot Operating instructions* for more detailed instructions.

The chapter also contains instructions for calibrating the monitors.

The start-up instructions in this chapter assume that the system has been correctly installed by GE personnel.

2.1 Starting the system

To start ÄKTApilot system:

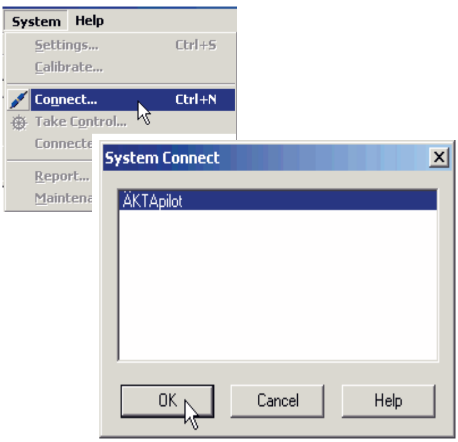
1 Switch on the separation unit by turning the mains power switch (located on the left side panel) to position ***1***.

The ***POWER*** indicator on the separation unit flashes rapidly for a few seconds during the internal communication test. After the test, the indicator flashes slowly.

2 Switch on power to the PC and the monitor.

3 Start and log on to UNICORN by double-clicking on the icon on the Windows desktop.

4 In the ***System Control*** module, select ***System:Connect...*** to connect UNICORN to the separation unit.



5 Select the appropriate system name and click ***OK***.

When the communication between UNICORN and the separation unit is established:

- There is a constant light on the ***POWER*** indicator on the separation unit.
- The green ***RUN*** indicator in the status bar in UNICORN is lit.

2.2 Performing a run

2.2.1 Creating a method

See the UNICORN user manual *Getting Started* or the ÄKTApilot *Operating instructions* for step-by-step instructions to create a method using the Method Wizard.

2.2.2 Preparing the system

Before starting the method, check the following:

1 The inlet tubings are immersed in or connected to the correct containers.

2 There is sufficient buffer available

3 The waste containers are not full and will accept the volume diverted to them during the run.

4 The inlet tubing and the pumps are primed.

5 The pH electrode is calibrated (if required). Refer to section 2.4.2 Calibrating the pH electrode.

6 The correct column has been fitted and equilibrated (if not included in the method).

7 The outlet tubings are immersed in the correct containers for fraction collection and waste.

2.2.3 Running a method

See the UNICORN user manual *Getting Started* for step-by-step instructions to run a method.

2.3 After the run

Clean the system according to the instructions in section 3.2.1 Cleaning the system.

2.4 Calibration

The monitors in ÄKTApilot system need to be calibrated regularly for correct results. This section shows the type of calibrations that can be done, how to perform the calibrations and how often. The calibrations are performed from UNICORN by selecting ***System:Calibrate...*** in the ***System Control*** module.

Component	How often
Pressure sensors	Once a year or when changing the pressure sensor
pH electrode	Every day
Conductivity cell	
Temperature	Must be done when changing the cell.
Entering a new cell constant	Must be done when changing the cell.
Cell constant	Only necessary when specific conductivity with high accuracy is measured.
UV cell (length)	Only necessary when high accuracy in the absorbance measurement is desired

2.4.1 Calibrating the pressure sensors

In pressure sensor 2 (before the columns) and 4 (after the columns), the zero pressure-reading can be calibrated. The amplification in these two sensors is already calibrated at the factory.

In pressure sensor 1 (system pump) and pressure sensor 3 (sample pump), the zero pressure-reading as well as the amplification can be calibrated.

Calibrating pressure sensor 2

To calibrate the zero pressure-reading:

1. In the ***System Control*** module, select ***Manual:Flowpath...***
2. Set the instruction ***Waste2*** to ***Open***. Click ***Execute*** and then ***Close***.
3. Make sure that the end of the Waste2 tubing is open.
4. Select ***System:Calibrate...***
5. In the ***Calibration*** window, select ***Press2*** from the ***Monitor*** pop-up menu.
6. Wait for the pressure reading to stabilize and then click ***Start Calibrate***. When the calibration is finished, click ***Close***.
7. Click ***End*** in the Status bar to reset the valves.

Calibrating pressure sensor 4

To calibrate the zero pressure-reading:

1. In the ***System Control*** module, select ***Manual:Flowpath...***
2. Set the instruction ***Outlet*** to ***WasteF1***. Click ***Execute*** and then ***Close***.
3. Select ***System:Calibrate...***
4. In the ***Calibration*** window, select ***Press4*** from the ***Monitor*** pop-up menu.
5. Wait for the pressure reading to stabilize and then click ***Start Calibrate***. When the calibration is finished, click ***Close***.
6. Click ***End*** in the Status bar to reset the valves.

Calibrating pressure sensor 1

Note: *If using pressure sensor 2 for reading the pressure during the calibration, it must be calibrated first*.

To calibrate the zero pressure-reading:

1 In the ***System Control*** module, select ***Manual:Flowpath...***

2 Set the instruction ***Airtrap_Filter*** to ***Bypass***. Click ***Execute***.

3 Set the instruction ***Waste2*** to ***Open***. Click ***Execute***.

4 Select ***System:Calibrate...***

5 In the ***Calibration*** window, select ***Press1***.

6 Click ***Read value 1*** when the pressure is stable. The zero pressure-reading is now calibrated. Do not close the ***Calibration*** window!

To calibrate the amplification:

1 There are two alternatives to measure the pressure:

- If using an external pressure gauge, connect it to the outlet port on the 3-port union (Press4) using a 5/16" connector.
- If using pressure sensor 2, connect a stop plug to the outlet port on the 3-port union (Press4).

2 Immerse the tubing from valve 1, port 1, in distilled water.

3 In ***Manual:Flowpath...***, set ***InletA*** to ***InletA1*** and click ***Execute***.

4 Set ***InletB*** to ***InletB1*** and click ***Execute***.

5 Also set ***Waste2*** to ***Closed*** and ***Columns*** to ***Col1BypassCol2Bypass***.

6 In ***Manual:Pump...***, set ***Flow*** to 0.5 ml/min and click ***Execute***.
The system pump starts and the pressure increases slowly.

7 Set ***Flow*** to 0 ml/min. When the ***Press2*** reading in the ***Run data*** view pane (or the external pressure gauge) reaches 1.7–1.9 MPa, click ***Execute***. The system pump stops.

8 Type the pressure value in the ***Reference value 2*** field and click ***Read value 1***. The amplification is now calibrated. Click ***Close***.

9 Release the over-pressure by setting ***Waste2*** to ***Open***. Click ***Execute***.

10 Click ***End*** in the Status bar to reset the valves when overpressure is released.

Calibrating pressure sensor 3

Note: *If using pressure sensor 2 for reading the pressure during the calibration, it must be calibrated first*.

To calibrate the zero pressure-reading:

1 In the ***System Control*** module, select ***Manual:Flowpath...***

2 Set the instruction ***SampleInlet*** to ***Sample1*** and click ***Execute***.

3 Set the instruction ***Waste2*** to ***Open*** and click ***Execute***.

4 Select ***System:Calibrate...***

5 In the ***Calibration*** window, select ***Press3***.

6 Click ***Read value 1*** when the pressure is stable. The zero pressure-reading is now calibrated. Do not close the ***Calibration*** window!

To calibrate the amplification:

1 When calibrating the amplification, there are two alternatives to measure the pressure:

- If using an external pressure gauge, connect it to the outlet port on the 3-port union (Press4) using a 5/16" connector.
- If using pressure sensor 2, connect a stop plug to the outlet port on the 3-port union (Press4).

2 Immerse the tubing from valve 3, port 1, in distilled water.

3 Set ***Waste2*** to ***Closed*** and click ***Execute***.

4 Set ***Columns*** to ***Col1BypassCol2Bypass*** and click ***Execute***.

5 In ***Manual:Pump...***, set ***SampleFlow*** to 0.5 ml/min and click ***Execute***. The system pump starts and the pressure increases slowly.

6 Set ***SampleFlow*** to 0 ml/min. When the ***Press2*** reading in the ***Run data*** view pane (or the external pressure gauge) reaches 1.7–1.9 MPa, click ***Execute***. The system pump stops.

7 Type this pressure value in the ***Reference value 2*** field and click ***Read value 1***. The amplification is now calibrated. Click ***Close***.

8 Release the over-pressure by setting ***Waste2*** to ***Open***. Click ***Execute***.

9 Click ***End*** in the Status bar to reset the valves.

2.4.2 Calibrating the pH electrode

A good laboratory routine is to calibrate the pH electrode at least once a day, when the electrode is replaced and if the ambient temperature is changed. The pH electrode is calibrated using standard buffer solutions in a two point calibration. The two buffer solutions may have any pH value as long as the difference between them is at least 1 pH unit, and the expected pH during the run is within this interval.

The calibration procedure should be done with the pH electrode fitted in the cell holder (in-line) for best result. However, it is also possible to calibrate with the pH electrode out of the cell holder although the reading might be different due to the flow around the electrode.

Calibrating with the electrode fitted in the cell holder

To calibrate the pH electrode:

1 Prepare two standard buffer solutions – reference solution 1 (normally pH 7.0) and 2. The difference in pH value between them must be at least 1 pH unit. The expected pH value during the run should be within this interval.

2 Immerse the inlet tubing from inlet A1 in reference solution 1 and the inlet tubing from inlet A2 in reference solution 2.

3 In the ***System Control*** module, select ***Manual:Pump***. Select the instruction ***SystemPumpWash*** and choose ***InletA1***. Click on ***Execute***.
The system pump and the inlet tubing will now be filled with reference solution 1.

4 When the ***SystemPumpWash*** is finished, click the ***Flowpath*** button.
Check that the following instructions are set as listed below:

InletB – InletB1
InletA – InletA1
Airtrap_filter – Bypass
Columns – Col1_Col2_BypassHigh
Waste2 – Closed
Outlet – WasteF1

5 Click on the ***Pump*** button and select the instruction ***Flow***. Enter the flow rate that will be used later during the run and click ***Execute***.

6. In the ***System Control*** module, select ***System:Calibrate...*** and select ***pH*** from the ***Monitor*** pop-up menu.

7. The ***Measured value*** field shows the actual pH reading according to the previous calibration. Allow at least 35 ml of reference solution 1 to pass through the cell holder.

8. When the pH is stable, enter the known pH value of reference solution 1 in the ***1. Reference value 1*** field. Click ***Read value 1***.
   Stop the pump.

9. Switch to the second reference solution by selecting ***Manual:Pump*** and the instruction ***SystemPumpWash***. Select ***InletA2*** and click ***Execute***.

10. When the ***SystemPumpWash*** is finished, click the ***Flowpath*** button. Set the instruction parameters as in step 4 but change ***InletA*** to ***InletA2***.

11. Click on the ***Pump*** button and select the instruction ***Flow***. Enter the flow rate that will be used later during the run and click ***Execute.***

12. Repeat steps 7 and 8 for ***Reference value 2***.

13. After the calibration, values are automatically entered into the ***Asymmetry potential at pH7; mV*** and ***Calibrated electrode slope; %*** fields.

A new electrode has a slope of typically 95–102% and an asymmetry potential within ±30 mV. As the electrode ages, the slope decreases and the asymmetry potential increases.

As a rule, when the ***Asymmetry potential at pH7; mV*** value is outside of ±60 mV and the ***Calibrated electrode slope; %*** value is lower than 80%, and no improvement can be achieved by cleaning, the electrode should be replaced.

An electrode is still usable at lower slopes and higher asymmetry potentials but the response will be slower and the accuracy diminished.

Calibrating with the electrode outside the cell holder

When calibrating the electrode out of the cell holder and changing from one buffer to another, rinse the electrode tip with distilled water and dab it carefully with a soft tissue to absorb the remaining water. Do NOT wipe the electrode as this may charge it and give unstable readings.

1. In the ***System Control*** module, select ***System:Calibrate...***.

2. Select ***pH*** from the ***Monitor*** pop-up menu in the ***Calibration*** window.

3. Prepare two reference buffer solutions, the first normally pH 7.0. The difference in pH value between them must be at least 1 pH unit. The expected pH value during the run should be within this interval.

4. Remove the pH electrode from the cell holder and immerse the electrode in the first reference solution.

5. Enter the known pH value of the solution in the ***1. Reference value 1*** field.

6. The pH reading is shown under ***Measured value***. When the pH value has stabilized, click ***Read value 1***.

7. Rinse the electrode tip with distilled water and then immerse the electrode in the second reference solution (e.g. pH 4.0 or 9.0).

8. Enter the known pH value of the second reference solution in the ***3. Reference value 2*** field.

9. When the pH value has stabilized, click ***Read value 2***.
   The calibration is finished.

To evaluate the calibration result, see the previous section.

Before use, rinse the pH electrode using distilled water.

2.4.3 Calibrating the conductivity cell

Entering a new cell constant
After replacing the conductivity cell, the cell constant has to be set. This requires recalibration.

1. In the ***System Control*** module, select ***System:Calibrate...*** and then ***Cond_Cell*** in the ***Monitor*** pop-up menu.

2. Enter the cell constant in the ***1. Reference value 1*** field.

3. Click ***Read value 1***. The new cell constant is updated. Click ***Close***.

Calibrating the temperature sensor
Calibration of the temperature sensor in the conductivity cell is only necessary when the cell is used in high accuracy measurements or if the cell is replaced.

1. Place the conductivity cell together with a precision thermometer inside a box or empty beaker and ensure that they are not exposed to draft. Leave them for 15 minutes to let the temperature stabilize.

2 In the ***System Control*** module, select ***System:Calibrate...*** and choose ***Temp*** in the ***Monitor*** pop-up menu.

3 Read the temperature on the thermometer.

4 Enter the temperature in the ***1. Reference value 1*** field.

5 Click the ***Read value 1*** button.

Setting up conductivity temperature compensation

The conductivity of a buffer is temperature dependent. To relate conductivity to concentration and/or compare conductivity values, temperature compensation should be used. The compensation consists of a compensation factor together with a reference temperature. All conductivity values will then automatically be converted to the set reference temperature.

1 In the ***System Control*** module, select ***System:Settings...*** and click the ***Monitors*** radio button.

2 Choose the instruction ***CondTempComp***.

3 The factor is expressed in percentage increase of conductivity per °C increase in temperature. If the temperature compensation factor is unknown, a general approximate value of 2% can be set for many common salt buffers.

   If no compensation is desired, set the ***CompFactor*** to 0%.

   Enter an appropriate percentage value in the ***CompFactor*** field.

4 Choose the instruction ***CondRefTemp***.

5 Select the reference temperature to which the measured conductivity values will be converted (normally 20 or 25 °C).

   Enter an appropriate temperature in the ***RefTemp*** field.

6 Click ***OK***.

Calibrating the cell constant

Normally it is not necessary to adjust the cell constant as the cell is pre-calibrated on delivery. Adjustment is only necessary when replacing the conductivity cell with a cell whose cell constant is unknown, or when changing strategy. We also recommend that the conductivity cell is recalibrated after cleaning.

Note: *The conductivity temperature compensation must not be used when adjusting the cell constant. Set the compensation factor to 0 (see section Setting up conductivity temperature compensation).*

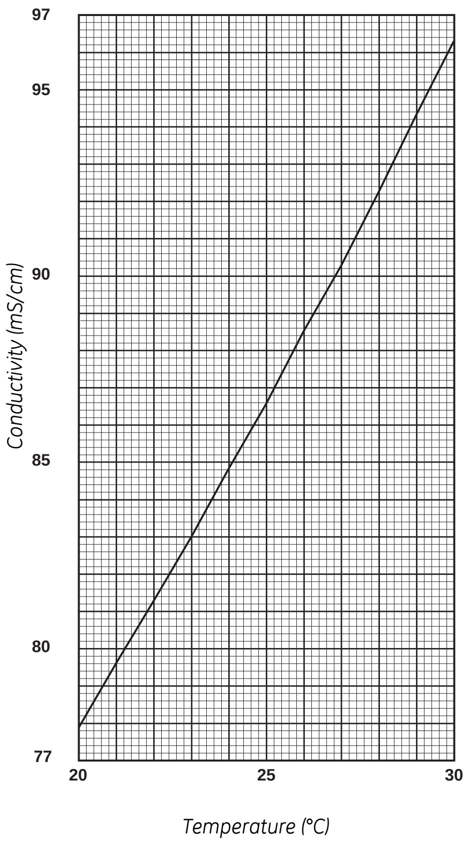
The temperature sensor must be calibrated before adjusting the cell constant (see section Calibrating the temperature sensor).

1. Prepare a calibration solution of 1.00 M NaCl, 58.44 g/1000ml. Let the solution reach room temperature. This is important for exact measurements.
2. Fill the cell completely with the calibration solution by pumping at least 15 ml through the cell using the system pump. Set the system to Pause to stop the pump.
3. When finished, wait for 15 minutes until the temperature is constant in the range 20–30 °C.
4. In the ***System Control*** module, select ***System:Calibrate...***
   Select ***Cond_Cal*** in the ***Monitor*** pop-up menu.
5. Read the conductivity value displayed under ***Measured value*** and compare it with the theoretical value from the graph opposite at the temperature of the calibration solution.

   If the displayed value and the theoretical value correspond, no further action is required.

   If the values differ, proceed with step 6 and 7.
6. Enter the theoretical conductivity value according to the graph in the ***1. Reference value 1*** field.
7. Click the ***Read value 1*** button. The new cell constant is automatically calculated and updated.

Conductivity of 1.00 M NaCl at 20-30°C



2.4.4 Measuring the real UV cell length

The real cell length in the UV cell might differ from the nominal length (1, 2 or 5 mm, 2mm being default), which can interfere with the calculation of the protein concentration in the eluate. To achieve normalized absorbance (±5%), the exact cell length in the UV cell can be measured and used for calibration.

Equipment required

To measure the real UV cell length you need:

- UV-900 Calibration kit for the cell length used . Use GE UV Linearity test kit, 18-1129-63, for the standard 2mm UV cell and 280 nm. (18-1129-69 for 5mm path length). The kit contains 5 bottles with different concentrations of Iron(III)Sulphate dissolved in 0.1 M sulfuric acid.

- UV-900 Cell Calibration Excel-file
  (found in folder ***UV-900 Calib*** on the UNICORN CD).

Preparing for measurement

1. Disable any previous calibration by first selecting ***System:Settings...*** in the ***System Control*** module. In the ***Instructions*** dialog, select ***Monitors:UVPathLengthCorrection***. Select ***Disabled*** and click ***Execute***.

2. For best results disconnect the tubing at the outlet from the UV cel and connect a 1.6mm O.D. capillary 0.75 mm I.D. approx. 20 cm long. Remove the short tubing between the conductivity cell and the pH cell. Dismount the clamp between UV cell and conductivity cell and dimount the conductivity cell and its holder. Dismount the left side holder for the rack for columns as well, it improves accecibillity for the UV cell. Use the connectors to attach the syringe to the UV cell inlet port. Fill the UV cell with the different solutions.

> **WARNING! HAZARDOUS CHEMICAL.** Sulfuric acid included in the test solutions is corrosive and therefore dangerous to health.
> Avoid spillage and use eye protection.

3. Unpack the calibration kit.

4. Connect a 5/16" male/M6 female (18-1169-16) to a connector TC 5/16" (18-1169-22) and connect to the UV cell TC connector. Do not forget the gasket.

5. Connect the short tubing supplied in the kit to the M6 female on the mounted connector and the luer connector to the other side of the tubing. Connect a syringe to the luer connector.

   Note: *Start with the lowest concentration, 0.1 M H_2SO_4, continue with increasing concentration. Wash the UV cell carefully with the solution,* *(i.e. inject a portion, ~1.2 ml, and redraw it to the syringe). Empty the syringe and fill up with fresh solution, ~2 ml, and inject that into the cell. For each reading perform a Snap Shot when UV signal is stable. Record the readings in the tables below:*

It is good to divide the UV values from the certificate, or the bottle, with the actual cell length in cm, it is enough with the nominal length, to indicate the level of the expected value.

When all concentrations are measured wash the cell with a portion of 0.1 M H_2SO_4 and wait until the monitor reading is close to zero. Rinse the cell with excess of water. Connect the cell to the flow path.

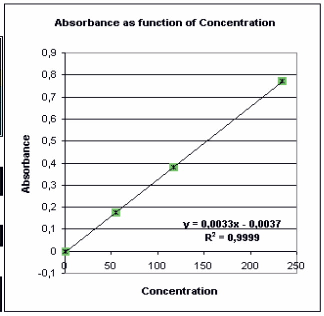
6 Open the UV-900 Cell Calibration Excel-file on the computer.

UV-900 cell calibration calculation

UV Test kit Concentration (mg/l)	UV Test kit Absorbance (AU/cm)	UV-900 Absorbance (AU)
0	0	0
55	0,828	0,1756
117	1,928	0,3795
234	3,84	0,7724

Real cell length	2,00
Coefficient of regression (R^2)	0,9999

Please observe that the coefficient of regression (R^2) shall be > 0,999
If this is not the case one or several measured values are faulty.



7 The bottles in the calibration kit are marked with the concentration value and the reference absorbance value for each solution.

- Enter the concentration of the solutions in increasing order in the column ***UV Test kit Concentration (mg/l)***.
- Enter the absorbance value in increasing order in the column ***UV Test kit Absorbance (AU/cm)***.

Performing the absorbance measurements

The absorbance will be measured and automatically compared to the reference value for each of the four test solutions.

WARNING! HAZARDOUS CHEMICAL. Sulfuric acid included in the test solutions is corrosive and therefore dangerous to health.
Avoid spillage and use eye protection.

1 To switch on the UV lamp, select ***Manual:Alarm&Mon...*** in the ***System Control*** module. Select ***AutoZeroUV*** and click ***Execute***.

2. Fill a 5 ml syringe (not included in the kit) with 4–5 ml of the 0 AU/cm solution. Make sure that there is no air in the syringe.
3. Fit the syringe in the Luer union and inject until the solution exits the UV cell. Do NOT remove the syringe!
4. Observe the actual absorbance value in the ***Run Data*** view pane and wait until it is stable.
5. Autozero the UV measurement by performing step 1 once again.
6. Remove the syringe.
7. Use a new syringe to inject the test solution (4–5 ml) having the second largest concentration.
8. Wait for a stable absorbance value and enter the measured value in the corresponding row in the column ***UV-900 Absorbance (AU)*** in the Excel sheet.
9. Repeat steps 6–8 for the remaining two solutions.
10. When all absorbance values have been entered, the exact cell length is shown next to the ***Real cell length*** square.

    The regressions coefficient should be higher than 0.999.
    If not, one or several measured values are faulty.

Entering the real cell length

1. Select ***System:Settings...*** in the ***System Control*** module.
2. In the ***Instructions*** dialog, select ***Monitors:UVPathLengthCorrection***.
3. If desired, enter the UV cell serial number.
4. Select the nominal cell length in the ***CellType*** menu.
5. Enter the ***Real cell length*** value (from the Excel-file) in the ***RealLength*** field.
6. Select ***Enabled*** and click ***Execute*** to activate the calibration.
7. If the calibration should not be activated, select ***Disable*** and click ***Execute***.

3 Maintenance

Regular maintenance is important for safe and trouble-free operation of ÄKTApilot system. The user should perform daily and monthly maintenance. Preventive maintenance should be performed on a yearly basis by qualified service personnel.

This chapter provides instructions for user maintenance and for replacing spare parts.

Contact your GE representative for more service information.

WARNING! NO SERVICEABLE PARTS INSIDE. Do not open covers. Service and planned maintenance should be performed by personnel authorized by GE.

WARNING! HAZARDOUS CHEMICALS. Ensure that the entire system has been flushed thoroughly with distilled water before service and maintenance.

WARNING! NaOH is corrosive and therefore dangerous to health. Avoid spillage and wear protective glasses.

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

WARNING! HEAVY OBJECT. The separation unit weighs 114 kg. Installation and moving must only be performed by personnel authorized by GE Healthcare.

WARNING! The mains cable must only be changed by personnel authorized by GE.

WARNING! Only use spare parts supplied or specified by GE.

3.1 User maintenance schedule

Table 3-1 lists the maintenance operations that should be performed by the user at regular intervals.

Interval	**Action**	**Instructions/reference**
Daily		
System	Clean the cover	Spray the system with 20% ethanol and wipe off. See section 3.2.1.
	Inspect the system for liquid leakage	Check that tubing and connectors are not damaged. Replace if necessary.
	Wash the system flow path	Avoid leaving the system filled with buffer overnight. Wash the flow path with distilled water. If leaving the system for a few days, use 20% ethanol. Make sure that all tubing and flow paths used are rinsed. See section 3.2.1.
	Check fan operation	Check that cooling air flows through the system, exiting at the rear of the system.
System pump Sample pump	Check for leakage	If there is sign of liquid leaking between a pump head and the system panel, or increased or decreased volume of rinsing solution, replace the piston assembly, ceramic tube, and/or O-rings in the pump head. See section 3.3.2.
	Remove air bubbles	When changing buffer, it is important to remove trapped air. Prime the pump using the PumpWash instruction, or prime it manually (see section 3.4).
pH electrode	Calibrate the pH electrode	See section 2.4.2

Interval	Action	Instructions/reference
Pump rinsing solution	Change rinsing solution	Always use 20% ethanol as rinsing solution. If the volume of rinsing solution in the storage bottles has increased, it can be an indication of internal pump leakage. Replace the piston assembly, ceramic tube, and/or O-rings in the pump head according to section 3.3.2. 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 piston, ceramic tube, and/or O-rings might be damaged. Replace according to section 3.3.2.
Every 3rd month		
UV cell	Check UV lamp run time	See section 3.2.4
Mains cable	Check the mains cable.	If the cable is damaged, contact GE Healthcare service personnel.
Every 6th month		
UV cell (or when required)	Clean the UV cell	Clean the cell and the optical fiber connectors to ensure proper UV monitoring. See section 3.2.4.
Conductivity cell (or when required)	Clean the conductivity cell	See section 3.2.6
pH electrode	Check the pH electrode	Check the pH electrode according to section 2.4.2. Replace the pH electrode if necessary. See section 3.3.9.

Interval	Action	Instructions/reference
Every year		
UV cell	Replace the O-rings	See section 3.3.8
Mixer	Replace the mixer chamber	See section 3.3.3
Air inlet filter	Contact GE service personnel.	
When required		
System pump Sample pump	Replace piston	See section 3.3.2
	Replace ceramic tube	See section 3.3.2
	Replace O-rings	See section 3.3.2
	Clean or replace the inlet and outlet check valves. Clean the rinsing system check valve.	See section 3.2.2 and 3.3.2
pH electrode	Clean the pH electrode	See section 3.2.5
Valves	Replace the membranes	See section 3.3.4
Tubing connectors	Replace the O-rings	
TC connectors	Replace the TC gaskets	
Air trap	Replace O-ring in vent connector	

Table 3-1. User maintenance schedule

3.2 User maintenance instructions

3.2.1 Cleaning the system

For proper function, the system should be kept dry and clean. Chemical stains and dust should be removed.

1 Spray the system with 20% ethanol to remove stains and wipe off.

The column should be by-passed (tubing S6) during cleaning of the system flow path.

At the end of the day

If the system will be used with the same buffers next day, rinse the pump and the system with distilled water as follows:

1 Submerge the appropriate inlet tubing in distilled water.

2 Replace the column with a by-pass tubing (S6).

3 Run the *System Wash* method found in the Method Wizard.
This method flushes the entire system flow path, including selected inlet and outlet tubings.

Leaving the system for a few days

1 Rinse the entire flow path with distilled water by, for example, using the System Wash method as described in the previous section.

2 Repeat with a bacteriostatic solution, for example, 20% ethanol, having first removed the pH electrode (see instruction below).

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

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

Column cleaning – CIP Column

Columns usually have flow rate and pressure limits that do not allow them to be cleaned with a normal cleaning procedure. CIP Column is a method specifically made for cleaning columns. The method is adapted to the column used when setting up the method in the Method Wizard, e.g. flow rate and pressure settings, column volume, etc.

Sanitization – CIP System

CIP System is a method that can be used for sanitizing the entire system flow path. Flow rates, exposure times, etc. have been optimized thoroughly in order to reduce the amount of contaminants and thus meet cGMP demands. Due to the high flow rate during the CIP System run, the column must be replaced by a by-pass tubing before starting the method.

During the CIP System run, 1 M NaOH is flushed through the selected inlet tubing, through the tubing and components in the system flow path, and out through the outlet tubing.

3.2.2 System pump and sample pump

Cleaning the inlet and outlet check valves

Faulty operation of the check valves is usually indicated by irregular flow, very low flow or unstable pressure traces. Probable causes are air or dirt in a check valve preventing it from closing to seal and hold the pressure.

Record the pressure and identify the faulty check valve by observing the pressure trace (see section 4.9 Checking the pump pressure). The flow rate should not exceed 10 ml/min.

To clean the check valves in-place on the pump head:

1 Pump distilled water at 50 ml/min for 2 minutes. This also prevents precipitation of crystals.

2 Pump 100% methanol for approximately 10 minutes.

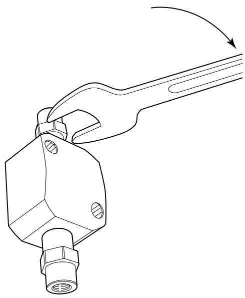
If this does not correct the problem, follow the instructions below for removing and then cleaning the valves.

Tools required: 16 mm torque wrench

Note: *Flush the check valves with distilled water before removing them.*

Note: *Before removing the check valves, check that all input buffer bottles are placed below the level of the pump heads to prevent siphoning.*

1 Disconnect and remove the tubing.



2 Use the 16 mm wrench to remove the valve from the pump head.

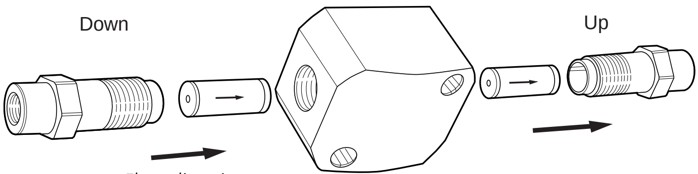
CAUTION! Handle the check valves with care when they have been removed from the pump heads to prevent loss of any internal components.

3 Use a syringe to flush distilled water through the valve to remove salt residues.

4 Immerse the complete valve in methanol and place in an ultrasonic bath for about 5 minutes.

Then repeat the treatment with distilled water.

5 Refit the check valves.



The inlet check valve is fitted to the lower side of the pump head. Tighten the valves using the torque wrench. See Fig. 3-2 for the tightening torque values.

CAUTION! Over-tightening might damage threads. Use a torque wrench to tighten the components.

6 Re-fit the tubing.

WARNING! Incorrectly fitted tubing might loosen, causing a jet of liquid to spray out. This is especially dangerous if hazardous chemicals are in use. Connect the tubing by first inserting the tubing fully, then tightening the connector fingertight.

7 Prime the pump carefully and check that the pumping action has been corrected.

CAUTION! Check valves have precision matched components and should only be disassembled further by a trained service engineer. If the problem cannot be corrected, the check valve should be replaced completely.

3.2.3 Membrane valves

If a membrane does not close or open properly (activating the valve alarm) or if internal leakage appears, the valve might require cleaning.

To clean a membrane valve:

1 Pump 15 ml of 1 M NaOH at 10 ml/min through the pressure sensor either by using a pump or a syringe.

2 Leave it for 15 minutes.

3 Rinse thoroughly with 500 ml de-ionized water.

§If this does not correct the problem, follow the instructions in section 3.3.4 Valve block to dismount the membrane and clean the valve as follows:

1 Immerse the connection block and the membranes in methanol and place in an ultrasonic bath for about 5 minutes.

2 Repeat the treatment with distilled water.

3 Re-assemble the membrane valve.

3.2.4 UV cell

Checking UV lamp run time

To check the total run time of the UV lamp:

1 In System Control, select ***System:Maintenance...***

2 Click the ***Info*** tab in the ***Maintenance Manager*** window.

3 Click on **+** next to ***System*** and then on **+** next to ***UV***.
The UV lamp run time ("on time") is shown in the right-hand field.

Cleaning the UV cell in-line

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

A clean cell and optical connectors are essential for ensuring proper operation of the UV monitor.

Pump a cleaning or sanitizing agent through the cell. The standard recommendation is 1 M NaOH for 30 minutes. Then wash out with buffer.

WARNING! NaOH is corrosive and therefore dangerous to health. Avoid spillage and wear protective glasses.

Cleaning the UV cell off-line

1 Flush a small amount of distilled water through the cell.

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

3 After five squirts, leave the detergent solution in the cell for at least 20 minutes.

4 Pump the remaining detergent solution through the cell.

5 Rinse the syringe and flush the cell with distilled water (30 ml).

Cleaning the optical fiber connectors

Clean the fiber tips with 30% isopropanol using lens paper.

CAUTION! Only use lens paper for touching the fiber tips.

3.2.5 pH electrode

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

Cleaning the pH electrode

Note: *The pH electrode has a limited life length and should be replaced every six months or when the response time is slow.*

To improve the response, clean the electrode using one of the following procedures:

- **Salt deposits**: Dissolve the deposit by immersing the electrode first in 0.1 M HCl, then in 0.1 M NaOH, and again in 0.1 M HCl. Each immersion is for a five-minute period. Rinse the electrode tip in distilled water between each solution.

WARNING! NaOH and HCl are corrosive and therefore dangerous to health. Avoid spillage and wear protective glasses.

- **Oil or grease films**: Wash the electrode tip in a liquid detergent and water. If the film is known to be soluble in a particular organic solvent, wash with this solvent. Rinse the electrode tip in distilled water.

- **Protein deposits**: Dissolve the deposit by immersing the electrode in a solution containing 1% pepsin in 0.1 M HCl. After five minutes, rinse with distilled water.

If these procedures fail to improve the response, try the following procedure:

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

2 Place the electrode tip in the heated KNO_3 solution.

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

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

pH electrode regeneration

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

3.2.6 Conductivity cell

WARNING! NaOH is corrosive and therefore dangerous to health. Avoid spillage and wear protective glasses.

If the conductivity measurements are not comparable to previous results, the electrodes in the conductivity cell might be contaminated and require cleaning.

To clean the cell:

1. Pump 15 ml of 1 M NaOH at 10 ml/min through the cell either by using a pump or a syringe.
2. Leave it for 15 minutes.
3. Rinse thoroughly with 500 ml de-ionized water.

Note: *If the cell is totally blocked, the blockage can be broken by carefully using a needle or a piece of string.*

3.2.7 Pressure sensors

WARNING! NaOH is corrosive and therefore dangerous to health. Avoid spillage and wear protective glasses.

If the pressure measurement seems to be inaccurate, the sensor might require cleaning.

To clean a pressure sensor:

1. Pump 15 ml of 1 M NaOH at 10 ml/min through the pressure sensor either by using a pump or a syringe.
2. Leave it for 15 minutes.
3. Rinse thoroughly with 500 ml de-ionized water.

If this does not correct the problem, dismount the pressure sensor according to the instructions in section 3.3.5 Pressure sensor (2-port or 3-port union) or 3.3.6 Pressure sensor (pump outlet manifold) and clean it as follows:

1. Immerse the tubing connection parts in methanol and place in an ultrasonic bath for about 5 minutes.
2. Repeat the treatment with distilled water.
3. Re-assemble the pressure sensor.

3.2.8 Air sensors

WARNING! NaOH is corrosive and therefore dangerous to health. Avoid spillage and wear protective glasses.

If the air sensor does not react on air passing the sensor, it might require cleaning.

To clean an air sensor:

1. Pump 15 ml of 1 M NaOH at 10 ml/min through the air sensor either by using a pump or a syringe.
2. Leave it for 15 minutes.
3. Rinse thoroughly with 500 ml de-ionized water.

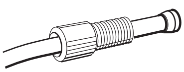
3.2.9 Air trap

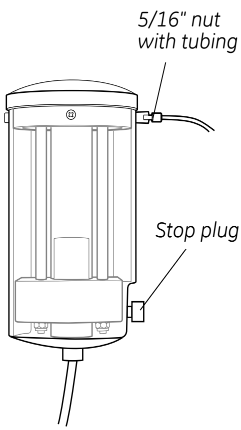
WARNING! NaOH is corrosive and therefore dangerous to health. Avoid spillage and wear protective glasses.

CAUTION! NaOH might cause the outer protection cylinder on the air trap to rupture. Avoid spillage.

To clean the air trap:

1. Fit a 5/16" stop plug in the outlet port.
2. Remove the vent connector at the top.
3. Connect a 5/16" connector with tubing at the top.
4. Fill the entire air trap with 1 M NaOH by using a pump or a syringe.
5. Leave it for 15 minutes.
6. Empty the air trap and rinse thoroughly with 1 l de-ionized water.



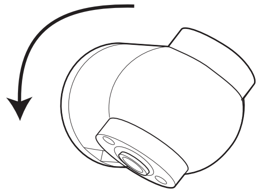


3.3 Replacing spare parts

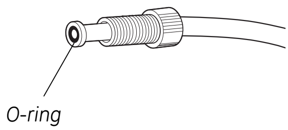
WARNING! Only use spare parts supplied or specified by GE.

3.3.1 General instructions

Some of the components are attached to the separation unit by a snap connection. These components are detached by turning a quarter of a turn and pulling them off the panel. To attach a component, fit it in the connection and turn it a quarter of a turn until it snaps into position.



Note: *Always make sure that the O-ring does not come loose when disconnecting a 5/16" connector. An O-ring that is stuck in the connector port might cause leakage when re-fitting the connector.*



3.3.2 System pump P-907 and sample pump P-908

If there are signs of liquid leaking between the pump head and the housing side panel, or the volume of the rinsing solution has increased or decreased, replace the piston assembly, liquid chamber and/or ceramic tube including O-rings of the leaking pump head.

Other typical symptoms of a damaged piston are observed as excessive piston wear, unstable pressure, a reduction in the flow or, in some cases, noise as the piston moves. The piston should be removed, examined for damage or salt precipitation and then replaced with a new piston if necessary.

If a damaged piston has been in operation, the ceramic tube might be damaged as well and should also be replaced.

If cleaning of a faulty check valve does not improve its performance, it should be replaced.

CAUTION! Do not disassemble the pump head unless there is good reason to believe that there is an internal leakage. Always make sure that sufficient spare components are available before attempting to replace a spare part.

Note: *The power must be switched OFF when removing and refitting the pump heads.*

Note: *Always replace the piston on both pump heads at the same time.*

Spare parts and tools required:

Seal kit containing (see Ordering information for code no.):
- Piston assembly, 400 ml
- Seal kit, 400 ml (includes O-rings and sealings)
- Check valve, inner
- Check valve, outer
- Ceramic tube

- 3 mm Allen key
- 16 mm wrench
- 16 mm torque wrench
- Screwdriver, flat-headed, with torque adapter

Note: *Before disassembling the pump heads, move all input buffer bottles below the level of the pump heads to prevent siphoning.*

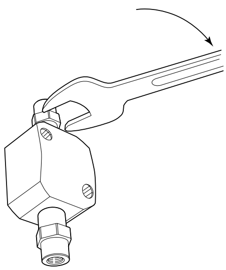
CAUTION! Read the following instructions carefully. Some individual parts of the pump head can be assembled incorrectly. Check the orientation of each part before continuing with the next instruction.

Removing the old piston assembly

1 Switch off the system with the mains power switch on the left-hand panel.

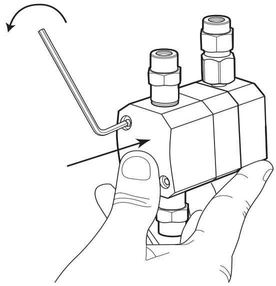
2 Remove the rinsing system tubing.

3 Remove the tubing connectors on the inlet and outlet check valves.

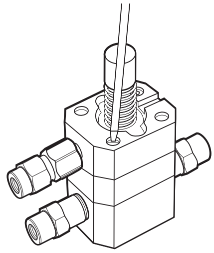


4 If the check valves are also to be checked/replaced, use the wrench to loosen the valves slightly. Do not remove them completely.

5 Using the Allen key, unscrew the two Allen screws locking the pump head in position. Loosen the screws cross-wise a half a turn at a time while pushing firmly on the front face of the pump head to compensate for the pressure of the piston return spring.



6 Carefully pull out the pump head and place it face down on the bench.



7 Using the flat-headed screwdriver, remove the two screws locking the piston assembly in position. Pull out the piston assembly.

8 Gently pull the ceramic tube off the piston.

9 Inspect the ceramic tube using a magnifying glass. Replace with a new ceramic tube if any scratches or cracks are found.

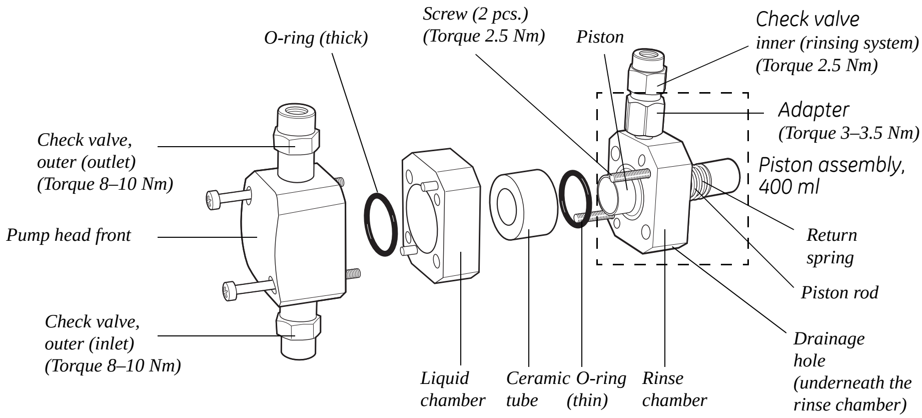


Fig 3-1. Pump head, exploded view

10 Inspect the piston, piston rod and return spring for signs of damage. If damaged, the piston assembly should be replaced.

11 Wipe the piston with a clean cloth. Inspect the piston with a magnifying glass for scratches. Replace the piston assembly if any scratches or cracks are found.

12 If salt solutions have been used, the piston rod or spring may be slightly corroded. This corrosion can be removed with a rubber eraser. If it cannot be wiped or rubbed clean, scrape off any deposits with a scalpel or razor blade.

Replacing the O-rings

1 Carefully remove and discard the old rinse chamber O-ring.

2 Insert a new O-ring (thin).

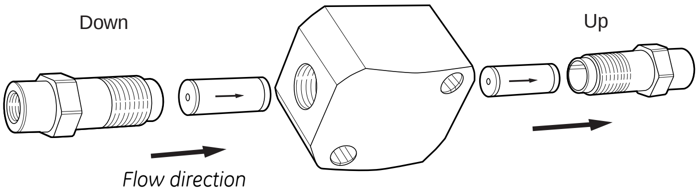
3 Lift off the liquid chamber.

4 Carefully remove and discard the old pump head front O-ring.

5 Insert a new O-ring (thick).

Replacing the outer check valves

1 Unscrew the two loose check valves. Note the direction of the tubes inside the valves. The flow always enters a tube through the round hole and exits through the triangular hole.



2 Inspect the nuts and the tubes for dirt or damage.

3 Replace with a new check valve if any dirt or damage is found and cleaning does not improve the performance of the old check valve (see section 3.2.2 System pump and sample pump).

Tighten the new check valve using the torque wrench. See Fig. 3-2 for the tightening torque value.

CAUTION! Over-tightening might damage threads. Use a torque wrench to tighten the components.

Replacing the inner check valve

1 Use the two wrenches to remove the metal nut of the check valve from the adapter. Note the direction of the tube inside the adapter; the round hole faces the rinse chamber.

2 Use a wrench to remove the adapter from the rinse chamber.

3 Inspect the nuts for dirt and damage. Replace the check valve and/or the adapter if required.

Tighten the new check valve and/or adapter using the torque wrench. See Fig. 3-2 for the tightening torque values.

CAUTION! Over-tightening might damage threads. Use a torque wrench to tighten the components.

Assembling the new piston head

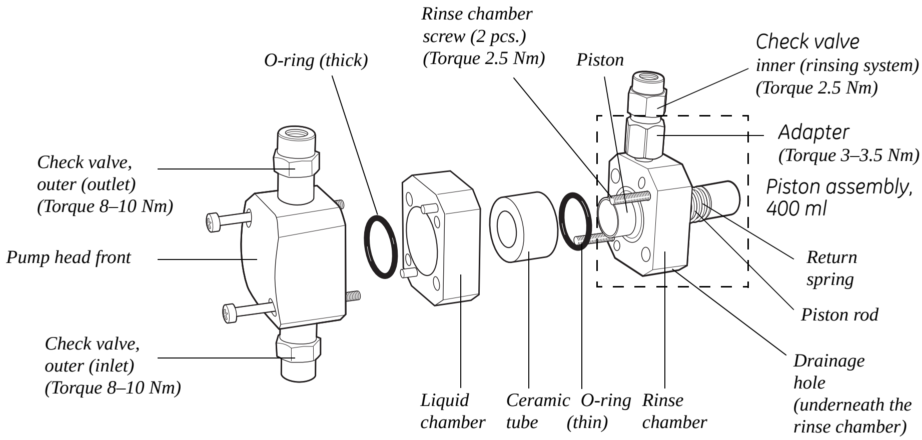
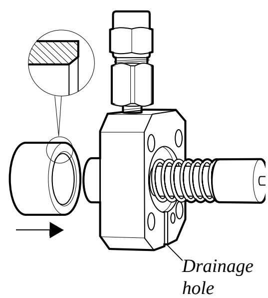


Fig 3-2. Pump head, exploded view

1. Make sure that the new O-rings are installed on the pump head front (thick O-ring) and the rinse chamber (thin O-ring).

2. With the pump head front facing downwards on the bench, place the liquid chamber onto the pump head front.

3. Carefully slide the ceramic tube onto the piston. Make sure that the tube end that has the largest bevel cutting on the inner edge faces the rinsing system chamber.



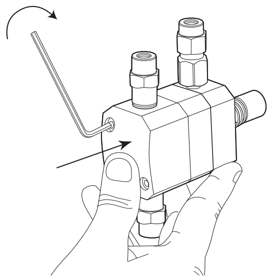
4. Insert the ceramic tube with the piston into the liquid chamber.

5. Use the two rinse chamber screws to lock the complete pump head together.

   Tighten the screws using the screw driver with a torque adapter. See Fig. 3-2 for the tightening torque value.

CAUTION! Over-tightening might damage threads. Use a torque wrench to tighten the components.

Installing the pump head



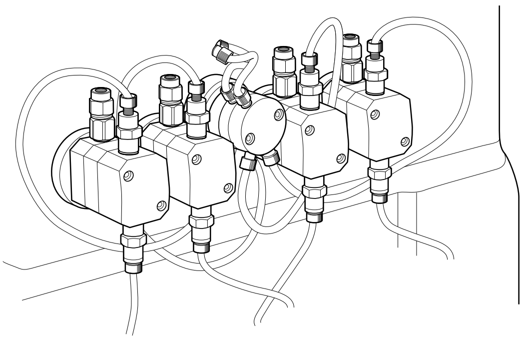
1. Turn the pump head so that **the inner check valve is facing upwards and the drainage hole downwards**. Mount the complete pump head over the locating pins on the front panel.

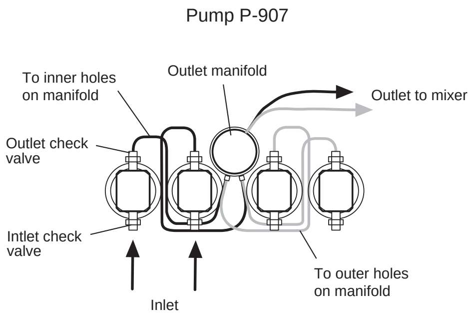
2. Press firmly on the pump head front and use the Allen key to fit and tighten the two retaining screws cross-wise a little at a time.

Connecting the tubing

> **WARNING!** Incorrectly fitted tubing might loosen, causing a jet of liquid to spray out. This is especially dangerous if hazardous chemicals are in use. Connect the tubing by first inserting the tubing fully, then tightening the connector fingertight.

1. Connect the outlet tubing between the outlet check valve and the manifold block. See also section 5.1.5 System flow path for the tubing configuration.





2. Connect the inlet tubing to the inlet check valve.

3. Connect the rinsing system tubing. See also section 5.1.6 Piston rinsing system for the tubing configuration.

4. The pump is ready to be primed. See section 3.4 Priming the system.

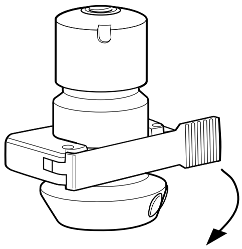
3.3.3 Mixer

Spare parts required:

- Mixer chamber, 5 ml (see Ordering information for code no.)
- Stirrer bar (see Ordering information for code no.)

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

1. Make sure that the pumps are stopped.
2. Make sure that the buffer bottles are placed lower than the mixer to prevent draining.
3. Disconnect the tubing.
4. Open the plastic lock holding the mixer chamber. A spring secures the mixer chamber in position when the lock is opened.



5. Gently pull out the mixer chamber.
6. Insert the new mixer chamber with the flat side facing the front panel and close the lock.
7. Connect the tubing.

3.3.4 Valve block

When replacing the wetted parts of a valve block, the connection block as well as all membranes should be replaced. At normal user maintenance, only the membranes need to be replaced.

Spare parts and tools required:

- Connection block (note that all connection blocks have separate code numbers, see Ordering information)
- Valve membrane containing 4 membranes (see Ordering information for code no.)

- 7 mm wrench

Removing the old connection block and membranes

1. Flush the valve thoroughly with distilled water.

2. Empty the air trap, for example, through Waste2 in valve V5.

3. Disconnect all tubing from the valve.

4. Disconnect the UniNet-1 cable between the separation unit and the computer.

   After approx. 1 min, the ***POWER*** LED on the front panel starts flashing slowly, which indicates that the communication between the computer and the separation unit is broken.

5. Remove the six attachment screws using the wrench.

6. Carefully loosen the old connection block.

7 Press the ***PAUSE*** and ***CONTINUE/RUN*** buttons on the front panel simultaneously for 3 seconds.

Note: *This action is enabled only if the* ***POWER*** *LED flashes.*

As a result, the membranes in all valves move to a position between open and closed ("service mode"), thus enabling their removal.

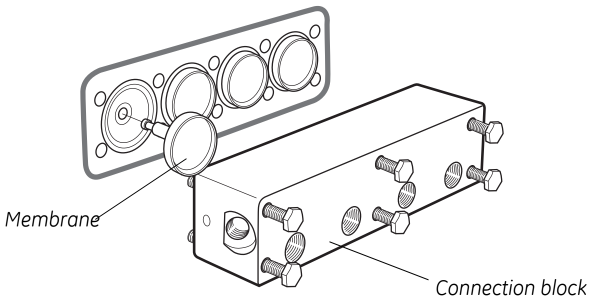


Fig 3-3. Valve block, exploded view

8 Pull out and discard the old membranes. Be careful not to scratch the mechanical housing of the valve!

Installing the new connection block and membranes:

1 Fit the new membranes into position.

2 Press the ***PAUSE*** and ***CONTINUE/RUN*** buttons simultaneously for 3 seconds.

Check that the new membranes retract to the open position.

3 Carefully fasten the new connection block using the attachment screws.

Note: *Make sure that the marking on the block is turned the right way round, i.e. not upside-down.*

4 Connect the tubing.

5 Connect the UniNet cable between the separation unit and the computer.

6 When the connection between the computer and the separation unit is established, the valves take up their normal positions in End mode.

3.3.5 Pressure sensor (2-port or 3-port union)

Spare parts and tools required:

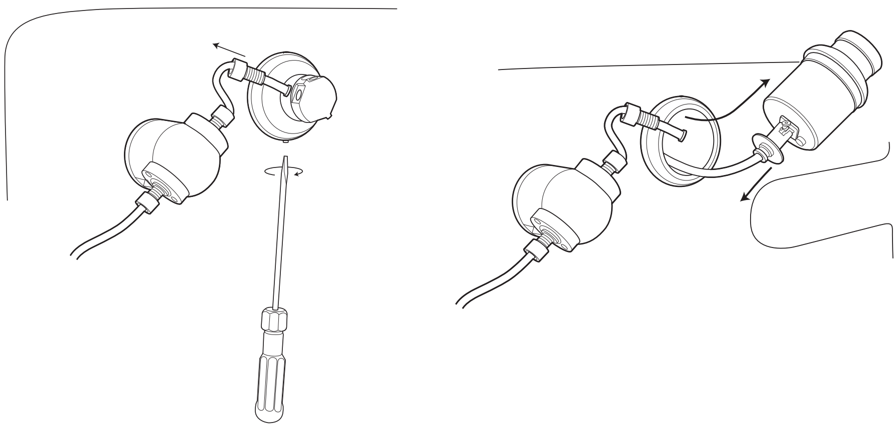
- Pressure sensor, 2-port, or
- Pressure sensor, 3-port (see Ordering information for code nos.)

- Screwdriver (small, flat-headed)

1 Flush the pressure sensor thoroughly with distilled water.

2 Switch off the system with the mains power switch.

3 Disconnect the tubing from the pressure sensor.



4 Loosen the locking screw at the lower side of the plastic holder using the small, flat-headed screwdriver.

5 Carefully pull out the pressure sensor from the holder.

6 Disconnect the signal cable from the pressure sensor.

7 Connect the signal cable to the new pressure sensor and insert it in the holder.

8 Tighten the locking screw.

9 Check the O-rings at the tubing ends and connect the tubing.

10 Calibrate the new pressure sensor according to section 2.4.1 Calibrating the pressure sensors.

To allow cleaning of the flow path inside the pressure sensor, loosen the four attachment screws at the rear of the sensor and remove the rear part.

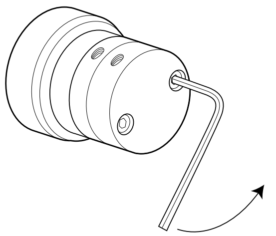
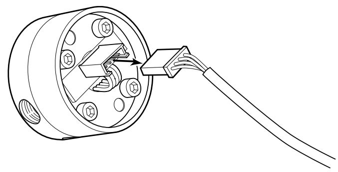
3.3.6 Pressure sensor (pump outlet manifold)

Spare parts and tools required:

- Pressure sensor incl. outlet manifold A (for P-907 and P-908)
- Outlet manifold B (for P-907 only)
  (see Ordering information for code nos.)

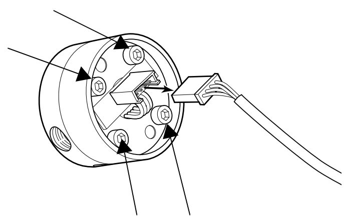
- 3 mm Allen key

1. Flush the outlet manifold thoroughly with distilled water.
2. Switch off the system with the mains power switch.
3. Disconnect all tubing from the outlet manifold.
4. Loosen the two attachment screws using the Allen key.

5. Gently pull out the outlet manifold.
6. Disconnect the signal cable.
7. Connect the cable to the new outlet manifold.
8. Fit the outlet manifold into position.
9. Carefully fasten the outlet manifold using the attachment screws. Tighten the screws cross-wise a little at a time.
10. Connect the tubing.
11. Calibrate the new pressure sensor according to section 2.4.1 Calibrating the pressure sensors.

To allow cleaning of the flow path inside the pressure sensor, loosen the four attachment screws at the rear of the sensor and remove the rear part.



3.3.7 Air sensors

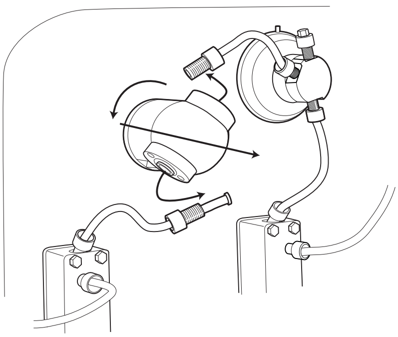
Spare parts required:

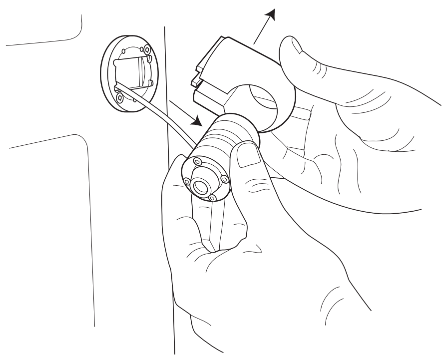
- Air sensor Cell 940 (at sample inlet), or
- Air sensor Cell 925 (at column inlet)
  (see Ordering information for code nos.)

1 Flush the air sensor thoroughly with distilled water and stop the pump.

2 Switch off the system with the mains power switch.

3 Disconnect the tubing from the air sensor.

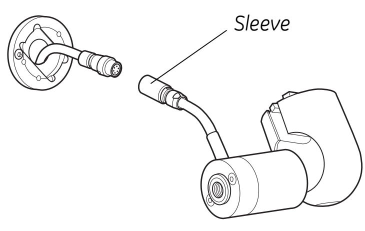




4 Release the air sensor holder from the panel by turning the holder a quarter of a turn.

5 Gently pull out the signal cable to reach the connector.

6 Disconnect the push-pull connector by pulling the sleeve and remove the air sensor.



7 Connect the cable from the new air sensor to the connector.

8 Fit the new air sensor in the holder.

9 Fasten the holder on the panel.

10 Connect the tubing.

3.3.8 UV cell

Spare parts and tools required:

- UV cell 1/2/5 mm
- O-ring kit (6 pcs.)
- O-ring (1 pc.)
- Shim plate 2 mm
  (see Ordering information for code nos.)

- 3 mm Allen key
- Phillips screw driver

Removing the UV cell

1 Flush the cell with distilled water.

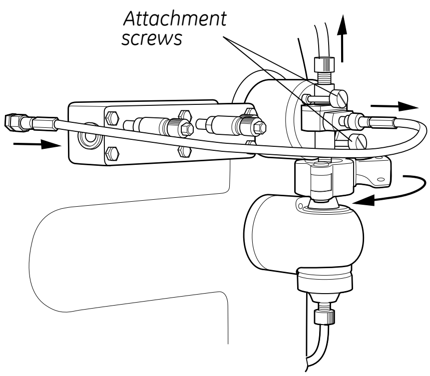
2 Switch off mains power to the system.

3 Disconnect the upper tubing from the UV cell.

4 Disconnect the optical fiber. The optical fiber is fragile and must be handled carefully!

5 Disconnect the TC clamp and the TC connector from the UV cell.

6 Loosen the two attachment screws to remove the UV cell from the front panel.



Installing the new UV cell

1 Make sure that the correct optical path length is set on the new UV cell with shim plates (see section Setting the optical path length).

2 Connect the TC connector to the lower port on the UV cell.

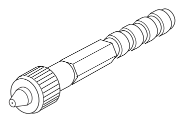
3 Fasten the UV cell to the panel using the attachment screws.

4 Connect the UV cell to the conductivity cell using the TC clamp.

5 Connect the upper tubing to the UV cell.

6 Connect the optical fiber to the UV cell.

Make sure that the end with a knurled nut is connected to the UV cell.



Changing the O-rings

Change the O-rings as follows:

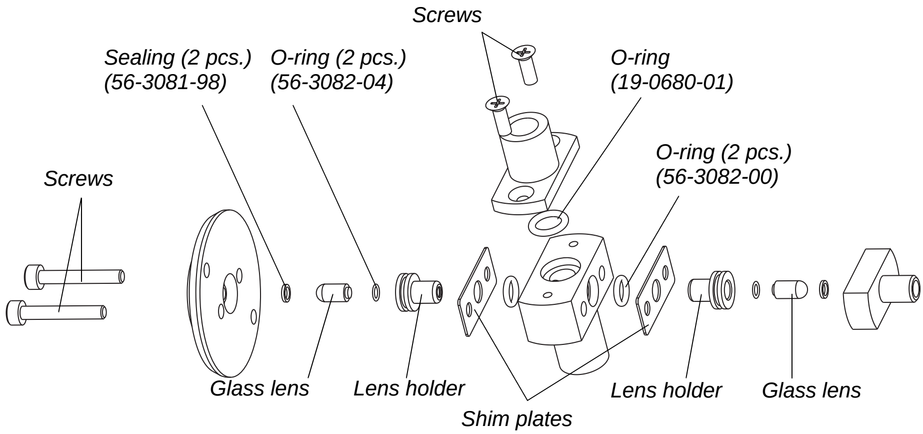


Fig 3-4. UV cell, exploded view

1. Loosen the four attachment screws on the UV cell (refer to the figure).
2. Take the UV cell apart completely. Be careful when taking the glass lenses out of the holders!
3. Replace the O-rings.
4. Re-assemble the UV cell using the four attachment screws.

Setting the optical path length

The optical path length is set with shim plates (see Fig. 3-4) according to the following table:

Path length [mm]	Shim plates [mm]
1	No shim plate
2	0.5
5	2

Table 3-2. Optical path length versus shim plate thickness

3.3.9 pH electrode

Spare parts and tools required:

- pH electrode, ÄKTApilot
- Cell holder, pH
  (see Ordering information for code nos.)

- Phillips screwdriver

Replacing the pH electrode

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

1 Switch off the system with the mains power switch.

2 Unscrew the cable connector at the top of the old pH electrode.

3 Unscrew the locking nut that secures the pH electrode.

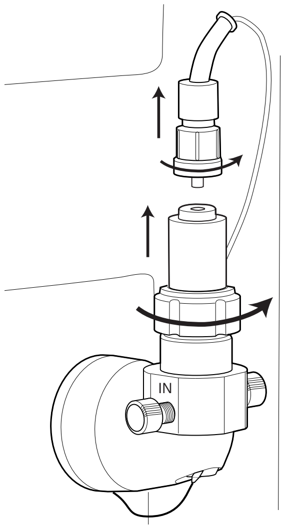
4 Remove the pH electrode.

5 Unpack the new pH electrode.

6 Remove the end cover. Make sure that it is not broken or dry.

7 Before using the electrode, immerse the glass tip in a pH 4 buffer solution for 30 minutes.

8 Carefully insert the electrode in the cell holder. Tighten the locking nut by hand to secure the electrode.



9 Fit the signal cable to the top of the pH electrode.

Replacing the cell holder

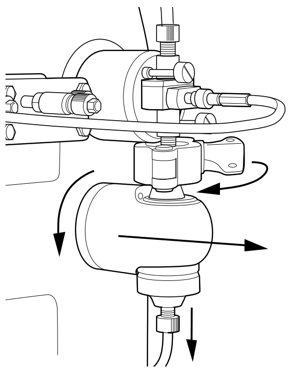
1. Flush the cell holder with distilled water.
2. Disconnect the tubing.
3. Disconnect the signal cable from the top of the pH electrode (if used).
4. Move the pH electrode or the dummy electrode to the new cell holder.
5. Remove the ground wire from the cell holder using the Phillips screwdriver.
6. Release the old cell holder from the panel by turning it a quarter of a turn.
7. Fasten the new cell holder on the panel.
8. Connect the tubing.
9. Connect the signal cable to the pH electrode (if used).

3.3.10 Conductivity cell

Spare parts and tools required:

- Conductivity cell (see Ordering information for code no.)

1. Flush the cell with distilled water.
2. Switch off the system with the mains power switch.
3. Disconnect the tubing and the TC clamp.
4. Release the conductivity cell holder from the panel by turning it a quarter of a turn. Gently push down the cell before releasing if necessary.
5. Gently remove the old conductivity cell from the holder and disconnect the push-pull cable connector.
6. Move the TC union to the new conductivity cell.



7. Connect the cable to the new conductivity cell and put the cell in the holder. Make sure that the screw head end on the conductivity cell faces the pH electrode!
8. Connect the tubing and the TC clamp.

3.3.11 Air trap

Spare parts and tools required:

- Air trap (see Ordering information for code no.)
- Phillips screwdriver

1 Flush the air trap thoroughly with distilled water.

2 Empty the air trap, for example, through Waste2 in valve V5.

3 Disconnect the tubing from the air trap.

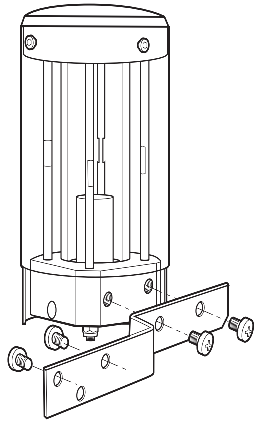
4 Use the Phillips screwdriver to unscrew the screws that attach the air trap bracket to the separation unit panel.

5 Remove the old air trap by unscrewing the screws that attach the air trap to the bracket.

6 Attach the new air trap to the bracket.

7 Attach the bracket with the air trap to the separation unit panel.

8 Connect the tubing.



3.4 Priming the system

3.4.1 Automated priming

To prime the pumps and the inlet tubing with buffer (or sample), use either of the pump wash instructions that are available in the System Control module:

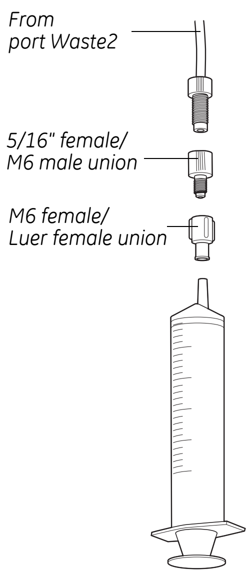
- SystemPumpWash
- SamplePumpWash

3.4.2 Manual priming

If a pump wash instruction fails to fill the pump or tubing, the flow path in the pump might be dry, which reduces the pumping capacity. If so, the pump has to be primed manually using a syringe and appropriate fittings.

To prime the pump and inlet tubing manually:

1. Fill a flask with distilled water and immerse the appropriate inlet tubing in the water.
2. Connect a tubing equipped with 5/16" connectors at both ends to port 3 (Waste2) in the flow direction valve V5.
3. Connect a 5/16" female/M6 male union to the other end of the tubing.
4. Connect a M6 female/Luer female union to the first union.
5. Fit an empty male Luer syringe (> 25 ml) to the Luer union.
6. In the ***System Control*** module, select ***Manual:Flowpath***.
7. Open the appropriate inlet valve and Waste2.
8. Use the syringe to draw water through the inlet tubing and pump until it starts to enter the syringe.



4 Troubleshooting

This chapter provides troubleshooting guidelines. The troubleshooting guide focuses on error symptoms related to monitor curves and operation of the individual components.

The chapter also contains instructions for running the Installation test, which is used for testing basic functions in the system.

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

4.1 UV curve

Error symptom	Possible cause	Corrective action
Ghost peaks	Dirt or residues in the flow path from previous runs	Clean the system according to section 3.2.1
	Residues in the column from previous runs	Clean the column according to the column instructions
	Poor mixing of the buffers	Check that the mixer works correctly. Check that the correct mixer chamber is being used. 5 ml is the default mixer chamber.

Error symptom	Possible cause	Corrective action
Noisy UV-signal, signal drift or instability	Bad cable connections	Check that the UV cell is properly attached to the panel
	Bad UV fiber connections	Check the connections of the UV cell optical fiber. Replace if necessary.
	Dirty UV cell	Clean the UV cell, refer to section 3.2.4
	The buffer may be impure	Check if the signal is still noisy with water

4.2 Conductivity curve

Error symptom	Possible cause	Corrective action
Baseline drift or noisy signal	Leaking tubing connections	Tighten the connectors. If necessary, replace the connectors.
	Column not equilibrated	Equilibrate the column. If necessary, clean the column.
	Bad pump or mixer	See sections 3.3.2 and 3.3.3
	Dirty conductivity cell	Clean the conductivity cell according to section 3.2.6
Waves on the gradient	Bad pump function	Check that the pump is operating and is programmed correctly
	Bad mixer	Check the motor operation. Place a finger on the mixer chamber and start it by starting the pump at a low flow rate. When the chamber is empty, you should both hear and feel the mixer motor and stirrer when they are spinning.
Conductivity measurement with the same buffer appears to decrease over time	Dirty conductivity cell	Clean the conductivity cell according to section 3.2.6
	The ambient temperature may have decreased	Use a temperature compensation factor, see section 2.4.3
Ghost peaks appear in the gradient profile	Air bubbles passing through the conductivity cell	Check for loose tubing connections

Error symptom	Possible cause	Corrective action
Non-linear gradients or slow response to %B changes	Dirt in the tubing	Make sure that the tubing has been washed properly
	Bad pump function	Check that the pump operates properly
	Bad mixer	Check the motor operation. Place a finger on the mixer chamber and start it by starting the pump at a low flow rate. When the chamber is empty, you should both hear and feel the mixer motor and stirrer when they are spinning.
Absolute conductivity value is wrong	Bad calibration	Calibrate the conductivity cell, see section 2.4.3
	Calibration solution, 1.00 M NaCl, not correctly prepared	Recalibrate using a new calibration solution
Incorrect or unstable reading	Bad pump or membrane valve function	Check the pump and the valves
	The temperature compensation not properly set	Check that the temperature sensor is calibrated, and that the correct temperature compensation factor is in use. See section 2.4.3.
	The column not equilibrated	Equilibrate the column. If necessary, clean the column.
	Poor mixing	Check the mixer by placing a finger on the mixer chamber and start it by starting the pump at a low flow rate. You should both feel and hear if the stirrer is rotating.

4.3 Pressure curve

Error symptom	**Possible cause**	**Corrective action**
Erratic flow, noisy baseline signal, irregular pressure trace	Air bubbles passing through or trapped in the pump	Check that there is sufficient buffer in the reservoirs
		Check all connections for leaks
	Inlet or outlet check valves not functioning correctly	Remove any dirt in the valves by cleaning according to section 3.2.2
	Piston assembly leaking	Replace the piston assembly, ceramic tube, and/or O-rings in the pump head. See section 3.3.2.
	Blockage or partial blockage of flow path	Flush through to clear blockage
		If necessary, replace tubing

4.4 System pump and sample pump

Error symptom	Possible cause	Corrective action
Liquid leaking between the pump head and the instrument panel	Piston, ceramic tube, liquid chamber or O-rings incorrectly fitted or worn	Replace the piston assembly, ceramic tube, and/or O-rings in the pump head. See section 3.3.2.
Low buffer flow and noise	Bad piston spring	Disassemble the pump head and examine the piston spring. Replace the piston assembly if necessary.
		If the spring is corroded, check piston, ceramic tube, and/or O-rings. Make sure that the rinsing system is always used when working with aqueous buffers with high salt concentration.
		If the piston is damaged, replace it according to section 3.3.2 Replace the piston assembly, ceramic tube, and/or O-rings with new components
Leakage around a tubing connector	Leaking connection and/or crystallized material around the tubing connector	Unscrew the connector and check if it is worn or incorrectly fitted. If required, replace the connector. Tighten the connector with your fingers only.
Erratic pump pressure	Air trapped in the pump heads	Check the pump function by observing the pressure curve in UNICORN. By observing the pressure trace, the pump head which is functioning abnormally can be identified. Some examples of normal and abnormal pressure traces, together with comments, are shown in section 4.9.
	Leaking connectors	
	Piston leakage	
	Piston damaged	
	Check valve malfunction	

4.5 Mixer

Error symptoms	Possible causes	Action
Leakage	Leaking tubing connections	Check the tubing connections. Re-tighten or replace if necessary
		Check the mixer chamber. Replace if liquid has penetrated the mixer chamber walls and sealings.
Non-linear gradients or slow response to %B changes	Poor mixing of the buffers	Check the mixer by placing a finger on the mixer chamber and start it by starting the pump at a low flow rate. You should both feel and hear if the stirrer is rotating. The mixer function can also be checked by running the installation test.

4.6 Membrane valves

Error symptoms	Possible causes	Action
External leakage	Leaking tubing connectors	Check the tubing connectors. Tighten or replace if necessary.
Internal leakage (can be detected underneath the valve body)	The valve membrane might be worn or damaged	Change the valve membrane. See section 3.3.4.
High back-pressure	Dirt in the flow path	Clean the valve according to section 3.2.3
		Change the valve membrane. See section 3.3.4.
Valve alarm	The valve membrane has not returned to its normal position from the “service position”	Restart the systemm and UNICORN.
	A valve membrane might be damaged	Change the valve membrane. See section 3.3.4.

4.7 Pressure sensors

Error symptoms	Possible causes	Action
External leakage	Leaking tubing connectors	Check the tubing connectors. Tighten or replace if necessary.
High back-pressure	Dirt in the flow path	Clean the pressure sensor according to section 3.2.7
		Change the pressure sensor. See section 3.3.5 or 3.3.6.

4.8 Air sensors

Error symptoms	Possible causes	Action
External leakage	Leaking tubing connectors	Check the tubing connectors. Tighten or replace if necessary.
High back-pressure	Dirt in the flow path	Clean the air sensor according to section 3.2.8
		Change the pressure sensor. See section 3.3.7.
The system is immediately set to Pause when enabling the air sensor	The air sensor is filled with air	Fill the air sensor with liquid before enabling

4.9 Checking the pump pressure

To check the pump function, check the pressure curve in UNICORN.

There can be several causes of an abnormal pressure recording, for example:

- Air trapped in the pump heads.
- Leaking connections.
- Piston seal leakage.
- Check valve malfunction.
- Piston damaged.

Some examples of abnormal pressure traces, together with comments, are shown in Table 4-1 .

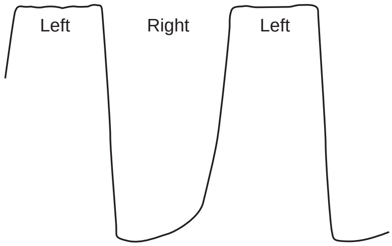
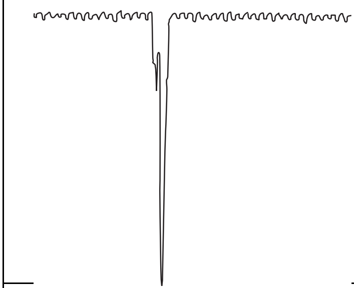
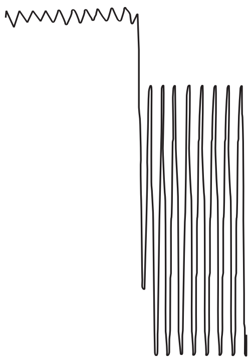
Result	Diagnosis
	Left pump head OK, right pump head not working. Possible causes: • Bad piston. • Major leakage in inlet check valve. • Inlet blocked. • Ceramic tube broken.
	Air bubbles passing through pump heads
	One pump head has stopped working. Possible causes: • Major leakage in one pump head. • Major leakage in inlet check valve. • Inlet blocked. • Bad pump calibration.

Table 4-1. Abnormal pressure traces

4.10 Running the installation test

The installation test checks the function of the liquid delivery and the UV and conductivity monitoring system of ÄKTApilot. It can be used at any time to check the condition of the system.
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 264, 254 and 280 nm and calculating the absorbance ratios 265/254 nm and 265/280 nm.

4.10.1 Testing pressure stability

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

1 Fit the dummy electrode in the pH cell holder.

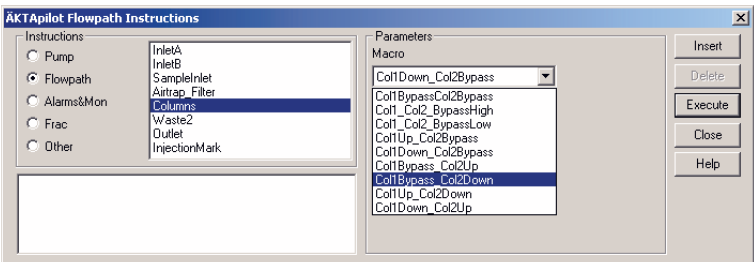
2 Make sure the pump has been primed properly.

3 Make sure that inlet tubing InletA1 and InletB1 are immersed in a distilled water flask.

4 Connect the IN port on flow restrictor FR-902 to valve 6, port 3.

5 Connect the other port on FR-902 to valve 7, port 3.

6 Set the **Columns** flow path to **Col1Bypass_Col2Down**.



7 Run 100 ml/min at 0%B (distilled water). Check on the pump display that the pressure reading is stable (variation < ±5%).

8 Run 100 ml/min at 100%B (distilled water). Check on the pump display that the pressure reading is stable (variation < ±5%).

9 If the pressure is not stable, consult the section 4.4 System pump and sample pump for troubleshooting instructions.

10 Click on **END**.

4.10.2 Preparing the installation test

Installation Test Method Guide	
Buffer Sample1:	0.4% acetone in 1 M NaCl
Buffer InletA1	Distilled water
Buffer InletB1	0.4% acetone in 1 M NaCl
Test flow rate	100 ml/min
Test run time	Approximately 25 minutes

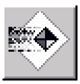
1. Prime the pumps with distilled water to remove any air trapped in the pump heads.
2. Prepare a flask containing 4.5 l of 0.4% acetone in 1 M NaCl.
3. Immerse inlet tubing InletA1 in a distilled water flask (> 2.5 l).
4. Immerse inlet tubing InletB1 in the acetone/NaCl flask.
5. Immerse inlet tubing Sample1 in the acetone/NaCl flask.

Note: *FR-902 should remain in place between valve 6 and valve 7.*

Note: *The Installation Test starts with priming the inlet tubing with the test liquids.*

4.10.3 Running the installation test

1. Start UNICORN as described in section 2 Operation.
2. In **UNICORN Main Menu**, select **File:Printer setup...**. Select the appropriate printer from the list and select **Landscape**. Click **OK** to acknowledge the chosen printer.



3. Click on the **Instant Run** button and then **Run** to start the Method Wizard.
4. Check the **Special Method** button and select **Installation Test.**
5. Click **Run**.
6. Click **Next** to go through the subsequent pages and make the appropriate selections.

   Note: *Check the* ***InstPilot*** *option in the* ***Evaluation Procedures*** *page to print the installation result automatically after the test.*

7. Click **START** to start the Installation Test.

To customize the **Curves** view pane in **System Control**:

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

2 Click the **Curves** tab.

3 Select the following curves to be displayed:

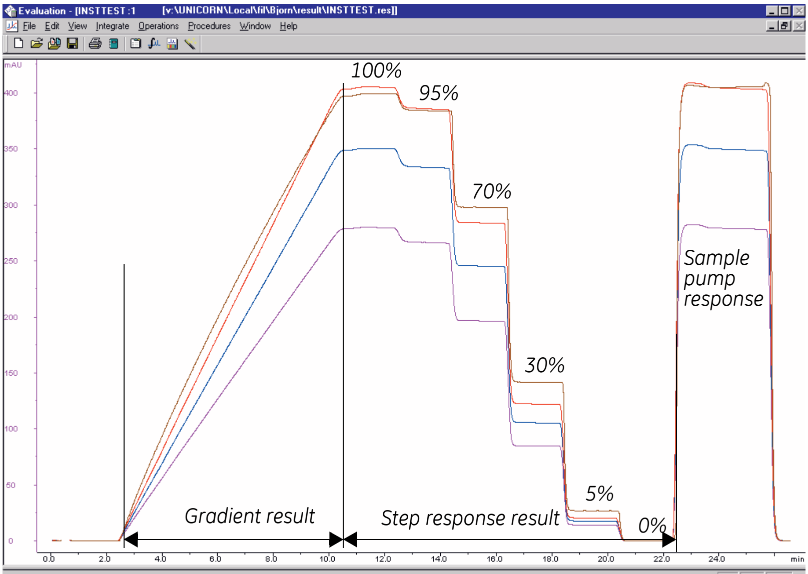
- **UV_265nm**
- **UV_254nm**
- **UV_280nm**
- **Cond**
- **Conc**

4 Click **OK**.

The curves are shown on the screen as the test progresses.
The installation test method run time is approximately 25 minutes.

Note: *The inlet Sample2 will be opened at the end of the run to flush out the salt solution. It can be avoided by changing this method instruction to Sample1.*

5 When the test is finished, the printer automatically prints the chromatogram and the test result (if **InstPilot** in **Evaluation Procedures** has been selected).



4.10.4 Evaluating the installation test results

Automatic evaluation

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 fall outside the specified range, go to section *4.10.5 Correcting faulty evaluation results.*

Manual evaluation

If you suspect that the automatic evaluation does not give a reliable result, a manual evaluation can be done.

1 In **UNICORN Main menu** click on  in the **Results** window.

2 Double-click on the **Installation Test** icon to open the result file.

3 Maximize the chromatogram window by clicking on  in the upper right corner.

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

5 Click on the **Curves** tab and select:

- **Installation Test01:1_UV1_265nm@01,SMTH**
- **Installation Test01:1_UV1_254nm@02,SMTH**
- **Installation Test01:1_UV1_280nm@03,SMTH**
- **Installation Test01:1_Conc**

6 Click **OK**.

7 Right-click in the chromatogram window, and select **Marker**.

8 Make sure that the UV1 curve value is displayed.

9 Read the absorbance for the steps corresponding to **Installation Test01:1_UV1_265nm@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 in section 4.10.6, leaving out the decimals.

10 Read the absorbance for the plateaus corresponding to 0% and 100% B for the curves (click on the curve name to change the curve reading):

- **Installation Test01:1_UV1_265nm@01,SMTH**
- **Installation Test01:1_UV1_254nm@02,SMTH**
- **Installation Test01:1_UV1_280nm@03,SMTH**

Enter the values in column 2 in the UV response table in section 4.10.6.

11 Click on **Print** under **File** to print the chromatogram.

Evaluating the gradient
Place a ruler along the gradient part of curve **Installation Test01:1_UV1_265nm@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 absorption plateau heights for curve **Installation Test01:1_UV1_265nm@01,SMTH** as follows:

1 Subtract the baseline value (0% B) from each of the values in column 2 in the Step response table in section 4.10.6 and enter the results in column 3.

2 Divide each value in column 3 by the baseline corrected value corresponding to 100% B, multiply by 100 and enter the results in column 4.

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

Evaluating the UV response
Calculate the UV response ratios in the following way:

- Subtract the baseline values (0% B) corresponding to each UV curve from the values in column 2 of the UV response table in section 4.10.6 and enter the results in column 3.
- Calculate the absorbance ratios 265/254 nm and 265/280 nm using the values of column 3 and enter the results in column 4.

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

Evaluating the sample pump function
The 100% step response of the three UV curves (265 nm, 280 nm and 254 nm) and the conductivity curve should not differ more than ±5% from the corresponding sample pump response.

4.10.5 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 mixer is faulty.
- Disturbances – may arise from air in the pump, pump valves or bad sealings in the pump. Refer to the section 4.4 System pump and sample pump.

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).

Faulty sample pump function
If values are faulty, make sure that the sample pump is properly primed and that all tubing fittings are tightened properly.

4.10.6 Test protocol

Gradient test result

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

Step response test result

1 Programmed Conc. %B	2 Value read [mAU]	3 Baseline corrected value	4 Normalized value	5 Allowed interval
100				
95				94 – 96
70				69 – 71
30				29 – 31
5				4– 6
0				

UV response test result

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					

5 Reference information

5.1 System description

5.1.1 ÄKTApilot system

ÄKTApilot is a high performance, automated liquid chromatography system. ÄKTApilot consists of a separation unit and a Windows-based computer running UNICORN version 4.11 or higher.

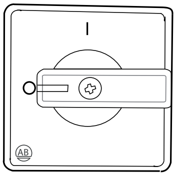
The system is designed for process development, process scale-up and scale-down, and small scale production. It can be used with pre-packed columns as well as with standard laboratory and pilot-scale columns packed with media. It can also be used for packing columns.

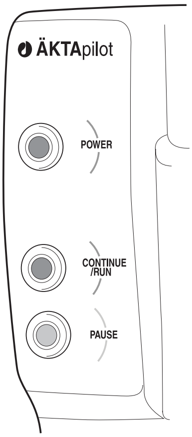
ÄKTApilot is biocompatible, hygienic and sanitizable. It meets all GLP and cGMP requirements for Phase I–III in drug development and final-scale production.

Fig 5-1. ÄKTApilot system

5.1.2 Indicators and controls on the separation unit

The separation unit is equipped with the following indicators, switch and push-buttons:





Indicator/Switch/ Push-button	Color	Description
POWER (switch)	–	Switches on/off power to the system. **Located on the left panel.**
POWER (indicator)	Green	Flashes rapidly for a few seconds during the internal communication test in the separation unit when switching on power to the unit. Flashes slowly when the communication test is finished. Steady light when UNICORN is connected to the separation unit.
CONTINUE/RUN (push-button/ indicator)	Green	Pressing this button with the system in Pause mode, causes the pump to continue at the set flow rate and gradient values. The button is lit in Run mode.
PAUSE (push-button/ indicator)	Yellow	Pressing this button, stops the pump but retains the set flow rate and gradient values. All inlet and outlet valves are closed. The button is lit in Pause mode.
Alarm buzzer	–	Indicates an alarm in UNICORN. **Located behind the front panel.**

Table 5-1. Indicators, switches and push-buttons on the separation unit.

Using the ***CONTINUE/RUN*** and ***PAUSE*** push-buttons to set the system in ”Service mode” (the valve membranes move to a position between open and closed):

1. Disconnect the UniNet-1 cable between the separation unit and the computer. Wait for the ***POWER*** indicator to start flashing slowly (after approx. 1 min).
2. Press ***CONTINUE/RUN*** and ***PAUSE*** simultaneously for 3 seconds.

5.1.3 Component location

The location of each of the main components of ÄKTApilot separation unit is shown in the following figure. The rinsing system tubing is not included.

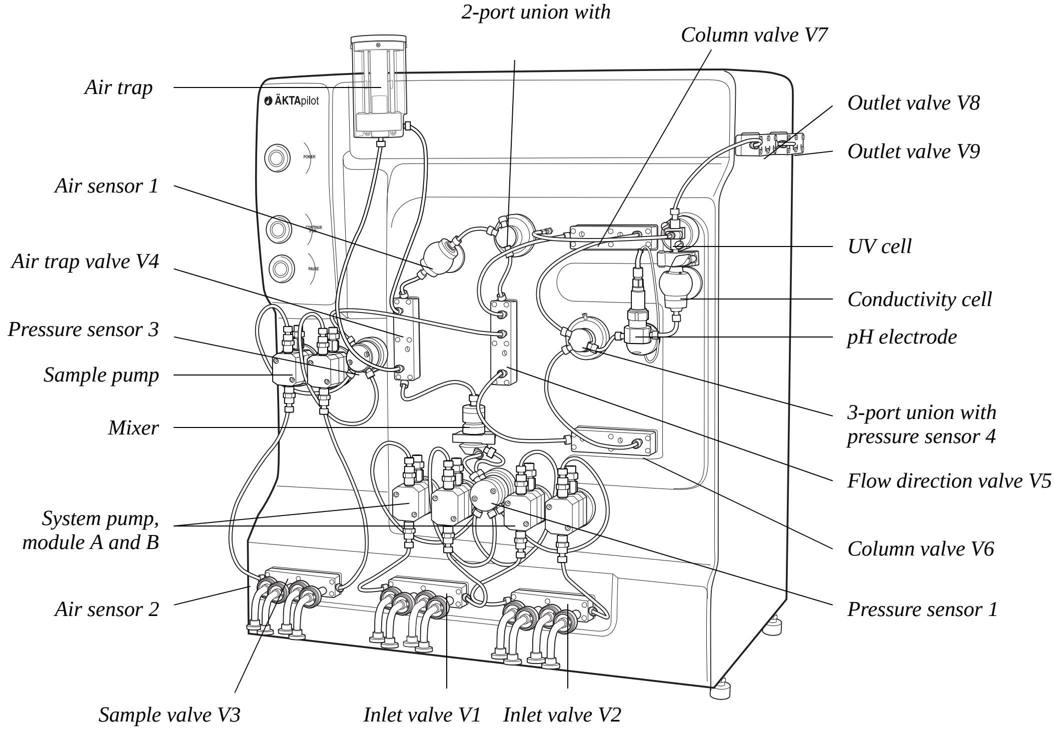


Fig 5-2. Location of the ÄKTApilot components

5.1.4 Electrical connections

Mains cable

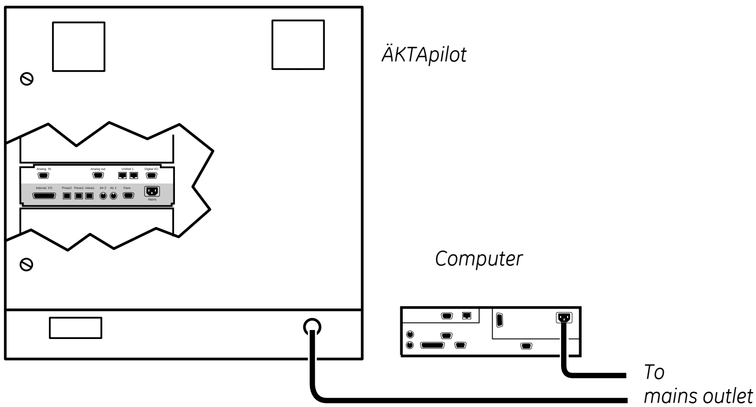


Fig 5-3. Mains cables

The mains cable is permanently connected inside the separation unit and routed through a conduit entry in the rear panel for connection to a mains outlet.

> **WARNING! HIGH VOLTAGE.** The mains cable must only be connected and changed by authorized service personnel. Faulty connection might result in live system parts that can give a lethal electric shock.

The cable wires are marked and connected as shown in Table 5-2 .

Wire marking	Connected to
Yellow/green	Ground
L1	Live
L2	Neutral

Table 5-2. Cable wires

Analog I/O and digital I/O

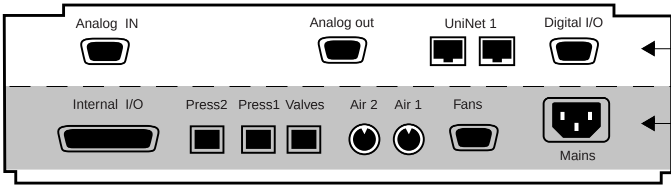
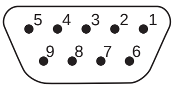


Fig 5-4. Controller upper rear panel

WARNING! HIGH VOLTAGE. The rear panel should only be accessed by authorized service personnel. Faulty connection might result in live system parts that can give a lethal electric shock.

The upper rear panel on the controller inside the separation unit is equipped with three D-Sub connectors – ***Analog IN***, ***Analog out*** and ***Digital I/O*** – intended for signals to/from auxiliary equipment.

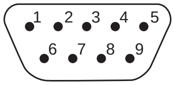
- ***Digital I/O*** (D-Sub, 9-pole, female)



Pin	Signal	Remark[1]
1	Positive input 1	Active level > 4.5 V between pin 1 and 2 Inactive level < 2.5 V between pin 1 and 2 Impedance: ~900 ohm Max. 25 V
2	Negative input 1	
3	Positive input 2	Active level > 4.5 V between pin 3 and 4 Inactive level < 2.5 V between pin 3 and 4 Impedance: ~900 ohm Max. 25 V
4	Negative input 2	
5	Reference ground	
6	Output 1	On/off relay between pin 6 and 7 Max. 100 mA. Max. 25 V.
7	Output 1	
8	Output 2	On/off relay between pin 8 and 9 Max. 100 mA. Max. 25 V.
9	Output 2	

[1] Section 5.3.1 Technical specifications provides a full specification of the Digital I/O interface.

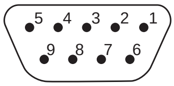
- ***Analog IN*** (D-Sub, 9-pole, male)



Pin	Signal	Remark[1]
1–6	Reference ground	
7	Negative input	
8	Positive input	0–20 mA or ±5 V (selectable)
9	Reference ground	

[1] Section 5.3.1 Technical specifications provides a full specification of the Analog In interface.

- ***Analog OUT*** (D-Sub, 9-pole, female)



Pin	Signal	Remark[1]
1–2	Reference ground	
3	Positive output	4–20 mA
4–6	Reference ground	
7	Negative output	
8–9	Reference ground	

[1] Section 5.3.1 Technical specifications provides a full specification of the Analog Out interface.

The reference ground pins within the connectors are common. Reference grounds between the connectors as well as to the chassis and internal grounds are separated.

The signal interfaces are opto-isolated.

pH electrode and conductivity cell

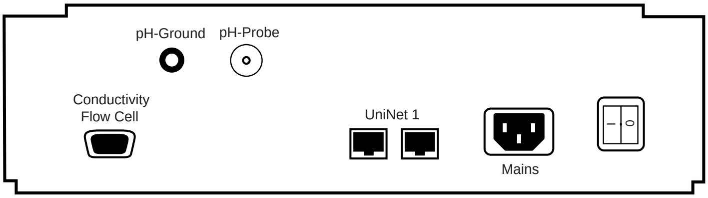


Fig 5-5. Controller mid rear panel

WARNING! HIGH VOLTAGE. The rear panel should only be accessed by authorized service personnel. Faulty connection might result in live system parts that can give a lethal electric shock.

The sockets for the electrical signals to/from the pH electrode and the conductivity cell are located on the mid rear panel on the controller inside the separation unit.

Connector	Function
pH-Ground	Ground from the pH flow cell housing
pH-Probe	Signal cable from the pH electrode
Conductivity Flow Cell	Connection cable to the conductivity cell. D-Sub, 9-pole, female

5.1.5 System flow path

The following flow diagram shows the positions of the components and tubing in the ÄKTApilot liquid flow path.

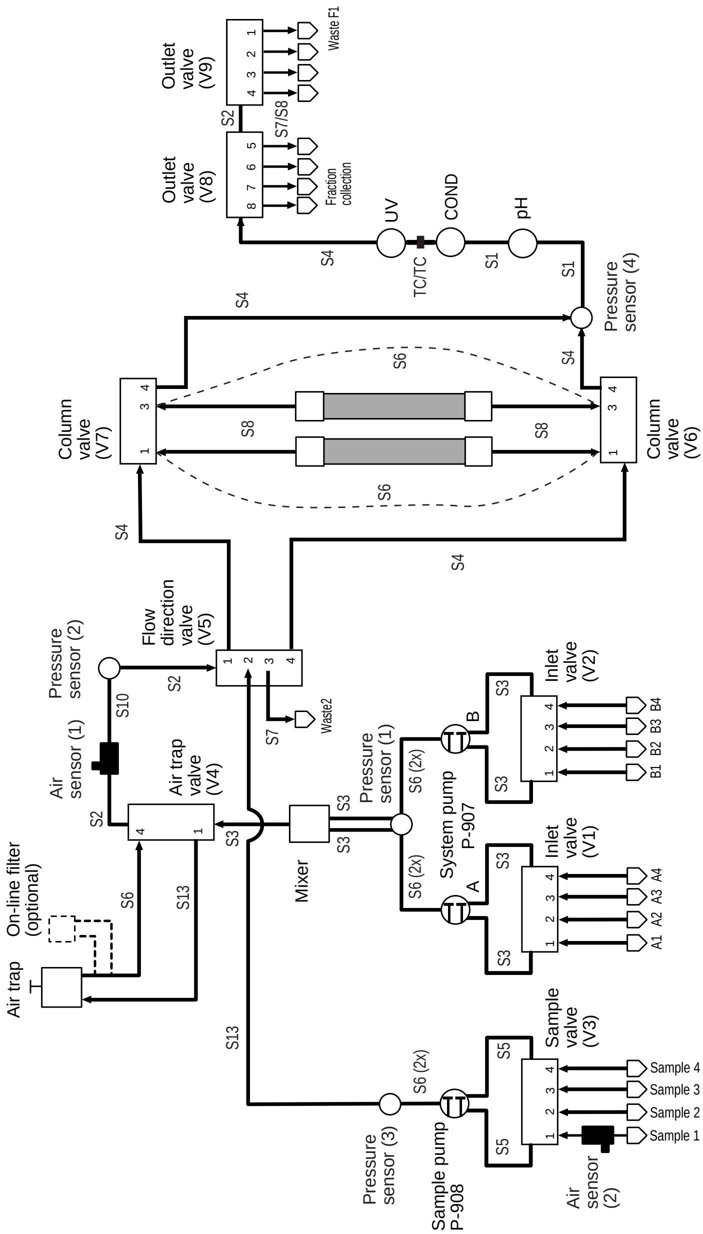


Fig 5-6. Liquid flow path

Tubing	Length [mm]	i.d. [mm]	Material	Location From	Location To
S1	85	2.9	ETFE	Pressure sensor 4	pH cell holder (IN)
				pH cell holder (OUT)	Conductivity cell (lower)
S2	120	2.9	ETFE	Valve V4 (upper port)	Air sensor 1
				Valve V8 (right port)	Valve V9 (left port)
				Pressure sensor 2	Valve V5 (upper port)
S3	200	2.9	ETFE	Valve V1 (left port)	P-907 A (left, lower)
				Valve V1 (right port)	P-907 A (right, lower)
				Valve V2 (left port)	P-907 B (left, lower)
				Valve V2 (right port)	P-907 B (right, lower)
				Pressure sensor 1 (inner ring, upper)	Mixer (IN)
				Pressure sensor 1 (outer ring, upper)	Mixer (IN)
				Mixer (OUT)	Valve V4 (lower port)
S4	300	2.9	ETFE	Valve V6 (port 4)	Pressure sensor 4
				Valve V7 (port 4)	Pressure sensor 4
				UV cell (upper)	Valve V8 (left port)
				Valve V5 (port 4)	Valve V6 (left port)
				Valve V5 (port 1)	Valve V7 (left port)
S5	350	2.9	ETFE	Valve V3 (left port)	P-908 (left head, lower)
				Valve V3 (right port)	P-908 (right head, lower)
S6	400	2.9	ETFE	P-907 A (left, upper)	Pressure sensor 1 (inner ring, lower, left)
				P-907 A (right, upper)	Pressure sensor 1 (inner ring, lower, right)
				P-907 B (left, upper)	Pressure sensor 1 (outer ring, lower, left)
				P-907 B (right, upper)	Pressure sensor 1 (outer ring, lower, right)
				P-908 (left, upper)	Pressure sensor 3 (lower, left)
				P-908 (right, upper)	Pressure sensor 3 (lower, right)
				Air trap (OUT)	Valve V4 (port 4)
				Valve V6 (port 1), by-pass	Valve V7 (port 1)
				Valve V6 (port 3), by-pass	Valve V7 (port 3)

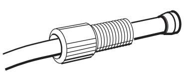
Table 5-3. Tubing description (continues on the next page)

Tubing	Length [mm]	i.d. [mm]	Material	Location	
				From	To
S7	2000	2.9	ETFE	Valve V5 (port 3)	Waste 2
				Valve V9 (port 1)	Waste F1
S8	1200	2.9	ETFE	Valves V9, V8 (ports 2–8)	Fraction collection
				Valve V6 (port 1)	Column
				Valve V7 (port 1)	Column
S10	95	2.9	ETFE	Air sensor 1	Pressure sensor 2
S13	470	2.9	ETFE	Valve V4 (port 1)	Air trap (IN)
				Pressure sensor 3 (OUT, upper)	Valve V5 (port 2)

Table 5-4. Tubing description (continued)

The tubings S1–S8 are pre-flanged. Each tubing end is equipped with a UNF 5/16" male connector and a perfluororubber O-ring.

A selection of unions and connectors is included on delivery. It contains TC connectors, M6 connectors, UNF 5/16" connectors, and unions. They can be used with various types of column fittings and for connecting the inlet and outlet tubing.



Optional tubing configuration

Tubing with i.d. 1.6 mm can be used in the system flow path instead of the i.d. 2.9 mm tubing in order to enhance system performance at low flow rates. See Ordering information for code numbers of i.d. 1.6 mm tubing and connectors.

Note: *When using i.d 1.6 mm tubing, the system flow path might not be sanitizable.*

5.1.6 Piston rinsing system

The piston rinsing system tubing is connected to the rearmost holes on the pump heads. The following flow diagram and table show the tubing configuration of the piston rinsing system.

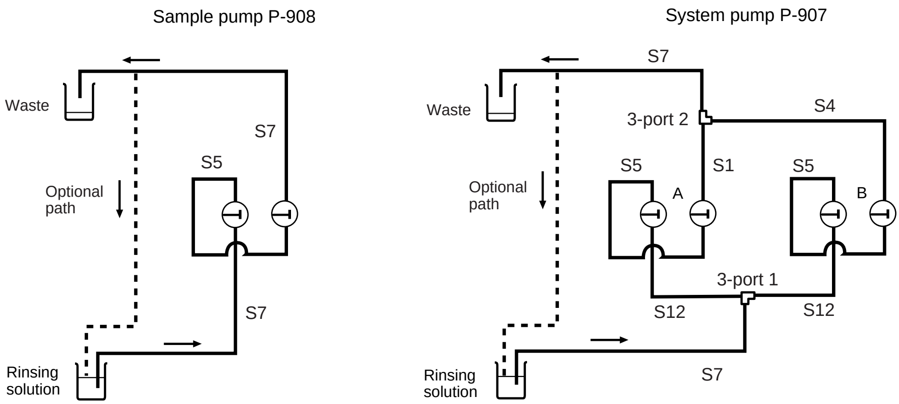


Fig 5-7. Piston rinsing system

Note: *Use the holders on the left panel intended for the containers.*

Tubing	Length [mm]	i.d. [mm]	Material	Location	
				From	To
S1	85	2.9	ETFE	3-port union 2	P-907 A (right, upper)
S4	200	2.9	ETFE	3-port union 2	P-907 B (right, upper)
S5	350	2.9	ETFE	P-908 (left head, upper)	P-908 (right head, lower)
				P-907 A (left, upper)	P-907 A (right, lower)
				P-907 B (left, upper)	P-907 B (right, lower)
S7	2000	2.9	ETFE	Rinsing solution container	P-908 (left head, lower)
				P-908 (right head, upper)	Waste (or back to the rinsing solution container
				Rinsing solution container	3-port union 1
				3-port union 2	Waste (or back to the rinsing solution container
S12	150	2.9	ETFE	3-port union 1	P-907 A (left, lower)
				3-port union 1	P-907 B (left, lower)

Table 5-5. Tubing description (piston rinsing system)

Note: *To eliminate the risk of re-introducing bacteria, etc. always use a separate waste container. Do not recirculate the same rinsing solution! Note also that a small amount of fluid remains in the pump and this comes into* *contact with fluid on the other side of the pump.*

5.1.7 Column tubing, inlet tubing, and outlet tubing

This section contains information on the column tubings, inlet tubings, and outlet tubings that are included in the ÄKTApilot system delivery. It also describes the additional tubing components needed to fully equip the system.

Column tubing kit

Item	Component	Code No.	Quantity/ pack	Number of packs included at delivery
1	Tubing S8 1200 mm	18-1169-75	1	2
2	Connector TC 5/16" female	18-1169-22	2	2
3	TC-Gasket 25/4 mm	18-1169-24	2	1
4	Union M6 female 5/16" female	18-1169-17	2	2
5	Union M6 female 5/16" male	18-1169-16	2	1

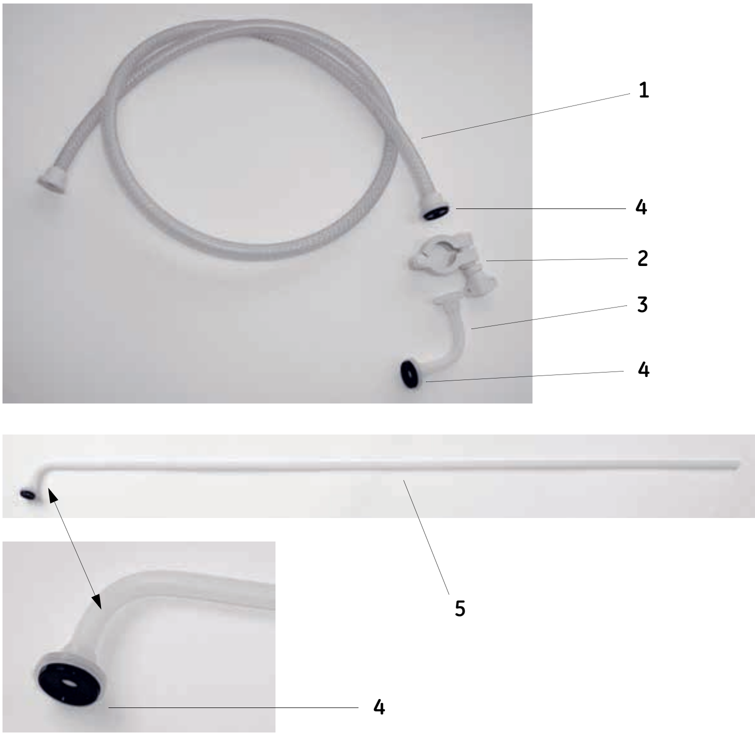
One complete Column tubing kit is included at delivery. Additional kits can be ordered (see Ordering information).

Inlet tubing

Item	Component	Code No.	Quantity/ pack	Aditional number of packs to fully equip the inlets
1	Tubing with sanitary pre-flanged fitting TC25 i.d. 6 mm, L=125 cm, PVC	18-0005-44	1	11
2	Clamp TC25	18-1169-18	4	2
3	Elbow 90° TC25-6, PP	18-1169-19	1	10
4	TC-Gasket 25/6.5 mm	18-1169-25	4	5
5	Elbow 90° TC25-6, long, PP	18-1169-20	2	5

The following inlet tubings are included at delivery:

- One complete Tubing with sanitary fitting (items 1-4)
- Two complete Elbow 90° TC25-6, long (items 4 and 5)



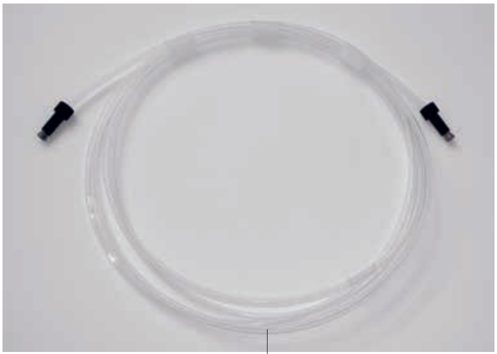
Outlet tubing

Item	Component	Code No.	Quantity/ pack	Addittional number of packs to fully equip the outlets
1	Tubing S7 2000 mm	18-1169-71	1	6
2	Tubing S8 1200 mm	18-1169-75	1	6
3	Connector TC 5/16" male	18-1169-23	2	4

The following outlet tubings are included at delivery:

- Two pieces of Tubing S7 (item 1)
- Two pieces of Tubing S8 (item 2)

1



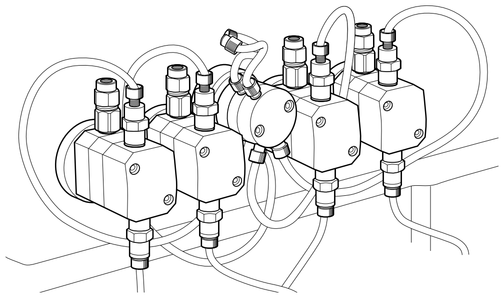
2

3

5.2 Component description

This section describes the components in the liquid flow path of the ÄKTApilot separation unit.

5.2.1 Pump P-907 and P-908



Pump P-907 and P-908 are high performance laboratory and pilot-scale pumps for use in liquid chromatography where accurately controlled liquid flow is required.

A buffer in an external vessel is drawn in by the action of the pump. Twin reciprocating pump heads then work in unison to deliver a smooth, low-pulsation flow from the pump outlet manifold.

P-907 is used as system pump; P-908 as sample pump.

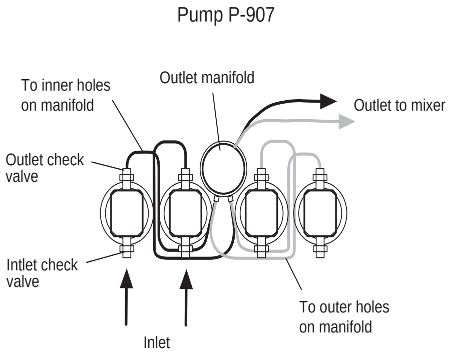
Pump P-907 and P-908 features:

- Pressure range: 0–2 MPa (20 bar, 290 psi).
- Flow rate range: 4–400 ml/min in isocratic and gradient mode.
  Double mode flow rate range: Up to 800 ml/min. The gradient performance is limited above 400 ml/min.
- P-907 is equipped with 2 pump modules; A and B, with two pump heads each. This permits binary gradients.
- Each pump houses a pressure sensor in the outlet manifold.
- Low pulsation.

Pump head

P-907 consists of two pump modules, A and B. P-908 has only one pump module. Each pump module consists of two pump heads. The individual heads are identical but are actuated in opposite phase to each other by individual stepper motors controlled by a microprocessor. This gives a continuous, low pulsation liquid delivery.

Each pump head is equipped with an inlet check valve and an outlet check valve for the liquid flow. In addition, each pump head has an outlet check valve for the rinsing system flow.



Solvent is drawn up into the pump head through a non-return inlet check valve by the action of the piston being withdrawn from the pump chamber.

On the delivery stroke of the piston, the inlet check valve is sealed by the pressure developed and buffer is forced out through a similar check valve at the outlet.

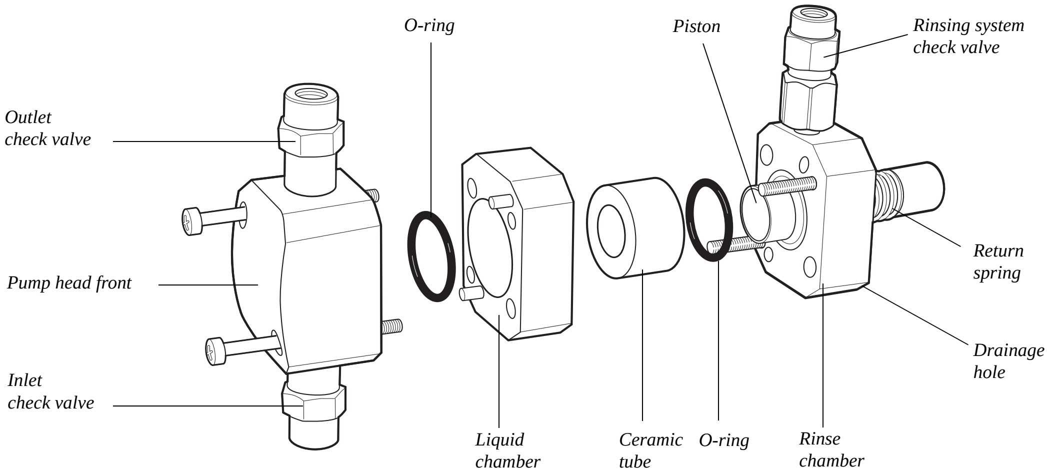


Fig 5-8. Pump head, exploded view

The pistons are actuated by cams (eccentrics) driven by the motors. Force for the retraction of the pistons is provided by coil springs. The length of stroke of the pistons is fixed and changes in the flow rate are made by varying the speed of the drive motor.

Leakage between the pump chamber and the drive mechanism is prevented by a piston. The piston is continuously lubricated by the presence of buffer. To prevent any deposition of salts from aqueous buffers on the pistons, the low pressure chamber behind the piston can be flushed continuously with a low flow of 20% ethanol.

The pump head is made of titanium alloy.

Pump principle

Each piston is driven by a simple robust cam (eccentric). These cams are driven by stepper motors via timing belts. The motor speed is varied to achieve linear movement and compensation for compressibility. This produces the particular motor sound. This system guarantees an accurate, low pulsation flow over the entire flow rate range, independent of the back pressure. When an increase in flow rate is programmed, the motor speed accelerates gradually, giving a soft start and building up speed to the flow rate required. When a decrease in flow rate is programmed, the motor speed slows rapidly to the lower flow rate.

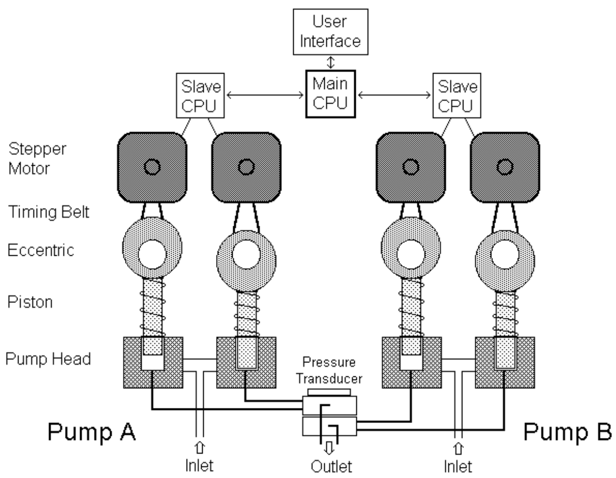
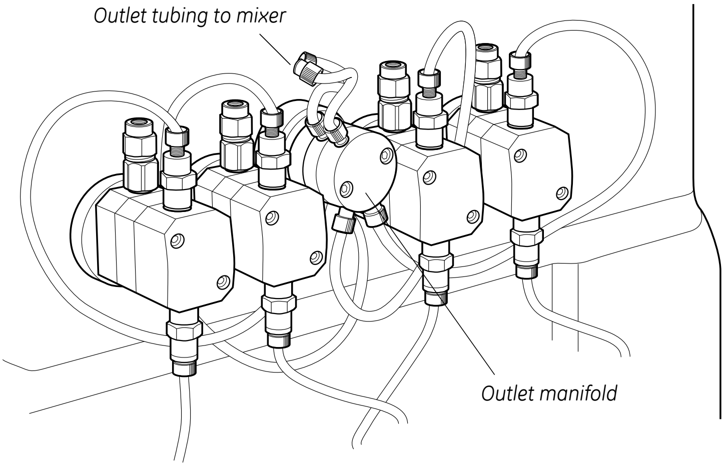


Fig 5-9. P-907 pump principle

Outlet manifold

The outlet of each pump head is connected by tubing to an outlet manifold block where the liquids from both heads are combined to give continuous buffer delivery. The outlet manifold also houses the outlet connections to the mixer.

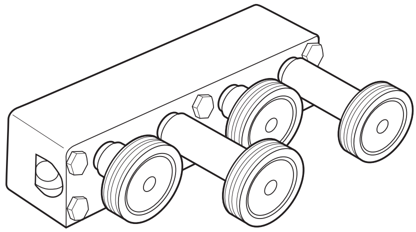


Each outlet manifold also contains a pressure sensor. On P-907, the pressure sensor, which is located in the rearmost part of the outlet manifold, is connected to the left-hand pump module (A).

The outlet tubing is made of PTFE and the outlet manifold is made of PEEK.

5.2.2 Membrane valve

The system uses stepper motor-actuated membrane valves for controlling the liquid flow in the separation unit. The valves in the system are located in nine valve blocks.



A valve block consist of a connection block containing the ports and the membranes, and a mechanical housing containing the stepper motors, cams and actuating pistons.

The membrane in a valve is actuated by a stepper motor via a cam and a piston. The piston is attached to a rubber membrane which is either retracted from or pressed against the valve seat. Consequently, the membrane either opens or closes the valve. An electrical switch indicates if the valve is open or closed. No power is required to keep the valve in the open or closed position.

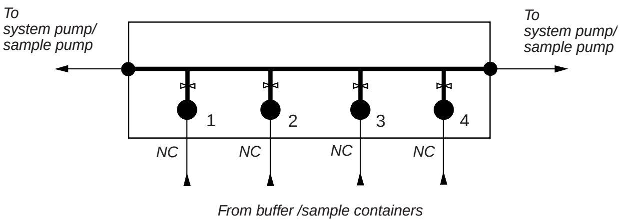
The valve seats have two different diameters. The smaller valve seats are used in valves where the operating pressure is higher than in other parts of the system.

The connection block is made of PEEK and the membranes of FFKM (perfluororubber). The connection block and the membranes are replaceable.

Valve block types

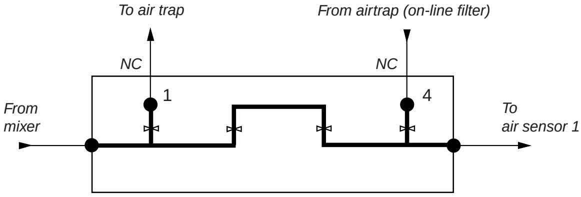
There are six different types of membrane valve blocks. The following illustrations show the flow path in the valve blocks and where the valves are located. The illustrations also show whether the valves are normally open (NO) or closed (NC).

- Inlet valve V1 and V2 and sample valve V3

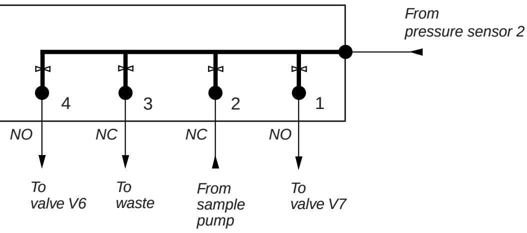


In V1, V2 and V3, only one valve in a block can be open at a time.

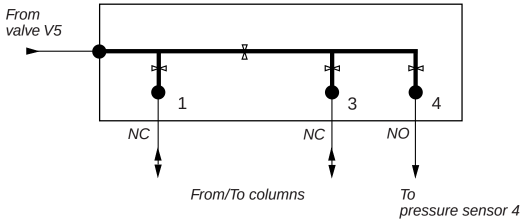
- Air trap valve V4



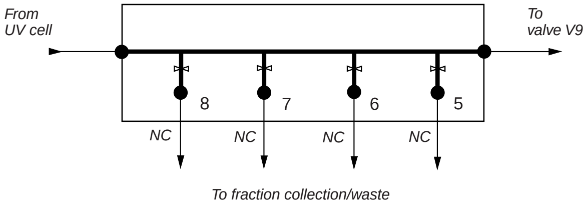
- Flow direction valve V5



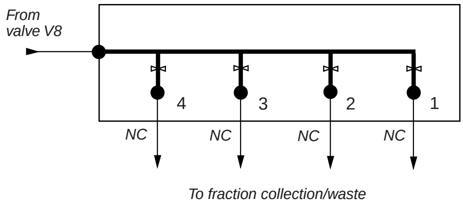
- Column valve V6 and V7



- Outlet valve V8



- Outlet valve V9



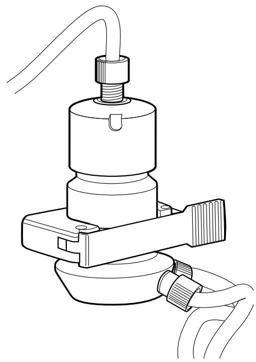
The table below shows the size of the valve seats in the valve blocks.

Valve block	Valve seat size
V1–V3, V5, V8, V9	Large
V4, V6, V7	Small

Table 5-6. Valve seat sizes

The valve blocks have UNF 5/16" female ports except for the inlets on valve V1–V3, which have G 1/8" female inlet ports.

5.2.3 Mixer M-905

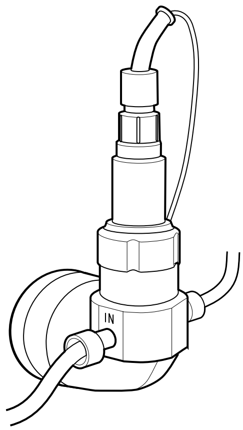


The mixer is a dynamic, single-chamber mixer positioned directly after the outlet manifold of system pump P-907.

A mixer motor behind the ÄKTApilot front panel spins a magnet at 600 rpm, which causes the stirrer in the mixing chamber to rotate.

The system comes with a 5 ml mixer chamber, which is replaceable. The wetted part in the chamber is made of PEEK. The stirrer is coated with PTFE.

5.2.4 pH electrode and cell holder



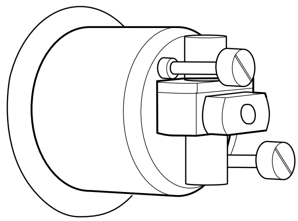
The pH electrode is optimized for continuous pH measurement in the ÄKTApilot flow path. The electrode is of the sealed combination double junction type. It contains a sealed Ag/AgCl reference, which cannot be refilled, an internal electrolyte bridge of 4 M KCl saturated with Ag/AgCl, an outer electrolyte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

The pH electrode has a glass tip and the cell holder is made of titanium. The whole assembly is replaceable.

The pH electrode should be calibrated regularly. This procedure is described in section 2.4.2 Calibrating the pH electrode.

5.2.5 Monitor UV-901 and UV cell

The UV cell is designed for continuous measurement of UV absorbance. The UV monitoring system provides high performance detection for wavelengths between 190 and 700 nm. Three wavelengths can be detected simultaneously. The optical pathlength – 1, 2 or 5 mm – is set with shim plates.The default length is 2mm.



The UV cell housing is made of PEEK, other wetted parts are made of glass and titanium.

Monitor principle

A xenon flash lamp gives a high intensity, continuous spectrum throughout the range 160–2000 nm. The light enters a monochromator which includes a condensing system, blocking filter, entrance slit and a concave aberration-corrected holographic grating. Monochromatic light from the grating is directed to an optical fibre. The grating is turned by a stepping motor.

For wavelengths between 360–700 nm, a blocking filter is moved into the light path to filter out unwanted light, of a half wavelength from the second order spectrum, before entering the monochromator.

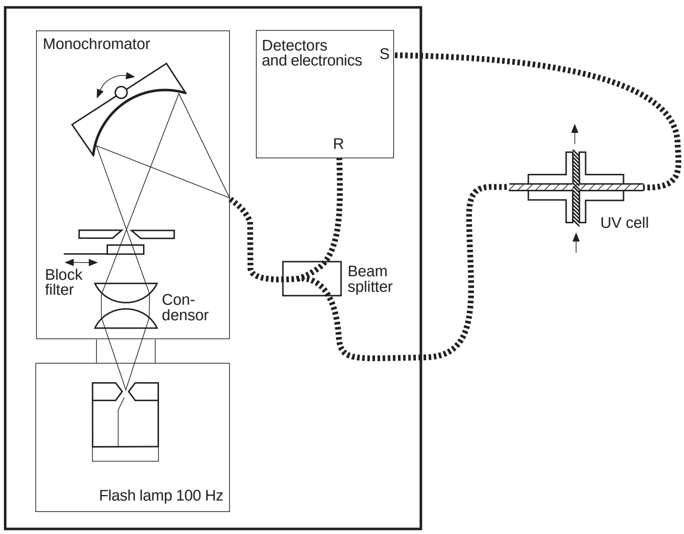


Fig 5-10. UV monitor principle

The light passing from the monochromator to the cell and from the cell to the detector electronics is guided by optical fibers which focus its full intensity on the liquid flow path, maximizing the sensitivity of the monitoring. Before entering the cell, the monochromatic light is split in a beam splitter, with 50% of the light passing through the sample fibre (S) and the cell, and 50% being directed through the reference fiber (R). Two photodiodes with identical characteristics monitor the intensities of the measuring and reference beams.

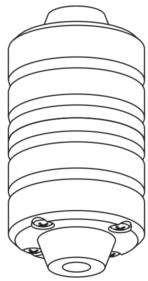
The detection system is very stable since the optical unit is located away from the lamp and electronics so that noise and drift caused by temperature variations is avoided.

At calibration, the instrument automatically finds 2 persistent lines in the spectrum of xenon. The wavelengths of these known lines are used to calibrate the stepper motor that turns the grating.

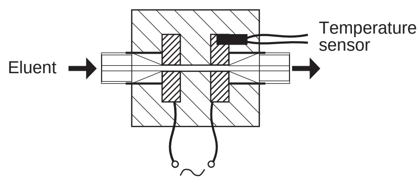
Note: *Using two or three wavelengths results in increased wear on the monochromator mechanics. To reduce the required maintenance use only one wavelength if this is sufficient for the application.*

5.2.6 Conductivity cell

The cell has two cylindrical titanium electrodes positioned in the flow path of the cell. An alternating voltage is applied between the electrodes and the resulting current is measured and used to calculate the conductivity of the buffer. The system controls the AC frequency and increases it with increasing conductivity between 50 Hz and 50 kHz, giving maximum linearity and true conductivity values.

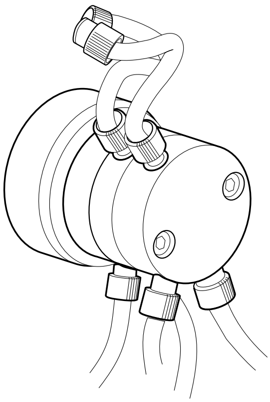


The conductivity is automatically calculated by multiplying the measured conductance by the cell constant of the cell. The cell constant is pre-calibrated on delivery but can be measured with a separate calibration procedure. This procedure is described in section 2.4.3 Calibrating the conductivity cell.

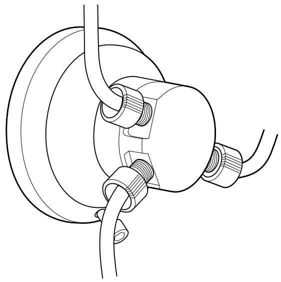


One of the electrodes has a small temperature sensor for measuring the temperature of the buffer in the cell. Temperature variations influence the conductivity and in some applications, when very precise conductivity values are required, it is possible to program a temperature compensation factor that recalculates the conductivity to a set reference temperature.

5.2.7 Pressure sensor



P-907 pressure sensor



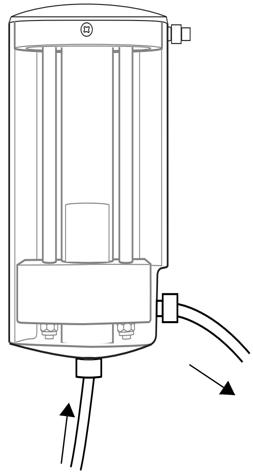
3-port union pressure sensor

The pressure in the flow path is continuously monitored by the four pressure sensors. Two of them are housed in the outlet manifold blocks of the pumps. In the system pump P-907, the outlet tubing from pump module A is connected to the pressure sensor (the inner block of the outlet manifold). The pressure is almost the same in pump module A and B. The other two pressure sensors are located before and after the columns.

The liquid chamber in the pressure sensor housing is equipped with a thin steel membrane. A strain gauge is attached to the rear side of the steel membrane. When the liquid pressure increases, the steel membrane bulges, which is detected by the strain gauge. The pressure is shown on the computer display. To protect the column, a maximum and minimum pressure limit can be set in UNICORN.

The pressure sensor has a pressure range of 0–25 bar (2.5 MPa, 362 psi), however the readings are restricted to 0-20 bar. The pressure sensor housing is made of PEEK, other wetted parts are made of titanium and FFKM (perfluororubber).

5.2.8 Air trap



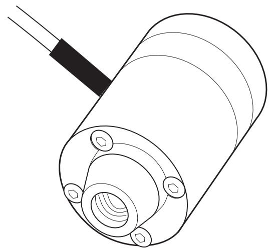
The liquid flow in the separation unit can be routed through an air trap in order to remove air from the liquid. Preventing air from entering the column is important since air might reduce the chromatographic performance of the column.

Through the air trap valve V4, the flow is routed to the inlet port at the bottom of the air trap. The flow enters the air trap cylinder through a pipe which reaches about 3 cm above the bottom of the cylinder. The air in the liquid is trapped in the cylinder while the liquid exits the cylinder through a hole at the bottom. The liquid then continues through the outlet port back to the V4 (or to the optional on-line filter).

The air trap also has an air vent connector at the top, which is used for adjusting the liquid level in the air trap to suit the flow rate that is used.

The air trap volume is 81 ml, and the max. pressure limit 2 MPa. The air trap cylinder is made of glass, the bottom and top parts are made of polypropylene, and the outer protective tube is made of polycarbonate.

5.2.9 Air sensor 925 and 940



The air sensor is a high precision monitor designed for continuous monitoring of air bubbles in the flow path. The air sensor is made of PEEK.

There are two different air sensors in the systems; one for sample application and one for column protection. When air is detected, the system is either paused, or performs an action that is set in the method.

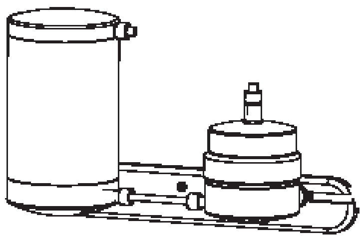
The air sensor 940 used for sample application should be connected on the sample inlet tubing between a selected inlet valve (V1, V2 or V3) and the sample vessel. The air sensor is used to detect when the sample vessel is emptied, and to protect the column from air bubbles. The flow path in this air sensor has i.d. 4 mm.

The air sensor 925 used for column protection is connected before the columns. It is important to protect the column from air since air might damage the packed medium or cause poor chromatographic performance. The flow path in this air sensor has i.d. 2.5 mm.

5.2.10 Online Filter (optional)

The optional online filter comes in two different kits, Sartorius (18-1171-06) and Millipore (18-1173-45).
The following is recommended with regards suitable filters to the filter kits:

Filter Kit	*Cartridge*	*Connection*
Sartorius	Minicartridge Size 7	Connector 15
Millipore	Millidisk 10	Code M



Sartorius filter kit

5.2.11 Frac 950 (optional)

Fraction collector Frac-950 (18-6083-00) is an automated fraction collector for use in liquid chromatography as part of an ÄKTAdesign chromatography system. Its design allows x-y fractionation but without x-y movements.

Frac-950 is equipped with an accumulator to eliminate spillage at high flows. A drop sensor that can be used to control tube changes at low flows is also included.

Frac-950 is delivered with Rack A (18-6083-11) which contains the following vessels:

120 x 18 mm tubes
8 x 30 mm tubes

Different types of racks accommodating different sizes and types of tubes are available as options. Accessories for collecting extra large fraction volumes are also available.

Frac-950 has a maximum flow rate of 100 ml/min and a fraction size of 0.1 - 99999.99 ml in Volume mode and 0.1 - 99999.99 min in Time mode.

5.3 Specifications

5.3.1 Technical specifications

ÄKTApilot separation unit

Mains voltage	100–240 V~, 50–60 Hz
Power consumption	800 VA
Fuse specification	min. T 10A L (SB) up to max. T 16A L (SB) on external mains supply (no user-replaceable fuse in the separation unit)
Degree of protection	IP 24
Dimensions, separation unit	900 × 750 × 540 mm (H × W × D)
Weight, separation unit	114 kg (monitor arm not included)
System hold-up volume	32 ml (from one of the pump head inlets on the system pump to valve V9, port 8)
System Frac-delay volume	3.5 ml (from the UV flow cell to valve V9, port 2)
Ambient temperature *operation* *storage*	 +4 - +40 °C -25 - +60 °C
Relative humidity, operation	20%–95% for temperatures up to 31°C decreasing linearly to 50% relative himidity at 40 °C
Cabinet material	Stainless steel 304
Digital output *protection* *max. voltage*	2 channels (opto-isolated relays) Automatic reset fuse, max. current 140 mA 25 V between output pins, and between outputs and chassis ground
Digital input *active level* *passive level* *impedance* *max. voltage*	2 channels (opto-isolated) > 4.5 V < 2.5 V ~ 900 ohm 25 V between input pins, and between inputs and chassis ground

Analog output *protection* *range* *resolution* *accuracy* *max. voltage*	Opto-isolated Automatic reset fuse, max. current 50 mA 4–20 mA 0.001 mA 0.2% 25 V between output pins and chassis ground
Analog input *voltage mode* *range* *accuracy* *impedance* *current mode* *range* *accuracy* *impedance* *max. voltage*	Opto-isolated ±0–5 V (max. ±25 V) 0.15% of full scale 1 Mohm 0–20 mA (max. 50 mA) 0.2% 250 ohm 25 V between input pins and chassis ground or reference ground
Compliance with standards	The declaration of conformity is valid for the instrument only if it is: • used in laboratory locations • used in the same state as it was delivered from GE except for alterations described in the user documentation • 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 610101-1 • IEC 61010-1 • CAN/CSA-C22.2 No. 61010-1 • UL61010-1

EMC standards	This instrument 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 instrument complies with part 15 of the FCC rules (emission). Operation is subject to the following two conditions: 1 This instrument may not cause harmful interference. 2 This instrument must accept any interference received, including interference that may cause undesired operation.

Computer and monitor

See the specifications provided by the manufacturer of the computer and monitor.

System pump P-907 and sample pump P-908

Flow rate *isocratic and gradient mode* *double mode*	 4–400 ml/min Up to 800 ml/min (for gradient specification, see below)
Increment	0.1 ml
Pressure	0–2 MPa (20 bar, 290 psi)
Flow rate accuracy	< ±2% (4–400 ml/min, 0.1–2 MPa)
Flow rate reproducibility	rsd < 0.5% (4–400 ml/min, 0.1–2 MPa)
Viscosity	Max. 5 cP
Gradient composition *accuracy* *reproducibility*	 ±3% rsd < 0.5% (8–400 ml/min, 10–90% linear gradient, 5–95% step gradient
Internal volume *maximum volume* *minimum volume*	 1760 µl/pump head 615 µl/pump head

UV measurement, Monitor UV-901

UV cell path length	1/2/5 mm
Internal volume	300 µl
Max. pressure	2 MPa (20 bar, 290 psi)
Wavelength accuracy	±2 nm
Wavelength precision	±0.01 nm
Noise [1,2] *Single wavelength* *short-term (0.5–1 min)* *long-term (1–10 min)* *Dual wavelengths* *short-term (0.5–1 min)* *long-term (1–10 min)*	 $< 6 \times 10^{-5}$ AU at 230 nm $< 6 \times 10^{-5}$ AU at 230 nm $< 2 \times 10^{-4}$ AU at 230 nm and 254 nm $< 2 \times 10^{-4}$ AU at 230 nm and 254 nm
Linearity	< 2% deviation up to 2 AU at 280 nm (iron(III)-sulphate in 0.1 M sulphuric acid)
Drift [2]	$< 4 \times 10^{-4}$ AU/hour at 230 nm
Absorbance range	0.001–3.5 AU

1 Measured with water at 150 ml/min, time constant 1s, 2 mm UV cell path length
2 Typical values at room temperature after warm-up.

Mixer M-905

Max. flow rate	800 ml/min
Max. pressure	2.5 MPa (25 bar, 362 psi)
Internal volume	4.2 ml
Mixing principle	Chamber with a stirrer bar

pH measurement, Monitor pH/C-901

pH range	0–14 (spec. valid between 2 and 12)
Accuracy *temperature compensated* *not temperature compensated*	 ±0.1 pH units within 4–40 °C ±0.2 pH units within 15–25 °C ±0.5 pH units within 4–15 °C and 25–40 °C
Response time	Max. 10 s (0–95% of step)
Long-term drift	Max. 0.1 pH units/10 h
Flow rate sensitivity	Max. 0.1 pH units within 0-100 ml/min.
Max. pressure	0.5 MPa (5 bar, 72 psi)
Internal volume, pH cell holder	240 µl

Conductivity measurement, Monitor pH/C-901

Conductivity range	1 µS/cm to 999.9 mS/cm
Deviation from theoretical conductivity	Max. ±2% of full scale calibrated range or ±10 µS/cm whichever is greater in the range 1 µS/cm to 300 mS/cm
Reproducibility *short-term* *long-term*	 Max. ±1% or ±5 µS/cm Max. ±3% or ±15 µS/cm
Noise	Max. ±0.5% of full scale calibrated range
Response time	Max. 3 s (0–95% of step)
Temperature sensor *accuracy* *drift*	 ±2.0 °C ±0.5 °C per 10 h
Flow rate sensitivity	±1% within 0–400 ml/min
Max. flow rate	800 ml/min
Max. pressure	2 MPa (20 bar, 290 psi)
Internal volume, conductivity cell	180 µl

Membrane valve

Max. pressure	2.2 MPa (22 bar, 320 psi)
Back-pressure	0.015 MPa at 400 ml/min
Valve principle	Stepper motor-controlled membranes

Pressure sensor

Pump oulet manifolds	
Pressure range	0–2.5 MPa
Offset error	< 0.02 MPa
Accuracy	< ±2% (FSD)
Internal volume	202 µl
2-port and 3-port union	
Pressure range	0–2.5 MPa
Offset error	< 0.005 MPa
Accuracy	< ±2% (FSD)
Internal volume *2-port union* *3-port union*	 331 µl 346 µl

Air sensor 925 and 940

Max. flow rate	800 ml/min
Max. pressure *Cell 925* *Cell 940*	 2.5 MPa (25 bar, 362 psi) 2.5 MPa (25 bar, 362 psi)
Internal volume *Cell 925* *Cell 940*	 190 µl 480 µl

Tubing connections

Inlet A1–A4 (V1) Inlet B1–B4 (V2) Sample 1–4 (V3) *dimension (i.d.,o.d.)* *flange type/size*	 6.0 mm, 9.8 mm TC 25
Outlet F2–F8 (V8, V9) *dimension (i.d.,o.d.)* *flange type/size*	 2.9 mm, 3/16" UNF 5/16"
WasteF1 (V9) *dimension (i.d.,o.d.)* *flange type/size*	 2.9 mm, 3/16" UNF 5/16"
Waste2 (V5) *dimension (i.d.,o.d.)* *flange type/size*	 2.9 mm, 3/16" UNF 5/16"
Column 1, Column 2 (V6, V7) *dimension (i.d.,o.d.)* *flange type/size*	 2.9 mm, 3/16" UNF 5/16"
Internal tubing connections between inlet and outlet *dimension (i.d.,o.d.)* *flange type/size*	 2.9 mm, 3/16" UNF 5/16"

Note: *The i.d. 2.9, o.d. 3/16" tubing can be replaced by i.d. 1.6 mm, o.d. 1/8" tubing (sanitizability not guaranteed).*

Liquid feed requirements

Media feed requirements	
Inlet	-1 – 0 m height difference [1]
Outlet	0 – 1 m height difference [1]
Pump wash feed requirements	
Inlet	+0-1 m height difference [1]
Outlet	+0-1 m height difference [1]

[1] Valid for water. If using liquids with higher density or higher viscosity than water, the distance will decrease.

Table 5-7. ÄKTApilot technical specifications

5.3.2 System performance specifications

System flow rate isocratic and gradient flow double mode	 4–400 ml/min Up to 800 ml/min (limited gradient performance above 400 ml/min)
Operating pressure	Up to 2 MPa (20 bar, 290 psi) between system pump and column inlet
UV wavelength range	190–700 nm in steps of 1 nm, 3 wavelengths simultaneously
pH range	0–14 (spec. valid between 2 and 12)
Conductivity range	1 µS/cm to 999.9 mS/cm
System back-pressure *system pump – V6, port 4* *V6, port 4 – V9, port 1*	 0.060 MPa at 400 ml/min 0.045 MPa at 400 ml/min

Table 5-8. ÄKTApilot performance specifications

5.3.3 ÄKTApilot component materials

The wetted materials of ÄKTApilot are listed below:

	PEEK	PTFE	FFKM	ETFE	CTFE	PP	UHM WPE	Titanium	Boro-silicate (glass)	Ceramic	Suprasil 2	Elgiloy	Ruby/ sapphire
System pump Sample pump	X		X		X		X	X		X		X	X
UV cell	X		X					X			X		
pH cell holder/ dummy electrode		X	X					X					
Conductivity cell					X			X					
Mixer	X	X											
Membrane valve	X		X										
Air trap	X		X					X	X				
Pressure sensors	X		X					X					
Air sensors	X												
Tubing			X	X									
Unions/ Connectors	X		X			X							

FFKM = perfluororubber (PFR91 Resel 77045)
CTFE = chlorotrifluoroethylene (KelF81)
PEEK = polyetheretherketone (PEEK 450G)
PTFE = polytetrafluoroethylene
ETFE = ethylenetetrafluoroethylene
PP = polypropylene
UHMWPE = ultrahigh molecular weight polyethylene
Suprasil = quartz

Table 5-9. Wetted materials in ÄKTApilot

5.4 Chemical resistance guide and chemical compatibility

The chemical resistance of ÄKTApilot 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 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 < 1 day***	***Exposure up to 2 months***	***Comments***
Acetaldehyde	OK	OK	
Acetic acid, < 5%	OK	OK	
Acetic acid, 70%	OK	OK	
Acetonitrile	OK	OK	FFKM, PP and PE swell
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, CTFE, 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	
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	

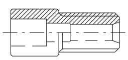
Chemical	*Exposure < 1 day*	*Exposure up to 2 months*	*Comments*
Hexane	OK	Avoid	Silicone not resistant Pressure limit for PEEK decreases
Hydrochloric acid, 0.1 M	OK	OK	Silicone not resistant
Hydrochloric acid, > 0.1 M	OK	Avoid	Silicone not resistant Titanium is affected by long-term use
Isopropanol	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, aluminium oxide and glass 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, 2 M	OK	Avoid	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
Tetrahydrofuran	Avoid	Avoid	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	PP and PE are affected by long-term use

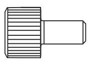
5.5 Ordering information

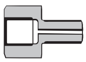
A selection of inlet and outlet tubing and connectors are included in the delivery of ÄKTApilot. The items listed in below are accessories and spare parts that can be ordered from your local GE representative.

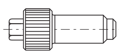
Item	Quantity/ pack	Code no.
System pump/ Sample pump		
Pump head 400 ml cpl., including piston assembly, O-rings (2 pcs.), ceramic tube, liquid chamber, pump head front, 3 check valves	1	29-0310-81
Piston assembly, 400 ml, cpl., including rinsing chamber, piston, sealing, O-ring, check valve adapter	1	18-1169-90
Ceramic tube	1	29-0239-78
Check valve, inner, cpl.	1	18-1169-88
Check valve outer, cpl.	1	18-1169-89
Seal kit, 400 ml	1	18-1169-91
pH monitoring		
pH electrode, ÄKTApilot, including O-ring and nut	1	18-1168-77
Cell holder, pH	1	18-1170-02
Dummy pH electrode, including O-ring and nut	1	18-1169-11
O-ring	1	18-1118-60
Conductivity monitoring		
Conductivity cell, i.d. 2.5 mm	1	18-1169-00
Cell holder, air sensor/cond	1	18-1170-01
UV monitoring		
UV cell, 1/2/5 mm	1	18-1168-83
Shim plate, 2 mm	2	18-1168-84
O-ring kit	6	18-1134-88
O-ring	10	19-0680-01
Optical fiber	1	18-1170-17
Mixer		
Mixer chamber, 5 ml, 5/16"	1	18-1168-98
Stirrer, diam. 15.7 mm	1	18-1114-32

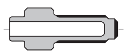
Item	Quantity/ pack	Code no.
Air sensors		
Cell-925	1	18-1167-70
Cell-940	1	18-1159-54
Connector TC–G1/8", PEEK (for Cell-940)	2	18-1171-02
Air trap		
Air trap, complete	1	18-1170-97
Membrane valve		
Connection block V1	1	18-1169-01
Connection block V2	1	18-1169-02
Connection block V3	1	18-1169-03
Connection block V4	1	18-1169-04
Connection block V5	1	18-1169-05
Connection block V6	1	18-1169-06
Connection block V7	1	18-1169-07
Connection block V8	1	18-1169-08
Connection block V9	1	18-1169-09
Valve membrane	1	18-1169-10
Inlet connectors, short/long, PEEK (for inlet valves)	4 (2+2)	18-1170-16
Elbow 90° TC 25–6, PP	1	18-1169-19
Elbow 90° TC 25–6, long, PP	2	18-1169-20
Inlet connection kit, incl. M27 nut, washer and circlip (for Elbow 90°)	4	18-1169-21
Elbow 90° kit, including inlet connection kit, Elbow 90° and TC gasket 25/6.5	1	18-1170-60
Pressure sensors		
Pressure sensor, 2.5 MPa, including outlet manifold A (for P-907 and P-908)	1	18-1169-79
Outlet manifold B (for P-907 only)	1	18-1169-86
Pressure sensor, 2-port	1	18-1169-78
Pressure sensor, 3-port	1	18-1170-19
Connectors and unions		
Union M6 female–UNF 5/16" male, PEEK	2	18-1169-16
Union M6 female–UNF 5/16" female, PEEK	2	18-1169-17











Item	Quantity/ pack	Code no.
Connector TC–5/16" female, PEEK	2	18-1169-22
Connector TC–5/16" male, PEEK	2	18-1169-23
Connector TC–5/16" male, short, PEEK	2	18-1170-08
Connector 5/16" female–5/16" female, PEEK	2	18-1173-51
Clamp TC 25	4	18-1169-18
TC gasket 25/4 mm, FFKM	4	18-1169-24
TC gasket 25/6.5 mm, FFKM	4	18-1169-25
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
Union Luer female/M6 female, polypropylene	2	18-1027-12
Union 5/16" female/M6 male, PEEK	3	18-1127-76
Stop plug, 5/16", PEEK	5	18-1112-50
Union 5/16" female/HPLC male	8	18-1142-08
On-line filter		
Filter kit Sartorius, including filter house, mounting plate (air trap/filter), tubing, spring clip, O-ring	1	18-1171-06
Filter house, including filter house, tubing,	1	18-1173-47
Miscellaneous		
Wetted parts kit, including all wetted parts in the ÄKTApilot flow path	1	18-1171-07
Tool kit ÄKTApilot	1	18-1174-57
Air inlet filter	1	18-1169-58
Tubing		
Tube S1 cpl. incl. 2 nuts and 2 O-rings	1	18-1169-59
Tube S2 cpl. incl. 2 nuts and 2 O-rings	1	18-1169-66
Tube S3 cpl. incl. 2 nuts and 2 O-rings	1	18-1169-67
Tube S4 cpl. incl. 2 nuts and 2 O-rings	1	18-1169-68
Tube S5 cpl. incl. 2 nuts and 2 O-rings	1	18-1169-69
Tube S6 cpl. incl. 2 nuts and 2 O-rings	1	18-1169-70
Tube S7 cpl. incl. 1 nut and 1 O-ring	1	18-1169-71
Tube S8 cpl. incl. 2 nuts and 2 O-rings	1	18-1169-75
Tube S10 cpl. incl. 2 nuts and 2 O-rings	1	18-1169-72

Item	Quantity/ pack	Code no.
Tube S12 cpl. incl. 2 nuts and 2 O-rings	1	18-1169-73
Tube S13 cpl. incl. 2 nuts and 2 O-rings	1	18-1169-74
Column tubing kit, incl. tube S8, TC–5/16" connector, M6–5/16" unions, TC gasket	1	18-1167-68
TC connection 5/16"–24 (for rinsing system)	1	18-1170-59
O-ring 3 x 1 (for system tubing and air trap vent connector)	25	18-1169-12
Air trap vent tubing, incl. Luer fitting	2 m	18-1171-01
PTFE tubing, i.d. 1.6 mm, o.d. 1/8"	3 m	18-1121-16
PTFE tubing, i.d. 2.9 mm, o.d. 3/16"	3 m	18-1112-47
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
UV Test kits		
UV-900 1 mm Calibration kit	1	18-6324-01
UV-900 2 mm Calibration kit	1	18-6324-02
UV-900 5 mm Calibration kit	1	18-6324-04

Table 5-10. Ordering information

Index

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For contact information for your local office,
please visit
www.gelifesciences.com/contact

www.gelifesciences.com

GE Healthcare Bio-Sciences AB
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751 84 Uppsala
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Amersham Place, Little Chalfont, Buckinghamshire, HP7 9NA, UK

GE Healthcare Bio-Sciences Corp
800 Centennial Avenue, P.O. Box 1327, Piscataway, NJ 08855-1327, USA

GE Healthcare Europe GmbH
Munzinger Strasse 5, D-79111 Freiburg, Germany

GE Healthcare Japan Corporation
Sanken Bldg. 3-25-1, Hyakunincho, Shinjuku-ku, Tokyo 169-0073, Japan

Instrument Handbook 18-1162-95 AH 02/2014