ÖÄKTA design

Optional Configurations ÄKTA explorer

User Manual



Important user information

All users must read this manual to fully understand the safe use of the components.

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 clearly understood and met.

Note

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

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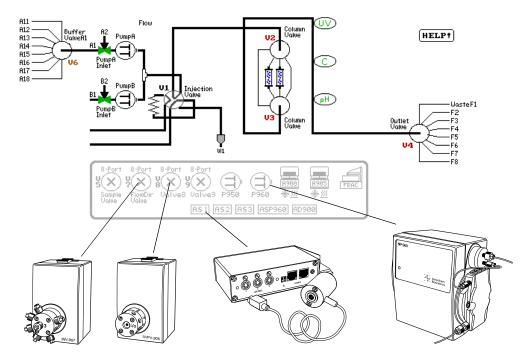
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1 Introduction

1.1 General

 $\ddot{A}KTA^{^{\mathrm{TM}}}$ explorer standard system configurations can be changed to optional configurations. This flexibility in $\ddot{A}KTA$ explorer $^{^{\mathrm{TM}}}$ system strategies allows the user to enhance already used purification methods and also to develop new, more complex methods.



When optional equipment is connected to a standard chromatographic system, new sets of instructions to control the optional equipment become accessible for the user.

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

Optional configurations are monitored and controlled via methods run by the $UNICORN^{TM}$ control system in the same way as $\ddot{A}KTA$ explorer standard system configurations.

Optional configurations are shown in the flow scheme displayed in the System Control in UNICORN. Components used in an optional configuration are highlighted in the flow scheme, whereas components not installed are hidden. The status of the components is also shown, e.g., valve position.

Note: The actual location of, for example, an optional valve is not shown in its proper flow path position. This must be taken into account by the user. However, a new flow path picture can be ordered with the optional configuration parts included, and installed together with the additional software to reflect the optional system configuration.

Generally speaking, there are two types of optional configurations. Completely user-defined configurations and dedicated configurations partially pre-defined and prepared at the factory, requiring user actions in their final implementation.

Components used in completely user-defined configurations are designated with general alpha-numeric position tags. For example, Valve8 refers to the valve corresponding to the component identity code 8 in UNICORN.

Components used in dedicated configurations are referred to in plain language, e.g. OutletValve, which is the same designation as the actual instruction name used in UNICORN.

1.2 Scope of manual

To support the process of implementing optional configurations, general guidelines regarding installation and operation are given in this manual.

For specific information regarding function, maintenance, trouble-shooting and spare parts, refer to the User Manual and/or Instruction for the respective component, as well as the System Manual for your ÄKTAexplorer system.

This manual is divided into a number of main sections reflecting the optional configurations available for ÄKTAexplorer system.

Select the main section describing the installation and operation of the optional configuration you have decided to use.

On the back page, there is a short instruction giving guidelines for the general steps in adding and removing optional configurations to your ÅKTAexplorer system.

1.3 Summary of optional configurations

An overview of all optional configurations available for ÄKTAexplorer is summarized below.

Optional configurations supported by ÄKTAexplorer are:

- Connecting Fraction collector Frac-901.
- Connecting Fraction collector Frac-950.
- Connecting Autosampler A-900 (and A-900 with Cooling) and Autosampler A-905.
- Connecting a flow direction valve.
- Connecting a sample selection valve.
- Connecting up to 2 motorized multi-port optional function valves. The actual use is completely user-defined.
- Connecting on-line pH measurement.
- Connecting sample pump P-960.
- Connecting up to four air sensors.
- Connecting external equipment using digital input/output signals through the system pump P-900 REMOTE connector.
- Connecting AD-900.
- Connecting a Superloop[™].

1.3.1 Fraction collector Frac-901

Fraction collector Frac-901 is an automated fraction collector for use in ÄKTAexplorer chromatography systems under UNICORN control. The functionality and performance is aimed for mid-range usage.

1.3.2 Fraction collector Frac-950

Fraction collector Frac-950 is an automated fraction collector for use in ÄKTAexplorer chromatography systems under UNICORN control. The functionality and performance is aimed for high-end usage.

1.3.3 Autosampler A-900 and Autosampler A-905 Autosampler A-900 used in ÄKTAexplorer enables:

- Automated, multiple sample injections from 1 μ l to 1 ml of sample solution.
- Sample stored in tubes in a circular, rotating rack.
- Quantification of amount, concentration and molecular size
 - External standard quantification.
 - Internal standard quantification.
 - · Standard addition.
 - Recovery calculations.
 - Molecule size calculations.

Quantification uses peak data from standards to produce calibration curves which can then be used to evaluate the amount and concentration of components in a sample. The molecular size function determines the molecular size of components in a sample using a molecular size curve prepared from one or more standards.

Note: Quantification requires additional software not included in the standard package of Autosampler A-900.

Autosampler A-900 with Cooling provides internal cooling of the sample solutions.

Autosampler A-905 used in ÄKTAexplorer enables:

- Automated, multiple sample injections from a few microliters up to 1 ml of sample solution.
- Sample stored in microplates, thus allowing samples collected in microplates in Frac-950 to be used directly in A-905.

Autosampler A-905 also provide cooling of the sample solutions.

1.3.4 Valves

- One INV-907 valve can be connected as a flow direction valve to select flow direction through the column.
- One PV-908 valve can be connected as a sample selection valve.
 This is commonly used in combination with an optional sample pump.
- Two optional valves, freely selected from INV-907, a seven-port, three-way valve that can be used, for example, as a second injection valve, and IV/PV-908 valves, which are eight-port rotary valves with different pressure limits and internal flow path diameters.

1.3.5 On-line pH measurement

pH measurement is accomplished in ÄKTAexplorer by a pH flow cell mounted in the main system flow path after the UV and conductivity flow cells. The pH flow cell is fitted with a pH electrode for on-line measurement. The pH electrode can be replaced by a dummy electrode when pH measurement is not required.

1.3.6 Sample pump P-960

Sample pump P-960 is a single-channel laboratory pump which can be connected to accomplish automatic sample application when using the same sample several times. It can also be used to apply samples directly on the column or for filling sample loops (including Superloop).

Pump P-960 uses plungers and check valves for transporting the liquid.

Its flow rate is controlled and the pump pressure monitored from UNICORN.

The sample pump is commonly used in combination with a multiport motorized valve PV-908 to enable automatic sample application of several different samples.

1.3.7 Air sensors

The use and location of the air sensors is user-defined. For example:

- One air cell can be used to detect air when automatic sample application using P-960 is performed.
- One air cell can be used to detect air when applying large amounts of sample with the system pump.

There are four types of air cells available. They are designated Air-912, Air-912N, Air-925 and Air-925N and differ in inner diameter. This makes them suitable to use with different sizes of capillary.

Air-912 has a smaller inner diameter and is suitable to use in the P-960 sample flow path.

Air-925 has a larger inner diameter and is suitable to use in the inlet flow path to the system pump P-900.

Air-912N (standard in Explorer 100 Air) and Air-925N can be connected directly to P-960.

Up to four detection points can be used in the process flow path. The detectors are designated AS1, AS2, AS3, and ASP960 in the flow scheme.

1.3.8 Auxiliary equipment via Pump P-900 REMOTE connection The system pump P-901/903 is provided with a digital input/output connection comprising four output and four input channels. The channels are opto-isolated and accept standard TTL-level signals.

Examples of use include:

- External alarm input/output.
- Start/stop of external equipment.

1.3.9 AD-900

AD-900 is an A/D-converter for connecting an external instrument to the ÄKTAexplorer system.

The module has one high resolution analogue input for monitoring e.g. pressure, UV monitor signals or other signals available as voltage outputs.

1.3.10 Superloop

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

1.4 Safety

- The components are designed for indoor use only.
- Do not use in a dusty atmosphere or close to spraying water.
- Operate in accordance with local safety instructions.



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



WARNING! Ensure that the entire chromatographic system has been flushed thoroughly with distilled water before removing any capillaries or components.



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



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



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

CAUTION! Make sure ÄKTAexplorer is switched off before installing the optional components. The mains power to ÄKTAexplorer must be switched OFF before disconnecting or connecting the UniNet-1 and UniNet-2 cables.

2 Installation guidelines

2.1 General

To use optional equipment in a chromatographic run, suitable instructions is selected in the Method Wizard when creating a new method. The general procedures for creating and editing methods are described in the *UNICORN User Manuals*.

Note: The system configuration delay volume must be recalculated and reset when the standard configuration after the UV flow cell is changed. Details are given in section 15 Configuration dependent delay volume.

2.2 Unpacking

Unpack the optional components and check against the supplied packing list. Inspect the items for obvious damage that may have occurred during transportation.

CAUTION! Read the following information carefully, as well as all the additional instructions supplied with the components, to ensure that the ÄKTAexplorer optional equipment is installed correctly.

2.3 Pre-requisites

- UNICORN version 4.12 or higher must be installed in the computer connected to your AKTAexplorer system.
- ÄKTAexplorer must be installed and fully tested before the optional components are installed. See the *Installation Guide* for your ÄKTAexplorer chromatography system.



WARNING! Ensure that the entire system has been flushed thoroughly with distilled water before removing any capillaries or components.

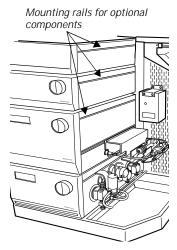
CAUTION! Make sure ÄKTAexplorer is switched off before installing the optional components. The mains power to ÄKTAexplorer must be switched OFF before disconnecting or connecting the UniNet-1 and UniNet-2 cables.

2.4 Principle installation of components

Some optional components can be mounted in the mounting rails on the side of the modules. Both sides of the modules are provided with rails for quick mounting of components.

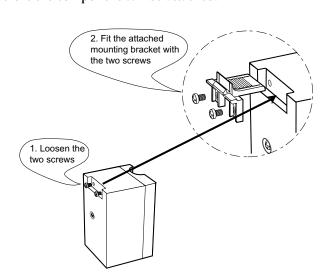
When selecting mounting location for the optional equipment it is important to choose a location which minimize the length of the capillaries used to connect the optional components to the rest of the system.

Many components that are attached to the mounting rails uses a snap-in bracket. The bracket is supplied separately with the component and needs to be fitted as shown below before the component can be attached.



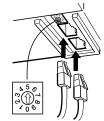






Capillaries are connected using unions as specified in chapter 17 Accessories and consumables.

All valves have a unique ID code which identifies them to the UNICORN control system at system start-up. This ID code should be checked before installation.



The ID codes for the different function valves and the corresponding instructions used in UNICORN to control them are as follows:

Valve function	ID code	Valve type	Instruction in UNICORN
Sample injection	1	INV-907	InjectionValve
Column selection	2	PV-908	ColumnPosition
Column selection	3	PV-908	(combined instruction controlling both valves)
Outlet fractionation	4	PV-908	OutletValve, OutletFractions (two instructions used for controlling different type of fractionation functions)
Sample selection	5	PV-908	SampleValve
Buffer selection	6	IV-908	BufferValveA1
Flow direction valve	7	INV-907	FlowDirectionValve
Optional function	8	INV-907, IV/PV-908	Valve8
Optional function	9	INV-907, IV/PV-908	Valve9

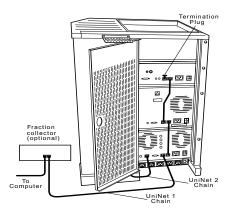
Components are connected to the UNICORN control system using either UniNet-1 or UniNet-2 cables*. Both the UniNet-1 and the UniNet-2 data communication chain in standard configuration is routed from the rear of P-900 via their respective components to the last component in the chain where it is terminated with a plug.

Note: When Pump P-960 is used, it is always installed as the last component in the UniNet-2 chain. Since the pump has an internal termination, no termination plug is needed.

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Frac-901, Frac-950, A-900 and A-905 are connected via the UniNet-1 chain. All other optional components are connected via the UniNet-2 chain.

Both the UniNet-1 and the UniNet-2 chain can be interrupted anywhere between P-900 and the termination plug to interconnect the optional components in the chain. The termination plug can be moved to the last component (furthest away from P-900), if motivated by cable routing considerations.



2.5 Components instruction overview

UNICORN must be informed about the insertion of an optional component. This is known as *selecting* the component, which makes its corresponding instructions available.

To print out a list of the general instructions, and the instructions corresponding to the selected components:

- 1 In the Method Editor, select File:Print.
- 2 Check the Instruction set box and clear the other boxes.
- 3 Click OK to print the instructions.

2.6 Preparation for use

To start your ÄKTAexplorer optional configuration system:

- 1 Switch on ÄKTAexplorer with the mains switch located at the front left on the system base.
- 2 Check that the computer and printer are switched on.
- 3 Log in (see chapter 2 in Making your first runs).
- 4 Make sure you have the correct strategy installed in your system, see 16 Strategies and Method Wizard. A new strategy is installed according to the software installation instructions given in the UNICORN User Manuals.
- 5 In UNICORN Main Menu, select Administration: System Setup.
- 6 Select System and then Edit. Click Component...



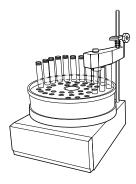
- 7 From the Component list, select the optional component(s) you have installed by checking the box(es).
- 8 Click OK twice and then Close.

The instructions associated to the new component appears when creating a new method or editing an existing method.

9 The correct tubing dimension for the column you intend to use must be installed. For most columns the standard tubing can be used. In complex configurations, using high flow rates, it may be necessary to replace the standard tubing with a larger i.d. PEEK tubing.

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

3 Fraction collector Frac-901



3.1 General

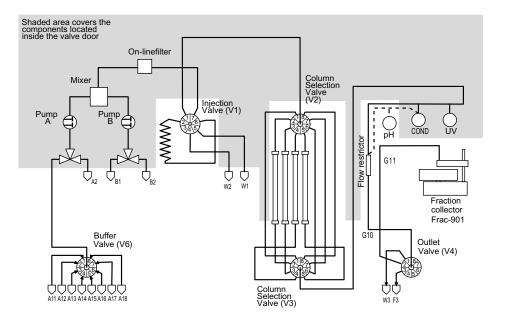
Frac-901 can be used for both small scale and preparative scale purifications with ÄKTAexplorer. It collects up to 175 fractions in 12 mm diameter tubes, up to 95 fractions in 10–18 mm diameter tubes, and up to 40 fractions in 30 mm diameter tubes.

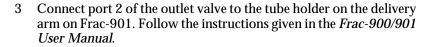
Frac-901 allows fixed volume fractionation and/or automatic peak fractionation. The latter function is based on peak detection using slope or level sensing. Fraction marks and fraction numbers make it easy to identify fractions and peaks. Fast tube change minimises spills between tubes, eliminating it entirely below flow rates of 5 ml/min.

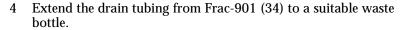
Drop synchronisation eliminates sample loss during tube change.

3.2 Installation

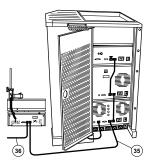
- 1 Unpack and assemble Frac-901 according to the instructions given in the *Fraction Collector Frac-900/901 User Manual*.
- 2 Place Frac-901 to the right of the system.



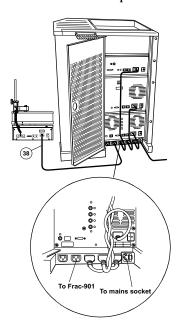


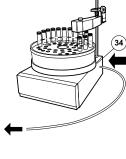






- Connecting a UniNet-1 cable (35) between Pump P-900 and Frac-901.
- Connecting a UniNet-1 cable (36) between Frac-901 and the computer.
- 6 Connect a mains cable supplied (38) between Frac-901 and a mains socket at the rear of ÄKTAexplorer.





3.3 Operation

3.3.1 Preparing for operation

- 1 Start your ÄKTAexplorer optional configuration system as described in 2.6 Preparation for use.
- 2 In Method Editor, go to the Instruction field and select Frac.
- 3 Check out the instructions available for Frac-901 to familiarize yourself with the new functions available. Refer also to the *Fraction collector Frac-900/901 User Manual* to learn more about using Frac-901.

3.3.2 Fractionation principles and definitions

Fraction collector Frac-901 is used for flowthrough fractionation before the elution, and for collecting fixed volumes and/or peak fractions during elution.

Fixed volume fractionation and peak fractionation can have different fractionation sizes. If both are activated simultaneously, the size set for peak fractionation is valid for collecting fractions during peaks and the size set for fixed volume fractionation is valid for collecting fractions during elution between peaks.

The instruction Fractionation_900 starts fixed volume fractionation. The instruction Peak_Fractionation_900 starts peak fractionation.

Fixed volume fractionation is terminated by issuing the command Fractionation_Stop_900 or by setting the fraction size to zero using the parameter FracSize. This will not terminate peak fractionation if the two are used simultaneously.

Peak fractionation is terminated by issuing the command Peak_FracStop_900 or by setting the peak fraction size to zero using the parameter PeakSize. This will not terminate fixed volume fractionation if the two are used simultaneously, even if the command is issued during a peak.

Tube change should occur (after the set delay volume) if a new fractionation command is issued during fixed volume or peak fractionation. The new fixed volume or peak fraction size is used instantly.

If a new peak fractionation command is issued during fixed volume fractionation, peak fractionation is valid during peaks and fixed volume fractionation before, between and after peaks.

If a new fixed volume fractionation command is issued during peak fractionation, peak fractionation is valid during peaks and fixed volume fractionation before, between and after peaks (this means that the new command becomes effective when the peak is ended).

3.3.3 Using Frac-901 in a method

Set the fraction collector parameters in the Method Wizard when creating the new method.

- Flowthrough fractionation and the fractionation volume is selected in the Wash Out Unbound Sample dialog. During the washout before elution fractionation, a specified volume is collected.
- Elution fractionation (Fixed Volume Fractionation and/or Peak Fractionation) is selected in the Elution Fractionation dialog.
 If fixed volume fractionation is selected, a fixed volume size set in this dialog is collected during the complete elution. If peak fractionation is selected, only the peaks will be collected. If fixed volume fractionation and peak fractionation is selected, the fixed volumes will be collected before, between and after the detected peaks.
- The peak fractionation parameters are specified in the Peak
 Fractionation dialog. Either the level or the slope of the UV curve,
 or the signal level from AD-900 (if used) can be used for detecting
 a peak.

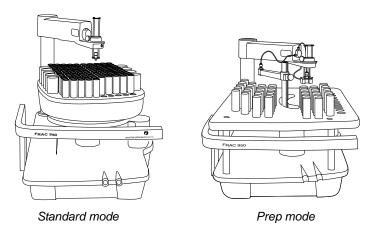
When using the UV curve level or the AD-900 signal level, the level for starting and finishing the fractionation, fraction size and minimum peak width (if not specified a column) should be specified.

When using the UV curve slope, the slope value for starting and finishing the fractionation, fraction size and minimum peak width (if not specified a column) should be specified.

Note: When using Frac-901 in ÄKTAexplorer 10 systems, we recommend a maximum flow rate of 3.5 ml/min with the i.d. 0.25 mm tubing kit. At higher flow rates, the drops will turn to a continuous liquid stream.

4 Fraction collector Frac-950

4.1 Generals



Frac-950 can be used for both small scale (standard mode) and preparative scale (prep mode) purifications with $\ddot{A}KTA$ explorer. Up to 392 fractions can be collected in time or volume base, as fixed volume and/or peak fractionations.

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

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

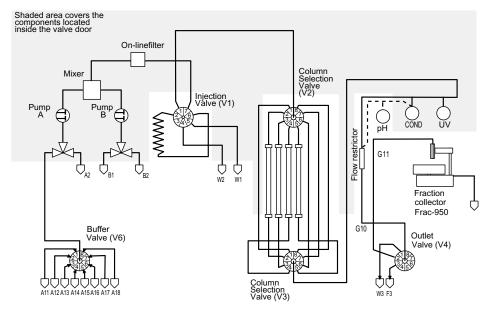
For **prep mode**, two sizes of racks for tube diameter of 30 mm tubes, and 250 ml bottles are available. A Funnel-to-flask kit for transporting the fractions to other vessels is also available. Using Frac-950 in prep mode requires a dispenser arm specially made for the prep mode racks.

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

4.2 Installation

CAUTION! Make sure ÄKTAexplorer is switched off before installing the optional components. The mains power to ÄKTAexplorer must be switched OFF before disconnecting or connecting the UniNet-1 and UniNet-2 cables.

- 1 Unpack and assemble Frac-950 according to the instructions given in the *Fraction Collector Frac-950 User Manual*.
- 2 Put Frac-950 on the workbench to the right of ÄKTAexplorer.



- 3 Locate the capillary kit included with Frac-950 and select if you want to include the accumulator in Frac-950 or not. Connect the outlet valve (port 2 is default in the Method Wizard) in ÄKTAexplorer to Frac-950 as described in the *Frac-950 User Manual*.
- 4 Connect the included waste tubing to the waste outlet on Frac-950 and route it to a suitable waste container.
- 5 Connect the UniNet-1 cables to Frac-950.
- 6 Connect the mains cable to Frac-950.
- 7 Check that all capillary and electrical connections are carried out correctly.

4.3 Operation

4.3.1 Preparing for operation

- 1 Start your ÄKTAexplorer optional configuration system as described in 2.6 Preparation for use.
- 2 In System Control, select Manual:Frac.
- 3 Check out the instructions available for Frac-950 to familiarize yourself with the new functions available. Refer also to the *Fraction collector Frac-950 User Manual* to learn more about using Frac-950.

4.3.2 Fractionation principles and definitions

Fraction collector Frac-950 is used for flowthrough fractionation before the elution, and for collecting fixed volumes and/or peak fractions during elution.

Fixed volume fractionation and peak fractionation can have different fractionation sizes. If both are activated simultaneuously, the size set for peak fractionation is valid for collecting fractions during peaks and the size set for fixed volume fractionation is valid for collecting fractions during elution before, between and after peaks.

The instruction Fractionation starts fixed volume fractionation. The instruction PeakFractionation starts peak fractionation.

Fixed volume fractionation is terminated by issuing the command FractionationStop or by setting the fraction size to zero using the parameter FracSize. This will not terminate peak fractionation if the two are used simultaneously.

Peak fractionation is terminated by issuing the command Peak_FracStop or by setting the peak fraction size to zero using the parameter PeakSize. This will not terminate fixed volume fractionation if the two are used simultaneously, even if the command is issued during a peak.

Tube change should occur (after the set delay volume) if a new fractionation command is issued during fixed volume or peak fractionation. The new fixed volume or peak fraction size is used instantly.

If a new peak fractionation command is issued during fixed volume fractionation, peak fractionation is valid during peaks and fixed volume fractionation before, between and after peaks.

If a new fixed volume fractionation command is issued during peak fractionation, peak fractionation is valid during peaks and fixed volume fractionation before, between and after peaks (this means that the new command becomes effective when the peak is ended).

If the parameter Start_at is set to Next line or Next group for peak fractionation and fixed volume fractionation, the fractionation during the next elution will start at the next line or group.

If the parameter Start_at is set to Next tube, the fractionation starts in the tube position after the previous fractionation. This is also valid when starting a new method, i.e. the last fractionation position in the previous run is stored and used in the next run, even if using another method. However, if the instruction Reset_position is ordered, or if the parameter Start_at is set to First tube, the fractionation will take place in the first position. The position is reset when changing racks as well.

If Last Tube is defined and the fraction collector run out of tubes, an alarm is generated and the fraction collector goes to waste. If:

- Continue is ordered, the fraction collector remains in the waste position.
- Ignore last tube and Continue are ordered, the fraction collector continues in next tube.
- a new fractionation command and Continue is ordered, the fraction collector continues with Start at tube number.

Note: Before executing a fractionation instruction, the outlet valve must manually be set to the position where the fraction collector is installed.

4.3.3 Using Frac-950 in a method

Set the fraction collector parameters in the Method Wizard when creating the new method.

- Flowthrough fractionation and the fractionation volume are selected in the Frac-950 Settings / Flowthrough Fractionation dialog. During the wash-out before elution fractionation, a specified volume is collected.
- Elution fractionation (Fixed Volume Fractionation and/or Peak Fractionation) is selected in the Elution Fractionation dialog. If fixed volume fractionation is selected, a fixed volume size set in this dialog is collected during the complete elution. If peak fractionation is selected, only the peaks will be collected. If fixed volume fractionation and peak fractionation is selected, the fixed volumes will be collected before, between and after the detected peaks.
- The peak fractionation parameters are specified in the Peak Fractionation dialog. Either the level or the slope of the UV curve, or the signal level from AD-900 (if used) can be used for detecting a peak.

When using the UV curve level or the AD-900 signal level, the level for starting and finishing the fractionation, fraction size and minimum peak width (if not specified a column) should be specified.

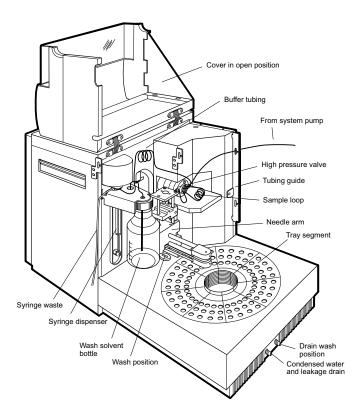
When using the UV curve slope, the slope value for starting and finishing the fractionation, fraction size and minimum peak width (if not specified a column) should be specified.

5 Autosampler A-900

5.1 General

Autosampler A-900 is intended for automated multiple sample injections.

A sequence of valve switching and syringe dispenser controlled sample withdrawal enables the A-900 to inject sample volumes in a range from a few microliters to 1 ml in a simple and reproducible manner.



Three different injection methods can be selected:

Flushed loop

The sample loop is completely (quantitatively) filled with sample resulting in extremely good reproducibility (better than 0.3%).

Partial loopfill

The sample loop is partially filled with sample giving low sample loss and allowing programmable injection volumes.

• µl pick-up

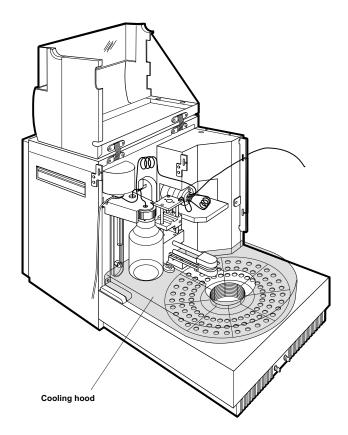
After aspiration from the vial, the sample volume is transported into the loop with transport liquid (mobile phase) from another vial. This eliminates sample loss.

The A-900 uses a syringe to aspirate the sample from a vial into the sample loop. To prevent contamination of the syringe, the A-900 is equipped with a buffer tubing between the syringe and the injection valve.

An integrated washing mechanism removes the sample from the buffer tubing, and sample needle, and also rinses both components.

5.2 Autosampler A-900 with Cooling

Autosampler A-900 with Cooling is purchased separately. It provides internal cooling of the sample vials. This is done by a Peltier element and a fan inside the autosampler, and a plastic hood covering the vials.

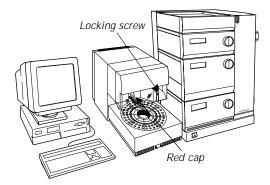


5.3 Installation

5.3.1 Preparations

A–900 needs approximately 28 cm of bench space and a mains connection of 220-240 V or 100-120 V \sim , 50/60 Hz, preferably taken from the ÄKTAexplorer base platform.

The recommended position is to the left of the separation equipment of ÄKTAexplorer, with approximately 3 cm spacing.



A-900 has two transportation safety devices which must be removed:

- Remove the locking screw holding the front cover. It is located at the right side of the front cover. Save the locking screw.
- A red plastic cap is attached over the needle washing mechanism below the front cover. Remove the red plastic cap and save it.

See also the installation instructions given in the *Autosampler A-900 User Manual, Chapter 2.*



5.3.2 Electrical connections

WARNING! A-900 must be connected to a grounded mains socket. It is recommended to use the mains socket in the system.

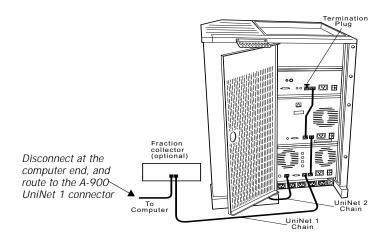


WARNING! For continued protection against risk of fire, replace only with fuses of specified type and rating. See Technical specifications in the A-900 User Manual for fuse data.

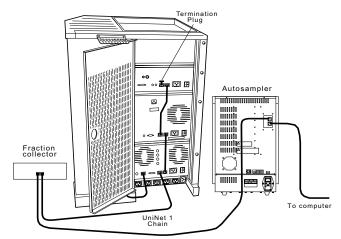
CAUTION! The mains power to ÄKTAexplorer must be switched OFF before disconnecting or connecting the UniNet-1 cables.

- 1 Make sure that the ÄKTAexplorer chromatography system is switched off.
- 2 Disconnect the UniNet-1 cable running between P-900 and the computer, in the computer end.

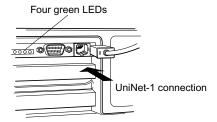
Note: If fraction collector Frac-901 or Frac-950 is used, disconnect the UniNet-1 cable running between Frac-901/ Frac-950 and the computer.



3 Connect this UniNet-1 cable to any of the two UniNet-1 connectors in A-900.



4 Connect a new UniNet-1 cable (supplied with the A-900) from the other UniNet-1 connector on the A-900 to the computer. The UniNet-1 connection to the computer must be made to the board with four green LEDs.



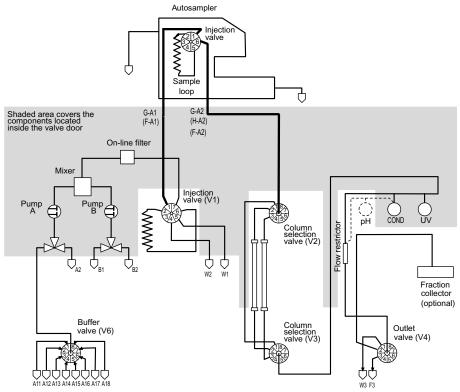
- 5 Check that the voltage setting of the A-900 matches the local mains supply voltage and main fuses. If the indicated voltage is not correct, select the proper voltage by removing, inverting, and then re-inserting the voltage selector cartridge. Check that the correct fuses are installed. If not, replace them with fuses as stated below:
 - For 110-120 VAC, use two 5 AT-fuses (slow).
 - For 220-240 VAC, use two 2.5 AT-fuses (slow).

CAUTION! DO NOT switch on any of the equipment yet!

When the voltage selection and fuses are correct for the local mains power source, plug in the supplied power cord in a free outlet in the base platform of the ÄKTAexplorer separation unit.

5.3.3 Capillary connections

The capillaries to be connected between A-900 and the ÄKTAexplorer separation unit are included in ÄKTA XT Tubing kit, no. 18-1122-92. The flow diagram below shows the location of A-900 in the ÄKTAexplorer flow path.



- 1 Remove the capillary marked G5, connecting the injection valve V1 port 1 with the column selection valve V2 center port.
- 2 Select the capillaries matching the tubing kit to be used in ÄKTAexplorer after the column. Use the capillaries marked G-A1 and G-A2 with the i.d. 0.25 mm tubing kit, G-A1 and H-A2 with the i.d. 0.50 mm tubing kit, and F-A1 and F-A2 with the i.d. 0.75 and 1.0 mm tubing kits.

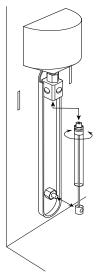
Note: See ÄKTAexplorer System Manual for general recommendations on selecting column and tubing kit.

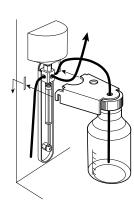
- 3 Both capillaries are routed in the space between the UV monitor UV-900 and the pH/conductivity monitor pH/C-900. The capillaries are supported in the mounting bracket of buffer valve V6 and are thread through the holes in the valve door below the valves they connect to. Start threading the capillaries from the valves, through the holes in the valve door, and further via the support and the space between the monitors to A-900.
- 4 Connect the capillary marked G-A1/F-A1 (l = 1.3 m) from the tubing kit between the injection valve V1 port 1 and the A-900 injection valve port 1 (marked "pump"). The end that is pre-bent is connected to the ÄKTAexplorer injection valve V1.
- 5 Connect the capillary marked G-A2/H-A2/F-A2 (l = 1.15 m) from the tubing kit between the A-900 injection valve port 6 (marked "column"), and the column selection valve V2 center port.
- 6 Check additional internal and waste tubing connections on A-900 according to the A-900 User Manual instructions.

5.4 General preparation

- 1 Start your ÄKTAexplorer optional configuration system as described in 2.6 Preparation for use. Make sure that AutoSampler A-900 is selected in the Component list.
- 2 Press the System Control button, and select System:Settings:Specials.
- 3 Check that the default sample loop volume (100 μ l), syringe volume (250 μ l) and needle height (2 mm) are selected.
- 4 Manually, run the SyringeEnd instruction under System Control:Manual:Flowpath:AutosamplerControl.
- 5 Remove the syringe from the syringe valve. Make sure that the Luer connector in the valve remains in place.
- 6 Fill the syringe with 20% ethanol in water from a vessel.
- 7 Replace the filled syringe. Fully tighten the syringe so that a gap between the plunger tip and the syringe end is obtained after having executed the SyringeHome instruction under System Control:Manual:Flowpath:AutosamplerControl.

Note: If the syringe holder is not fully tightened, i.e. no gap is obtained, the plunger tip moves forcefully into the syringe end which can damage the syringe.





- 8 Fill the wash solvent bottle with 20% ethanol in water. If the sample contains large amounts of salt, or if the sample is very sensitive to ethanol, use water as wash solvent instead (degas and change the water every day!).
- 9 Screw the bottle into the wash solvent holder and place the holder on the A-900.
- 10 Put the wash solvent tubing in the wash solvent bottle.
- 11 Fill the tubing using the SyringeEnd and SyringeHome instructions.

SyringeEnd draws a syringe volume of wash solvent from the wash solvent bottle and fills the wash solvent tubing.

SyringeHome dispenses the syringe contents to the syringe waste.

Repeat this action a number of times until the wash solvent tubing and the syringe are completely filled.

12 After the wash solvent tubing and the syringe are filled, use the InitialWash instruction to perform a standard wash. All tubing connected to the syringe valve will be filled and flushed with wash solvent.

5.4.1 Purging the syringe in A-900

- 1 Manually, run the SyringeEnd and SyringeHome instructions alternately.
- 2 Repeat until no more air is left in the syringe (more than 5 repeats can be necessary).
- 3 Finish by performing the InitialWash instruction.

- 5.4.2 Checking the needle height in A-900
- 1 Remove the tray segment next to (on the left side) the one directly below the needle arm.
- 2 Put a vial with a flat bottom, filled with e.g. water, in the tray segment directly below the needle arm. The bottom of the vial must be clearly visible.
- 3 Perform a manual injection by issuing the Manual:Flowpath:InjectionFlushed instruction in System Control.
- 4 Check visually that the needle stops approximately 2 mm above the vial bottom.
- 5 If not, go to System: Settings: Specials to adjust the needle height. The default setting is 2 mm.

Note: Check the needle height with 2 mm as default setting whenever the needle is replaced. The 2 mm setting is only a relative measure to ensure that the needle stops above the vial bottom regardless of which type of vial used. Adjust the final setting in small steps until a needle height suitable for the actual use is obtained (type of vial, sample volume available, etc.).

Installation test 5.5

The installation test performs a total check of the liquid processing parts of the ÄKTAexplorer separation unit. This is to establish that the integration of the optional components in ÄKTAexplorer has not disturbed the total system function.

Note: The installation test that includes the autosampler only applies to ÄKTAexplorer 10 systems. In ÄKTAexplorer 100 systems, the regular installation test should be used, which does not include the autosampler.

5.5.1 **Preparation**

- Verify that the Pump P-900 is primed and purged according to the instructions in the AKTAexplorer Installation Guide.
- 2 Prepare a flask containing 500 ml of 0.4% acetone in water solution.
- Fill two standard vials with this solution and seal them properly with the correct type of cap or septum.
- Put one vial in position 1, and the other vial in position 2 in a standard tray.
- Immerse the inlet tubing B1 into the rest of the 0.4% acetone solution.
- 6 Immerse the inlet tubing A11 into a flask containing distilled water.

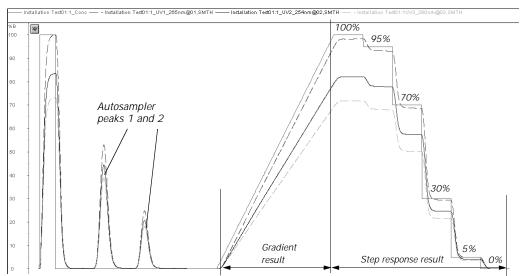
5.5.2 Starting the test

- In UNICORN Main menu select File:Printer setup... Select the appropriate printer from the list and select Landscape. Click OK to acknowledge the printer chosen.
- Click the Instant Run button 2



- In the InstantRun window, select system and click Run.
- In the Method Wizard, select Installation_Test in the Main Selection menu. Click Run.
- 5 In Evaluation Procedures, InstTest_Expl10_A90X. Click Next twice.
- Click START to begin the installation test.

7 The curves can now be monitored on the screen as the test progresses. The Installation test method run time is approximately 30 minutes.



8 When the test run is finished, the printer automatically prints out the chromatogram and the test result.

5.6 Evaluating the installation test result

5.6.1 Automatic evaluation

If your chromatography system includes no other optional components than the autosampler, the automatic evaluation will give a reliable result. When the test is finished, the result is printed automatically. It consists of a chromatogram and an evaluation of the test result.

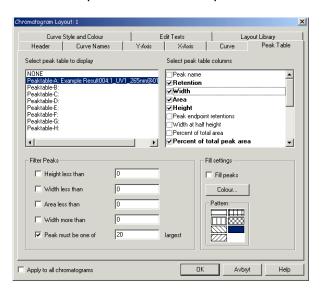
- If the gradient test result is OK, the print-out says "Gradient linearity accepted".
- If the step response test result is OK, the print-out says "Step response accepted".
- If the UV response test result is OK, the print-out says "UV response accepted".
- If the Autosampler peaks test result is OK,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

If any of the evaluated values falls outside the specified range, refer to 5.6.3 Correcting faulty evaluation results.

5.6.2 Manual evaluation

If your chromatography system includes other optional components than the autosampler, the automatic evaluation will not give a reliable result. If so, a manual evaluation must be done.

- 1 Click on the Main menu button in the Taskbar.
- 2 Click on default in the results panel and then double-click on the Wizard Generated 001 icon to open the result file.
- 3 Click in the chromatogram window with the right mouse button, and select Properties.
- 4 Click on the Curves tab and select the following curves:
 - Wizard Generated001:1_UV1_265nm@01,SMTH
 - Wizard Generated001:1_UV2_254nm@02,SMTH
 - Wizard Generated001:1_UV3_280nm@03,SMTH
- 5 Click Peak table. Check that Peak Table A is selected and select Percent of total peak area in the Select peak table columns. Click OK.



- 6 Right-click in the chromatogram window, and select Marker.
- Move the vertical bar to the constant section of each plateau by dragging it. Read the absorbance (in mAU) for the steps corresponding to Installation Test01:1_UV1_265nm@01,SMTH.
- 8 Enter the absorbance values (in mAU) in column 2 in the Step response table of the Test record, leaving out the decimals.
- 9 Read the absorbance for the plateaus corresponding to 0% and 100% B for the curves:
 - Wizard GeneratedOO1:1_UV1_265nm@01,SMTH
 - Wizard Generated001:1_UV2_254nm@02,SMTH
 - Wizard Generated001:1_UV3_280nm@03,SMTH

and enter the values in column 2 in the UV response table of the Test record.

10 Click Print under File to print the chromatogram.

Evaluating the gradient

- 1 Place a ruler along the gradient part of curve Wizard Generated001:1_UV1_265nm@01,SMTH in the printed report.
- 2 The curve should be linear between 10% B and 90% B and void of discontinuities.
- 3 Fill in the Test record.

Evaluating the step response

1 Calculate the relative adsorption plateau heights for curve Wizard Generated001:1_UV1_265nm@01,SMTH in the following way:

Subtract the base line value (0% B) from each of the values in column 2 in the Step response table of the Test record. Enter the results in column 3.

Divide each value in column 3 by the base line corrected value corresponding to 100% B. Multiply by 100 and enter the results in column 4.

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

Evaluating the UV response

1 Calculate the UV response ratios in the following way:

Subtract the base line values (0% B) corresponding to each UV curve from the values in column 2 of the UV response table of the Test record. Enter the results in column 3.

Calculate the absorbance ratios 265 nm/254 nm and 265 nm/280 nm using the values from column 3 and enter the results in column 4.

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

Evaluating the autosampler peaks

- In the printed report, read the Area and the Area/Peak area % values for peaks No. 1 and 2.
- 2 Fill in the Test record.
- 3 Check that the values fall within the given intervals.

The installation procedure is completed when all results fall inside the limits specified in the Test record.

5.6.3 Correcting faulty evaluation results

Faulty gradient

- The gradient is linear but the interval is too small. The mixer chamber is too large, or the mixer is faulty.
- Disturbances. These may arise from air in the pump. Purge again and repeat the test run. If persistent, faulty check valves or bad piston sealings in the pump may be the cause. Refer to the Pump P-900 User Manual.

Faulty step response

- All values are faulty. This may arise from air in the pump or a faulty pump. Purge again and repeat the test run. If persistent, a faulty pump may be suspected. Refer to the Pump P-900 User Manual.
- 5% and 95% faulty. This may be due to bad sealings in the pump (5% faulty=pump module B, 95% faulty=pump module A). Refer to the Pump P-900 User Manual.

Faulty UV response

• 0%-value too far from 0 AU (±10 mAU). Auto zero not properly performed. Repeat the test and re-evaluate. Refer to the Pump P-900 User Manual.

Faulty Autosampler peak values

• If values are faulty, make sure that the A-900 is properly purged and that all tubing fittings are properly tightened.

5.6.4 Test record

Gradient test result

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

Step response test result Step response table:

1 Programmed Conc. %B	2 Value read (mAU)	3 Base line corrected value	4 Normalised value	5 Allowed interval
100				
95				94 - 96
70				69 - 71
30				29 - 31
5				4 - 6
0	•			

UV response test result UV response table:

1 Wavelength (nm)	Value 100	2 e read 9%B 6 B	3 Base line corrected value	4 Absorbance ratio	5 Allowed interval
254					
265/254					1.11 - 1.26
265					
265/280					1.26 - 1.53
280					

Autosampler peak test result Autosampler peak table:

1 Peak no.	2 Area (mAU x min)	3 Area/Peak area (%)	4 Allowed interval (%)
1			66.1 - 67.2
2			32.8 - 33.9

5.7 Operation

5.7.1 General

The parameters for controlling Autosampler A-900 are set in the Sample Injection dialog in the Method Wizard when creating the method.

The A-900 can be used for automatic sample injection from a few microlitres to one millilitre of sample solution. To achieve optimal performance, selection of sample loop volume, syringe size and speed, plus the washing method between injections must be considered.

The sample injection techniques are as follows:

InjectionFlushed

The sample loop is completely (quantitatively) filled with sample resulting in extremely good reproducibility.

InjectionPartial

The sample loop is partially filled with sample giving low sample loss and allowing programmable injection volumes.

InjectionPickup

After aspiration from the vial, the sample volume is transported into the loop with transport liquid (mobile phase) from another vial resulting in virtually no sample loss.

Manual functions used for setting up and maintaining the A-900 are found in **S**ystem Control:Manual:Flowpath:AutosamplerControl.

Some of the available commands are as follows:

- InitialWash
- RotateTray
- SyringeHome/End
- NeedleHome/Front

The parameters for controlling Autosampler A-900 with Cooling are set in System Control:System:Settings:Specials (desired temperature), and in System Control:System:Settings:Alarms (alarm and warning levels).

These functions are described in the following sections.

5.7.2 InjectionFlushed

The InjectionFlushed instruction selects the flushed loop injection mode for A-900.

The syringe dispenser aspirates a flush volume of sample into the sample line. The A-900 injection valve is turned to LOAD position, and the sample loop is filled with 1.5–3 times the sample loop volume (depending in the loop volume). The injection volume equals the loop volume. When the sample loop size is changed, the loop volume setting must be changed in System:Settings (100 µl is the default loop volume).

After each injection, the needle is rinsed:

- If WashExt=NO is selected in the method, the needle is only washed internally.
- If WashExt=YES is selected in the method, the needle is also washed on the outside.

In both cases, the buffer tubing is also washed.

An air segment (Air segment=Air) is recommended to reduce the amount of the flush volume. The air segment is not injected.

5.7.3 InjectionPartial

The InjectionPartial instruction selects the partial loopfill injection mode for A-900.

The syringe dispenser aspirates a flush volume of sample into the sample line. The A-900 injection valve is turned to LOAD position, and the sample loop is filled by transporting the selected injection volume into the sample loop, which is partially filled with sample.

After each injection, the needle is rinsed:

- If WashExt=NO is selected in the method, the needle is only washed internally.
- If WashExt=YES is selected in the method, the needle is also washed on the outside.

In both cases, the buffer tubing is also washed.

An air segment (Air segment=Air) is recommended to reduce the amount of the flush volume. The air segment is not injected.

5.7.4 InjectionPick-up

The InjectionPickup instruction selects the μl pick-up injection mode for A-900.

The syringe dispenser aspirates transport liquid from the transport vial into the sample line. The A-900 injection valve is turned to LOAD position. The needle moves from the transport vial to a sample vial and aspirates the selected injection volume. The needle moves back to the transport vial and the sample is quantitatively transported into the sample loop with transport liquid.

After each injection, the needle is rinsed:

- If WashExt=NO is selected in the method, the needle is only washed internally.
- If WashExt=YES is selected in the method, the needle is also washed on the outside.

In both cases, the buffering tubing is also washed.

An air segment is not recommended for this injection mode (Air segment=NoAir) because the air segment at the front of the sample plug is injected.

5.7.5 InitialWash

The InitialWash instruction performs a complete wash of the needle and the syringe.

The syringe is first washed using one syringe volume. Then the needle is moved to its home position and washed internally.

Finally, the needle moves to its front position and is washed both internally and externally.

5.7.6 SyringeHome/End

The SyringeHome/End instructions are used when the syringe is replaced. They are also used for purging the syringe, i.e. to remove air.

SyringeHome empties the syringe contents to the syringe waste container.

SyringeEnd aspirates wash solvent into the syringe from the wash bottle.

Note: When the performed action is completed, make sure the syringe is run to the Home position before leaving the manual function.

5.7.7 RotateTray

The RotateTray instruction is used to rotate the tray, e.g. when new vials are loaded into the tray segments.

5.7.8 NeedleHome/Front

The NeedleHome/Front instructions are used when the sample needle is replaced.

NeedleHome moves the needle to its normal position.

NeedleFront moves the needle closer to the front of A-900.

5.7.9 AutoSampler_Temperature

When using A-900 with cooling function, the AutoSampler_Temperature parameter is used to enable the cooling function and to set the desired temperature.

The cooling function is enabled by deselecting the OFF check box.

The temperature range is 4–40 °C.

The cooling starts immediately when clicking on OK.

Note: To use Autosampler A-900 with Cooling, both AutoSampler and AutoSamplerThermo should be selected in the Components list in System Setup.

5.7.10 Alarm_AutoSampler

The Alarm_AutoSampler parameter is used to set alarm temperatures when using the cooling function.

The upper alarm level is set by the parameter HighAlarm, and the lower alarm level by LowAlarm.

5.7.11 Warning_AutoSampler

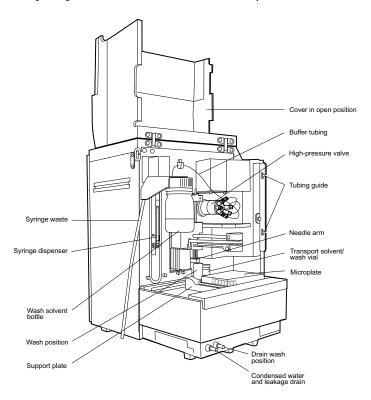
The Warning_AutoSampler parameter is used to set warning temperatures when using the cooling function.

The upper alarm level is set by the parameter HighWarn, and the lower alarm level by LowWarn.

6 Autosampler A-905 for ÄKTA

6.1 General

Autosampler A-905 for ÄKTA is intended for automated multiple sample injections of volumes down to 0.1 $\mu l.\,$

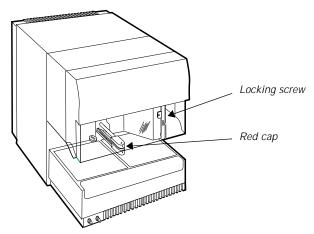


6.2 Installation

6.2.1 Preparations

A–905 needs approximately 28 cm of bench space and a mains connection of 220-240 V or 100-120 V~, 50/60 Hz, preferably taken from the ÄKTAexplorer base platform.

The recommended position is to the left of the separation equipment of ÄKTAexplorer, with approximately 3 cm spacing.



A-905 has two transportation safety devices which must be removed:

- Remove the locking screw holding the front cover. It is located at the right side of the front cover. Save the locking screw.
- A red plastic cap is attached over the needle washing mechanism below the front cover. Remove the red plastic cap and save it.

See also the installation instructions given in the *Autosampler A-905 User Manual, Chapter 2.*



6.2.2 Electrical connections

WARNING! A-905 must be connected to a grounded mains socket. It is recommended to use the mains socket in the system.

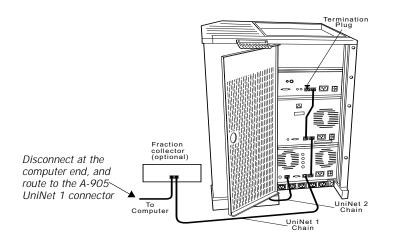


WARNING! For continued protection against risk of fire, replace only with fuses of specified type and rating. See Technical specifications in the A-905 User Manual for fuse data.

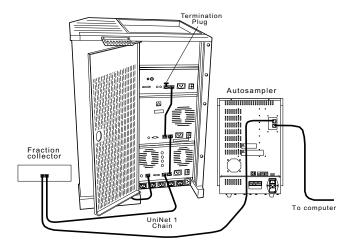
CAUTION! The mains power to ÄKTAexplorer must be switched OFF before disconnecting or connecting the UniNet-1 cables.

- 1 Make sure that the ÄKTAexplorer chromatography system is switched off.
- 2 Disconnect the UniNet-1 cable running between P-900 and the computer, in the computer end.

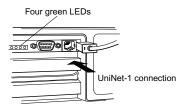
Note: If fraction collector Frac-901 or Frac-950 is used, disconnect the UniNet-1 cable running between Frac-901/ Frac-950 and the computer.



3 Connect this UniNet-1 cable to any of the two UniNet-1 connectors in A-905.



4 Connect a new UniNet-1 cable (supplied with the A-905) from the other UniNet-1 connector on the A-905 to the computer. The UniNet-1 connection to the computer must be made to the board with four green LEDs.



- 5 Check that the voltage setting of the A-905 matches the local mains supply voltage and main fuses. If the indicated voltage is not correct, select the proper voltage by removing, inverting, and then re-inserting the voltage selector cartridge. Check that the correct fuses are installed. If not, replace them with fuses as stated below:
 - For 110-120 V~, use two 5 AT-fuses (slow).
 - For 220–240 V~, use two 2.5 AT-fuses (slow).

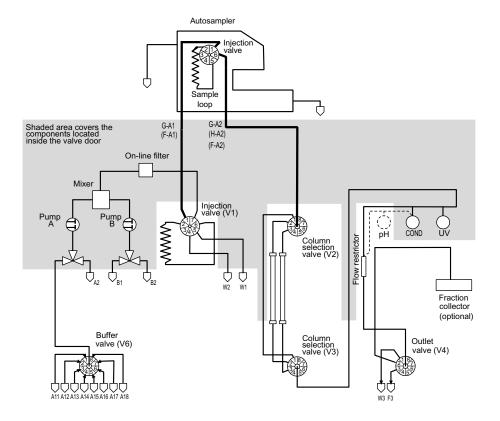
CAUTION! DO NOT switch on any of the equipment yet!

6 When the voltage selection and fuses are correct for the local mains power source, plug in the supplied power cord in a free outlet in the base platform of the ÄKTAexplorer separation unit.

6.2.3 Capillary connections

The capillaries to be connected between A-905 and the ÄKTAexplorer separation unit are included in ÄKTA XT Tubing kit, no. 18-1122-92.

The flow diagram below shows the location of A-905 in the ÄKTAexplorer flow path.



- 1 Remove the capillary marked G5, connecting the injection valve V1 port 1 with the column selection valve V2 center port.
- Select the capillaries matching the tubing kit to be used in ÄKTAexplorer after the column. Use the capillaries marked G-A1 and G-A2 with the i.d. 0.25 mm tubing kit, G-A1 and H-A2 with the i.d. 0.50 mm tubing kit, and F-A1 and F-A2 with the i.d. 0.75 and 1.0 mm tubing kits.

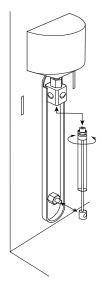
Note: See ÄKTAexplorer System Manual for general recommendations on selecting column and tubing kit.

- 3 Both capillaries are routed in the space between the UV monitor UV-900 and the pH/conductivity monitor pH/C-900. The capillaries are supported in the mounting bracket of buffer valve V6 and are thread through the holes in the valve door below the valves they connect to. Start threading the capillaries from the valves, through the holes in the valve door, and further via the support and the space between the monitors to A-905.
- 4 Connect the capillary marked G-A1/F-A1 (l = 1.3 m) from the tubing kit between the injection valve V1 port 1 and the A-905 injection valve port 1 (marked "pump"). The end that is pre-bent is connected to the ÄKTAexplorer injection valve V1.
- 5 Connect the capillary marked G-A2/H-A2/F-A2 (l = 1.15 m) from the tubing kit between the A-905 injection valve port 6 (marked "column"), and the column selection valve V2 center port.
- 6 Check additional internal and waste tubing connections on A-905 according to the A-905 User Manual instructions.

6.3 General preparation

- 1 Start your ÄKTAexplorer optional configuration system as described in 2.6 Preparation for use. Make sure that AutoSampler A-905 is selected in the Component list.
- 2 Click the System Control button, and select System: Settings: Specials.
- 3 Check that the default sample loop volume (1 ml), syringe volume (1 ml) and needle height (5 mm) are selected.
- 4 Run the AutosamplerControl_905:SyringeEnd_905 command in Manual:Flowpath.
- 5 Remove the syringe from the syringe valve. Make sure that the Luer connector in the valve remains in place.
- 6 Fill the syringe with 20% ethanol in water from a vessel.
- 7 Replace the filled syringe. Fully tighten the syringe so that a gap between the plunger tip and the syringe end is obtained after having executed the AutosamplerControl_905:SyringeHome_905 command in Manual:Flowpath.

Note: If the syringe holder is not fully tightened, i.e. no gap is obtained, the plunger tip moves forcefully into the syringe end which can damage the syringe.





- 8 Fill the wash solvent bottle with 20% ethanol in water. If the sample contains large amounts of salt, or if the sample is very sensitive to ethanol, use water as wash solvent instead (degas and change the water every day!).
- 9 Screw the bottle into the wash solvent holder and place the holder on the A-905.
- 10 Put the wash solvent tubing in the wash solvent bottle.
- 11 Fill the tubing using the SyringeEnd_905 and SyringeHome_905 instructions.

SyringeEnd_905 draws a syringe volume of wash solvent from the wash solvent bottle and fills the wash solvent tubing.

SyringeHome_905 dispenses the syringe contents to the syringe waste.

Repeat this action a number of times until the wash solvent tubing and the syringe are completely filled.

- 12 After the wash solvent tubing and the syringe are filled, use the InitialWash_905 instruction to perform a standard wash. All tubing connected to the syringe valve will be filled and flushed with wash solvent.
- 6.3.1 Purging the syringe in A-905
- 1 Manually, run the SyringeEnd_905 and SyringeHome_905 instructions alternately.
- 2 Repeat until no more air is left in the syringe (more than 5 repeats can be necessary).
- 3 Finish by performing the InitialWash_905 instruction.
- 6.3.2 Checking the needle height in A-905 Refer to the *Autosampler A-905 for ÄKTA User Manual*.

Installation test 6.4

The installation test performs a total check of the liquid processing parts of the ÄKTAexplorer separation unit. This is to establish that the integration of the optional components in ÄKTAexplorer has not disturbed the total system function.

Note: The installation test that includes the autosampler only applies to ÄKTAexplorer 10 systems. In ÄKTAexplorer 100 systems, the regular installation test should be used, which does not include the autosampler.

6.4.1 **Preparation**

- Verify that the Pump P-900 is primed and purged according to the instructions in the ÄKTAexplorer Installation Guide.
- 2 Prepare a flask containing 500 ml of 0.4% acetone in distilled water.
- 3 Prepare a flask containing 500 ml of distilled water.
- Fill A1 and A2 in a 96-well low microplate with the 0.4% acetone solution.
- Seal the microplate with a foil or a cap mat.
- Immerse the inlet tubing B1 into the rest of the 0.4% acetone solution.
- Immerse the inlet tubing A11 into the distilled water flask.

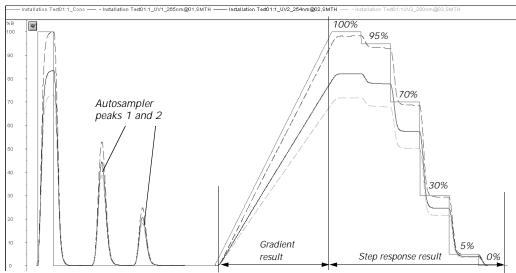
6.4.2 Starting the test

- In UNICORN Main menu select File:Printer setup... Select the appropriate printer from the list and select Landscape. Click OK to acknowledge the printer chosen.
- Click the Instant Run button



- In the InstantRun window, select system and click Run.
- In the Method Wizard, select Installation_Test in the Main Selection menu. Click Run.
- In Evaluation Procedures, select InstTest_Expl10_A90X. Click Next twice.
- 6 Click START to begin the installation test.

The curves can now be monitored on the screen as the test progresses. The Installation test method run time is approximately 30 minutes.



8 When the test run is finished, the chromatogram and the test result is printed automatically.

6.5 Evaluating the installation test result

6.5.1 Automatic evaluation

If your chromatography system includes no other optional components than the autosampler, the automatic evaluation will give a reliable result. When the test is finished, the result is printed automatically. It consists of a chromatogram and an evaluation of the test result.

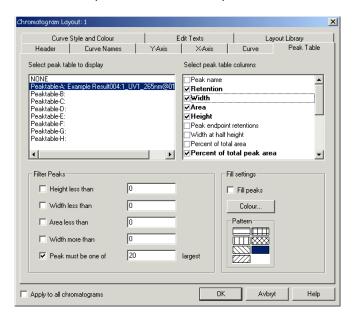
- If the gradient test result is OK, the print-out says "Gradient linearity accepted".
- If the step response test result is OK, the print-out says "Step response accepted".
- If the UV response test result is OK, the print-out says "UV response accepted".
- If the Autosampler peaks test result is OK,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

If any of the evaluated values falls outside the specified range, refer to 6.6 Correcting faulty evaluation results.

6.5.2 Manual evaluation

If your chromatography system includes other optional components than the autosampler, the automatic evaluation will not give a reliable result. If so, a manual evaluation must be done.

- 1 Click the UNICORN Main menu button in the Taskbar.
- 2 Click on default in the results panel and then double-click on the Wizard Generated 001 icon to open the result file.
- 3 Right-click in the chromatogram window and select Properties.
- 4 Click the Curves tab and select the following curves:
 - Wizard GeneratedO01:1_UV1_265nm@01,SMTH
 - Wizard Generated001:1_UV2_254nm@02,SMTH
 - Wizard Generated001:1_UV3_280nm@03,SMTH
- 5 Click Peak table. Check that Peak Table A is selected and select Percent of total peak area in the Select peak table columns. Click OK.



- 6 Right-click in the chromatogram window, and select Marker.
- Move the vertical bar to the constant section of each plateau by dragging it. Read the absorbance (in mAU) for the steps corresponding to Wizard Generated001:1_UV1_265nm@01,SMTH.
- 8 Enter the absorbance values (in mAU) in column 2 in the Step response table of the Test record, leaving out the decimals.
- 9 Read the absorbance for the plateaus corresponding to 0% and 100% B for the curves:
 - Wizard GeneratedO01:1_UV1_265nm@01,SMTH
 - Wizard GeneratedO01:1_UV2_254nm@02,SMTH
 - Wizard Generated001:1_UV3_280nm@03,SMTH

and enter the values in column 2 in the UV response table of the Test record.

10 Click Print under File to print the chromatogram.

Evaluating the gradient

- 1 Place a ruler along the gradient part of curve Wizard Generated001:1_UV1_265nm@01,SMTH in the printed report.
- 2 The curve should be linear between 10% B and 90% B and void of discontinuities.
- 3 Fill in the Test record.

Evaluating the step response

1 Calculate the relative adsorption plateau heights for curve Wizard Generated001:1_UV1_265nm@01,SMTH in the following way:

Subtract the base line value (0% B) from each of the values in column 2 in the Step response table of the Test record. Enter the results in column 3.

Divide each value in column 3 by the base line corrected value corresponding to 100% B. Multiply by 100 and enter the results in column 4.

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

Evaluating the UV response

1 Calculate the UV response ratios in the following way:

Subtract the base line values (0% B) corresponding to each UV curve from the values in column 2 of the UV response table of the Test record. Enter the results in column 3.

Calculate the absorbance ratios 265 nm/254 nm and 265 nm/280 nm using the values from column 3 and enter the results in column 4.

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

Evaluating the autosampler peaks

- In the printed report, read the Area and the Area/Peak area % values for peaks No. 1 and 2.
- 2 Fill in the Test record.
- 3 Check that the values fall within the given intervals.

The installation procedure is completed when all results fall inside the limits specified in the Test record.

6.6 Correcting faulty evaluation results

6.6.1 Faulty gradient

- The gradient is linear but the interval is too small. The mixer chamber is too large, or the mixer is faulty.
- Disturbances. These may arise from air in the pump. Purge again and repeat the test run. If persistent, faulty check valves or bad piston sealings in the pump may be the cause. Refer to the *Pump P-900 User Manual*.

6.6.2 Faulty step response

- All values are faulty. This may arise from air in the pump or a
 faulty pump. Purge again and repeat the test run. If persistent, a
 faulty pump may be suspected. Refer to the *Pump P-900 User Manual*.
- 5% and 95% faulty. This may be due to bad sealings in the pump (5% faulty=pump module B, 95% faulty=pump module A). Refer to the *Pump P-900 User Manual*.

6.6.3 Faulty UV response

• 0%-value too far from 0 AU (± 10 mAU). Auto zero not properly performed. Repeat the test and re-evaluate. Refer to the *Pump P-900 User Manual*.

6.6.4 Faulty Autosampler peak values

• If values are faulty, make sure that the A-905 is properly purged and that all tubing fittings are properly tightened.

6.6.5 Test record

Gradient test result

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

Step response test result Step response table:

1 Programmed Conc. %B	2 Value read (mAU)	3 Base line corrected value	4 Normalised value	5 Allowed interval
100				
95				94 - 96
70				69 - 71
30				29 - 31
5				4 - 6
0	_			

UV response test result UV response table:

1 Wavelength (nm)	Value 100	2 e read 9%B 6 B	3 Base line corrected value	4 Absorbance ratio	5 Allowed interval
254					
265/254					1.11 - 1.26
265					
265/280					1.26 - 1.53
280					

Autosampler peak test result Autosampler peak table:

1 Peak no.	2 Area (mAU x min)	3 Area/Peak area (%)	4 Allowed interval (%)
1			66.1 - 67.2
2			32.8 - 33.9

6.7 Operation

The parameters for controlling Autosampler A-905 are set in the Sample Injection dialog in the Method Wizard when creating the method.

The A-905 can be used for automatic sample injection from 0.1 μl to 1 ml. To achieve optimal performance, selection of sample loop volume, syringe size and speed, plus the washing method between injections must be considered.

Three different injection methods can be selected:

Flushed loop

The sample loop is completely (quantitatively) filled with sample resulting in extremely good reproducibility (better than 0.3%).

Partial loopfill

The sample loop is partially filled with sample giving low sample loss and allowing programmable injection volumes.

• µl pick-up

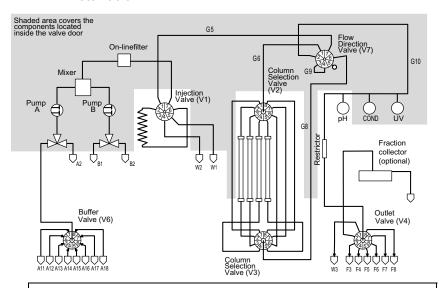
After aspiration from the vial, the sample volume is transported into the loop with transport liquid (mobile phase) from another vial. This eliminates sample loss.

The A-905 uses a syringe to aspirate the sample from a microplate into the sample loop. To prevent contamination of the syringe, the A-905 is equipped with a buffer tubing between the syringe and the injection valve.

An integrated washing mechanism removes the sample from the buffer tubing, and the sample needle, and also rinses both components.

7 Flow direction valve

7.1 Installation



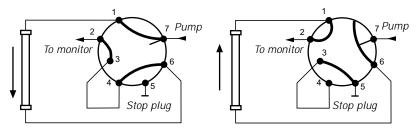
CAUTION! Make sure ÄKTAexplorer is switched off before installing the optional components. The mains power to ÄKTAexplorer must be switched OFF before disconnecting or connecting the UniNet-2 cables.

- 1 Mark the INV-907 valve with V7. Check that the ID code switch is set to 7.
- 2 Attach the valve to the valve door in the recess next to column valve V2. Use the mounting bracket and screws supplied with the valve.
- 3 Cut and mount the required PEEK capillaries. Use a capillary with the correct inner diameter as required by your system. The principle flow path through the valve is shown below in the following figure.

Reversed flow configuration

DOWNWARD FLOW, position 1

UPWARD FLOW, position 2



- 4 Use the attached UniNet-2 cables to connect the valve to the UniNet-2 communication link. The valve can be connected anywhere between the system pump P-900 and the termination plug.
- 5 Check that all capillary and electrical connections are carried out correctly.

7.2 Operation

7.2.1 Preparing for operation

- 1 Start your ÄKTAexplorer optional configuration system as described in 2.6 Preparation for use. Make sure that FlowDirectionValve V7 is selected in the Component list.
- 2 The flow direction valve is automatically recognised by UNICORN at system start-up. Check that the valve added is indicated in the Flow scheme in UNICORN.
- 3 Verify that flow direction valve V7 is functioning properly by issuing manual commands from UNICORN as follows:
 - In System Control, select Manual:FlowPath.
 - Select instruction FlowDirection.
 - Set FlowDirection to position DownFlow.
 - Manually, run Pump P-900 with distilled water at a flow rate of approximately 5 ml/min and check that water is flowing downwards the bypass capillary connected between ports 1 of the column selection valves V2 and V3.
 - Set FlowDirection to position UpFlow to check the upflow function in the same way.

7.2.2 Using the flow direction function in a run

The flow direction valve instruction is designated FlowDirection in the flow scheme. In the run data window, it is designated $V7_FlowDir$.

To use the flow direction valve in a method:

- 1 In the Method Editor, select File:Method Wizard and start creating a new method.
- 2 In the Elution dialog, select Segment Gradient Advanced under Elution Technique.
- 3 Click Next twice.
- 4 Select DownFlow or UpFlow under Flow Direction.
- 5 Save the method when finished.

8 General function valve

8.1 Installation

To install any optional valve, proceed as follows:

CAUTION! Make sure ÄKTAexplorer is switched off before installing the optional components. The mains power to ÄKTAexplorer must be switched OFF before disconnecting or connecting the UniNet-2 cables.

- 1 Mark the optional function valve with V8 or V9 as required. Check that the ID code switch is set to 8 or 9 respectively.
- 2 Mount the bracket and attach the valve to a rail on the system wall. Choose the best location to facilitate the routing of the tubing that connects the valve to the main system flowpath.
- 3 Cut and mount the necessary capillaries (see the *Instructions* of the valve). Restrict the length of the capillaries as much as practically possible to reduce band broadening effects.
- 4 Use the attached UniNet-2 cable to connect the valve to the UniNet-2 communication link. The valve can be connected anywhere between Pump P-900 and the termination plug.
- 5 Check that all capillary and electrical connections are carried out correctly.

8.2 Operation

8.2.1 Preparing for operation

- Start your ÄKTAexplorer optional configuration system as described in 2.6 Preparation for use. Make sure that Valve V8 or Valve V9 is selected in the Component list.
- 2 A connected valve is automatically recognised by UNICORN at system start-up. Check that the added valve is indicated in the flowscheme in UNICORN.
- 3 Verify that the valve is functioning properly by issuing manual commands from UNICORN as follows:
 - In System Control, select Manual:FlowPath.
 - Select instruction Valve X, where X=ID code for the valve.
 - Set the valve to position 3 (corresponds to the WASTE position in valve INV-907).
 - Manually, run Pump P-900 with distilled water at a flow rate of approximately 5 ml/min and check that water is coming out from port 3 on the valve (corresponds to port 5 in valve INV-907).

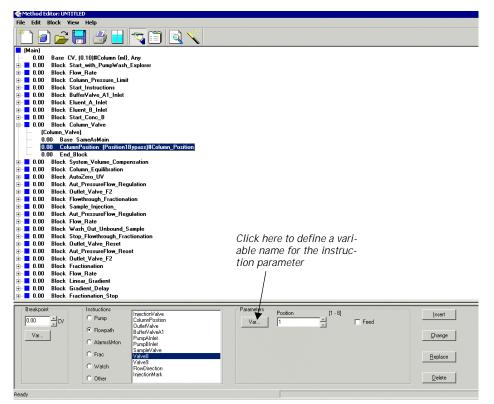
8.2.2 Using the general function valve in a run

General function valve instructions are designated Valve8 and Valve9 in the flow scheme and run data windows.

To use a valve in a method:

- 1 Create a new method by selecting File:Method Wizard in the Method Editor.
- 2 Select View:Text instructions to display the text instruction editor. Double-click on the instruction block where you want to add the general function valve instruction. Highlight the instruction below which you want to insert the valve instruction.
- 3 Click the Flowpath radio button.

4 From the Instructions list, select the valve you intend to use (Valve8 or Valve9). The valve instruction parameters allow you to change the position of the valves. For INV-907, only three positions can be selected (see the *Instructions* of the valve).

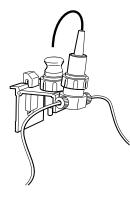


5 Define a variable name for the instruction parameter, e.g. Valvex_pos, where x=8 or 9 depending on the ID code for the valve used. This variable allows you to turn the position of the valve to the port you want.



- 6 Click OK. The defined variable is inserted in the method.
- Return to the Variables page by selecting View:Run setup to set the variable Valvex_pos to the desired position.
- 8 Save the method.

9 pH flow cell and pH electrode mounting



9.1 General

The pH electrode is optional in ÄKTAexplorer 10, 10 S and 10 XT. The pH electrode is to be mounted in a separate pH flow cell, housing the pH electrode itself, as well as a dummy pH electrode. The dummy pH electrode replaces the pH electrode whenever the pH electrode is to be removed from the system, e.g. for storage.

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

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

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

Note: Using the flow cell is not recommended when the peak volume is less than 1 ml. The volume in the flow cell decreases the resolution of the peaks.

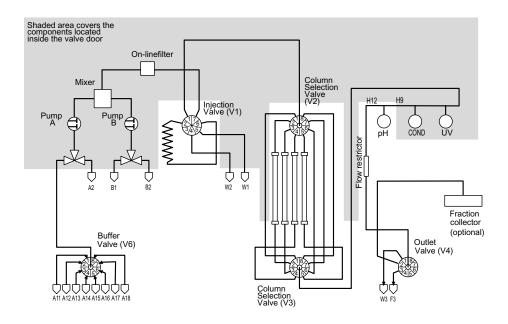
9.2 Installation

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

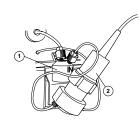
- 1 Install the pH flow cell holder (packed in Box-900) in the groove directly below the outlet valve (V4) on the outside of the valve door.
- 2 Push the slide clamp upwards to secure the pH flow cell holder.
- 3 If 0.25 mm i.d. tubing is installed, remove the capillaries G5 and G7–G11. Connect Tubing kit 0.50 as described from step 4.

If 0.50 mm i.d. tubing is already installed, remove capillary G9 only. Connect the new tubing according to steps 7 and 8.

- 4 Connect the center port of the injection valve V1 to the center port of the column selection valve V2 using capillary H5.
- 5 Connect the center port of the column selection valve V3 to the inlet (on top) of the UV flow cell using capillary H7.
- 6 Connect the outlet (at the bottom side) of the UV flow cell to the inlet of the conductivity flow cell (opposite to the screw head end) using capillary H8.



- 7 Connect the outlet of the conductivity flow cell (the screw head end) to the inlet of the pH flow cell using capillary H9.
- 8 Connect the outlet of the pH flow cell to the FR-902 flow restrictor (marked IN) using the H12 capillary.
- 9 Connect the outlet of the FR-902 flow restrictor to the center port of the V4 outlet valve using the H10 capillary.
- 10 Place the FR-902 flow restrictor (1) in the hole in the pH flow cell holder.



11 Mount the pH electrode (2) for experiments where pH is to be measured. Otherwise, mount the pH electrode dummy in the hole in the pH flow cell.

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

Note: For calibration of the pH electrode, refer to the UNICORN User Manuals.

9.3 Operation

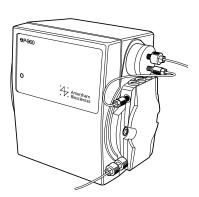
- 1 Start your ÄKTA optional configuration system as described in 2.6 Preparation for use. Make sure that pH CeII is selected in the Component list.
- 2 If fraction collection should be used, remember to change the delay volume. This setting is found in System Control, in System:Settings:Specials. See also 15 Configuration dependent delay volume.
- 3 Using pH measurement in a run is described in the ÄKTAexplorer System Manual. Also refer to the Monitor UV-900 User Manual.

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10 Sample pump P-960 and sample valve V5

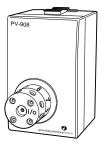
10.1 General

Pump P–960 is a laboratory pump for use as a sample pump to apply samples directly on the column and/ or to fill sample loops, including Superloop. Pumping action is provided by plungers driven by a stepper motor. The pump is a low pulsation pump and features a motor speed independent of temperature and load to give accurate and reproducible flow rates up to 50 ml/min at a pressure up to 2 MPa.



A sample valve PV-908, with a pressure limit of 25 MPa, can be used in combination with the sample pump. PV-908 is a motorised 8-way valve used for automatic sample application of up to 8 samples.

Pump P-960 and sample valve PV-908 are optional components in ÄKTAexplorer 10 and ÄKTAexplorer 10 XT.



Three sample application techniques can be performed by using Pump P–960 in the ÄKTAexplorer system:

- Loading a sample loop.
- Direct loading onto the column (default configuration)
- Loading a Superloop.

Each technique requires a specific configuration and different procedures to remove air from the sample flow path. This is described in the following sections.

Control of the sample pump can be achieved automatically from a method, or manually via the functions available in UNICORN.

Note: Make sure that any air trapped in the sample flow path is removed before use. Presence of air in the sample pump might cause pressure pulsation and inaccurate flow.

Sample pump P-960 kit and Sample valve kit includes the items needed for upgrading an ÄKTAexplorer 10 system for *Direct loading onto the column* with Pump P-960 and sample valve V5.

The following items are included in Sample pump P-960 kit:

- Pump P-960
- UniNet cable
- Holders for ÄKTAdesign systems
- Flow restrictor FR-902
- Purge kit
- Tefzel tubing i.d. 1.0 mm, 3 m
- PEEK tubing i.d. 0.75 mm, 2 m
- Finger-tight connectors and unions

The following items are included in Sample valve kit:

- Sample valve PV-908
- Sample holder SH-900
- UniNet cables
- Tefzel tubing i.d. 1.0 mm, 6 m
- Teflon tubing i.d. 1/16" o.d. 1/8", 6 m
- Finger-tight connectors, unions and stop plugs

ÄKTAexplorer 10S, 100 and 100 Air are supplied with *Loading sample loop tubing kit* as an accessory. The kit contains the tubing and connectors that are required for using an ÄKTAexplorer system for *Loading a sample loop*.

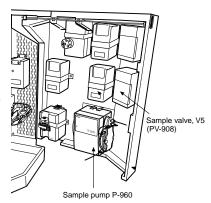
10.2 Installing the components

CAUTION! The mains power to ÄKTAexplorer must be switched OFF before disconnecting or connecting the UniNet-2 cables.

This section describes how to install the components in the system. To connect the capillaries, see the section describing that specific sample application method—*Loading a sample loop, Direct loading onto the column* or *Loading a Superloop*.

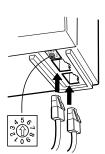
10.2.1 Installing Pump P-960

- 1 Make sure that the ÄKTAexplorer chromatography system is switched off.
- 2 Open the valve door.
- 3 Install Pump P-960 in the separation unit of ÄKTAexplorer.
 - Locate the fixing screws and the clamp supplied with the pump.
 - Fit the pump as described in the *Mounting kit for Pump P-950/P-960, Instructions* supplied.



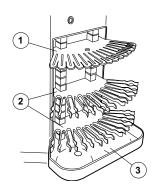
10.2.2 Installing the sample valve

- 1 Install the sample valve V5 (PV-908) above Pump P-960 (also refer to *IV/PV-908 Instructions*).
 - Set the rotary ID-switch at the bottom of the V5 valve to position 5.
 - Remove the black, circular plastic cap attached in the mounting hole.
 - Use the attached bracket and screws to fit the valve in the same way as the V1 valve.
 - Apply an identification number (5) from the labels supplied. Place the number so that it is easily readable from the front.
- 2 Close the valve door.



10.2.3 Installing Sample holder SH-900

- 1 Install the Sample holder SH-900 on the beige, plastic mounting plate below the sample valve V5.
- 2 Install the Tubing holder (1), the Tube holders (2) and the sample tray (3) by pushing them into the slits.

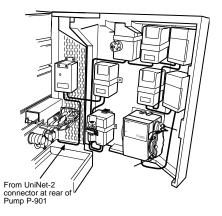


10.2.4 Installing UniNet-2 cables

Connecting P-960 and the sample valve V5

- 1 Remove the termination plug from the column selection valve V3.
- 2 Connect a new cable (supplied) between column selection valve V3 and sample valve V5.
- 3 Connect a new cable (supplied) between sample valve V5 and Pump P-960 (the leftmost socket).

Note: The termination plug is not required. P-960 has an internal termination.



Connecting P-960 only

- 1 Remove the termination plug from the column selection valve V3.
- 2 Connect a new cable (supplied) between V3 and Pump P-960 (the leftmost socket).

Note: The termination plug is not required. P-960 has an internal termination.



10.2.5 Installing Flowrestrictor FR-902

If the sample vessel is placed at a higher level than the end of the sample waste tubing, sample might accidently flow through the sample flow path to waste. Flow restrictor FR-902 supplied is used in the sample flow path to eliminate this effect by creating a back-pressure of 0.2 MPa. Thereby, sample vessels can be placed, for example, on top of the system.

The position of the flow restrictor in the flow path depends on how the sample is applied with the sample pump (see Table 10-1). The injection valve position refers to when sample otherwise might flow straight through to waste.

Sample application type	Flow restrictor port IN connected to*	Injection valve position
Filling a sample loop	Sample pump P-960 outlet port	LOAD
Direct loading onto a column	Injection valve V1, port 4	INJECT
Loading a Superloop**	Injection valve V1, port 5	INJECT

^{*} A tubing should be used between the flow restrictor and the port. See instruction below.

Table 10-1. Position of the flow restrictor

When connecting the flow restrictor to the sample pump (Filling a sample loop), use a 22 cm long tubing between the pump outlet and the flow restrictor IN port. Route the tubing through the hole in the door and connect the flow restrictor **outside** the door.

When connecting the flow restrictor to the injection valve, use a 10 cm long tubing between the flow restrictor IN port and the injection valve.

^{**}The flow restrictor is required only if filling the Superloop several times during the run.

10.3 Loading a sample loop

Automatic filling of sample loops can be useful when samples must be applied repeatedly, for example, in scouting runs. By using sample loops supplied by Amersham Biosciences, volumes between $0.1–2.0\,$ ml can be applied. The sample is drawn into the sample loop by the sample pump.

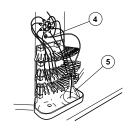
Note: The flow rate should not exceed 0.5 ml/min when filling the sample loop. Otherwise, cavitation may occur due to the resistance in the flow path.

10.3.1 Connecting the capillaries

- 1 Make sure that the sample pump and the sample valve (if included) are properly installed according to section 10.2 Installing the components.
- 2 Connect all capillaries using connectors and ferrules as indicated in the table and in the figures. We recommend using the P-960 Loading a sample loop kit.

Use the sample holder for storing sample tubes and sample waste vessels (5), and for routing the sample and waste capillaries (4).

3 Check that all capillary and electrical connections have been carried out correctly.



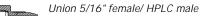


Fingertight connector 1/16"



Union fingertight female/ M6 male

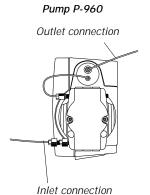


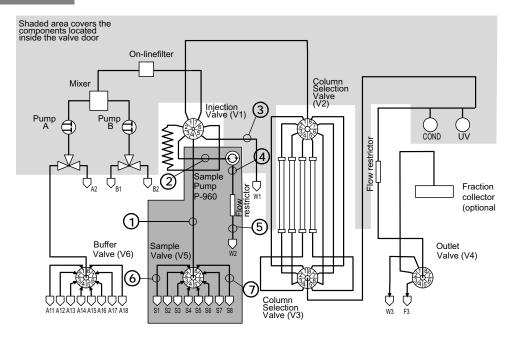






Tubing connector for 1/8" o.d. tubing with ferrule for 1/8" o.d. tubing





Capillary (max. length)	Connector	Connection points
1 PEEK i.d. 0.75 mm x 20 cm (Sample inlet tubing)	Fingertight connector 1/16", 18-1112-55	Injection valve, port 4
	Fingertight connector 1/16", 18-1112-55*	Sample valve, center port*
2 Tefzel i.d. 1.0 mm x 57 cm	Fingertight connector 1/16", 18-1112-55	Injection valve, port 3
	Fingertight connector 1/16", 18-1112-55. Union fingertight female/M6 male, 18-1112-57	Sample pump, inlet
3 Tefzel i.d. 1.0 mm x 130 cm	Fingertight connector 1/16", 18-1112-55	Injection valve, port 5
4 Tefzel i.d. 1.0 mm x 22 cm	Fingertight connector 1/16", 18-1112-55 Fingertight connector 1/16", 18-1112-55	Sample pump, outlet Flow restrictor, inlet port
5 Tefzel i.d. 1.0 mm x 108 cm	Fingertight connector 1/16", 18-1112-55	Flow restrictor, outlet port
6 Tefzel i.d. 1.0 mm x 32 cm	Fingertight connector 1/16", 18-1112-55	Sample valve, inlets S1-S7*
7 Teflon i.d. 1.6 mm x 130 cm	Tubing conn. 1/8" o.d. tubing, 18-1121-17 with ferrule for 1/8" o.d. tubing, 18-1121-18. Union 5/16" female/HPLC male, 18-1142-08	Sample valve, inlet S8*

^{*} Only if a sample valve is installed. Connect stop plugs to non-used ports in the sample valve.

10.3.2 Preparing for operation

- Start your ÄKTAexplorer optional configuration system as described in 2.6 Preparation for use. Make sure that Sample pump P-960 and (if required) SampleValve V5 are selected in the Component list.
- 2 Check that the sample pump and the sample valve (if included) are indicated in the flow scheme window.
- 3 Make sure the system is connected as previously described in section 10.3.1 Connecting the capillaries.

10.3.3 Testing the pump and sample valve

Verify that the sample pump is functioning properly by issuing manual commands from UNICORN as follows:

- 1 Fill a container with distilled water and submerge the sample inlet capillary S1 (or, for example, S7 if the sample valve is included) in the container.
- 2 Set the injection valve to position LOAD.
- 3 Remove air from the sample flow path using the purge tubing according to section 10.3.5.
- 4 In System Control select Manual:Flowpath.
- 5 If the sample valve is included, select the instruction SampleValve in the list and select S7.
- 6 Click on Execute.
- 7 Select Manual:Pump.
- 8 Select the instruction SampleFlow_960 in the instruction list.
- 9 Set Flowrate to 0.5 ml/min and click Execute.
- 10 Check that the sample pump is delivering flow through its outlet.
- 11 Stop the pump by clicking End in the System Control toolbar.

10.3.4 Removing air by running the sample pump

If there are only small amounts of air in the sample flow path, remove the air as follows:

- 1 Immerse the end of the sample inlet tubing in a suitable buffer (inlet S8, if using sample valve V5).
- 2 If using sample valve V5, set the valve to port 8 (buffer inlet).
- 3 Set the injection valve to LOAD.
- 4 Run the pump at 5 ml/min for 1 min.

If the pump fails pumping liquid, remove the air using a purge tubing according to section 10.3.5 Removing air by using a purge tubing.

10.3.5 Removing air by using a purge tubing

If there is plenty of air in the sample inlet tubings or if the sample pump is dry, use a purge tubing to fill the tubings and the pump.

Procedure for system including sample valve V5

This procedure describes how to first fill the sample inlet tubings and then the buffer inlet tubing.

To fill the sample inlet tubings in port 1–7:

- 1 Put the chosen sample inlet tubings from port 1–7 in sample valve V5 into the sample tubes.
- 2 Immerse the tubing from port 8 in V5 in a buffer vessel.
- 3 Set the valve to any of the chosen sample inlet ports 1-7.

Note: The sample inlet tubings should be filled before filling the buffer tubing connected to port 8.

4 Disconnect the connector fitted to the outlet port on the connection part of Pump P-960, i.e. before the pressure sensor. Connect the purge tubing to the same port.



- 5 Draw sample with the syringe until the liquid level has passed through the sample valve.
- 6 Set the injection valve V1 to LOAD.

7 Switch sample valve V5 to the next sample inlet tubing to be filled.

Note: If the syringe needs to be emptied, switch the sample valve V5 **before** removing the syringe to prevent sample from flowing back to the vessel.

8 Repeat step 6 and 7 for the remaining sample inlet tubings.

To fill the buffer inlet tubing in port 8:

- 1 Set the sample valve V5 to port 8.
- 2 Draw buffer with the syringe until the liquid level has passed through the sample pump.

The check valves in the sample pump will prevent the liquid from being withdrawn when removing the purge tubing.

- 3 Disconnect the purge tubing.
- 4 Fit the original connector to the port.
- 5 Flush the sample flow path tubing with buffer to remove any trapped air bubbles according to the procedure in section 10.3.4 Removing air by running the sample pump.

Procedure for system not including sample valve V5

- 1 Put the sample inlet tubing in a buffer vessel.
- 2 Disconnect the connector fitted to the outlet port on the connection part of Pump P-960, i.e. before the pressure sensor. Connect the purge tubing to the same port.
- 3 Set the injection valve V1 to LOAD.
- 4 Draw buffer with the syringe until the liquid level has passed through the sample pump.

The check valves in the sample pump will prevent the liquid from flowing back when removing the purge tubing.

- 5 Disconnect the purge tubing.
- 6 Fit the original connector to the port.
- 7 Flush the sample inlet tubing with buffer to remove any trapped air bubbles according to the procedure in section 10.3.4.
- 8 Gently move the sample inlet tubing to the sample vessel.

10.3.6 Preparing a method for filling a sample loop

To use the sample pump for filling a sample loop, select Sample Pump Loop Filling P-960 as sample application technique in the Method Wizard when creating the new method.

The sample pump properties are designated P960_Flow and P960_Press in the flow scheme and run data windows.

Note: When filling the sample loop, the resistance in the flowpath may cause cavitation if the flow rate exceeds 0.5 ml/min.

Note: For complete filling, an overfill of 2-5 times the loop volume is needed for maximum reproducibility between the runs.

10.3.7 Emptying the sample loop

When emptying the sample loop, use a buffer volume of approximately 5 times the sample loop volume to flush the loop and ensure that all sample is injected onto the column.

10.4 Direct loading onto the column

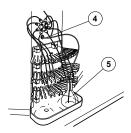
Loading sample directly onto the column is useful when large amounts of sample is to be loaded. The sample is pushed onto the column.

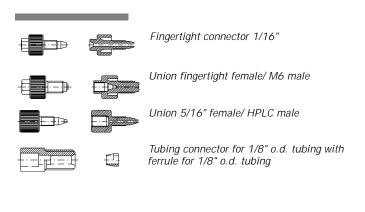
Note: In this configuration, some columns generate a back-pressure above 2.0 MPa at their recommended flow rates. If the back-pressure exceeds 2.0 MPa (the upper pressure limit of Pump P-960), the pump will stop immediately. To achieve a back-pressure below 2.0 MPa when using these columns, we recommend decreasing the flow rate.

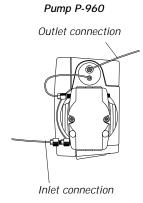
We particularly recommend decreasing the flow rate of the system pump during equilibration, before sample application, and before switching the sample pump in-line. The sample pump will not start if the back-pressure exceeds 2.0 MPa.

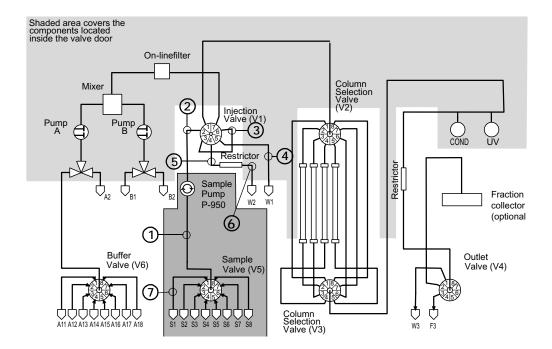
10.4.1 Connecting the capillaries

- 1 Make sure that the components are properly installed according to section 10.2 Installing the components.
- 2 Connect all capillaries using connectors and ferrules as indicated in the table and in the figures.
 - Use the sample holder for storing sample tubes and sample waste vessels (5), and for routing the sample and waste capillaries (4).
- 3 Check that all capillary and electrical connections have been carried out correctly.









Capillary (max. length)	Connector	Connection points
1 Tefzel i.d. 1.0 mm x 40 cm (Sample inlet tubing)	Fingertight connector 1/16", 18-1112-55	Sample valve, center port*
	Fingertight connector 1/16", 18-1112-55 Union fingertight female/ M6 male, 18-1112-57	Sample pump, inlet
2 Tefzel i.d. 1.0 mm x 50 cm	Fingertight connector 1/16", 18-1112-55	Sample pump, outlet
	Fingertight connector 1/16", 18-1112-55	Injection valve, port 2
3 PEEK i.d. 0.75 mm x 15 cm	Fingertight connector 1/16", 18-1112-55	Injection valve, port 3
	Fingertight connector 1/16", 18-1112-55	Injection valve, port 6
4 Tefzel i.d. 1.0 mm x 130 cm	Fingertight connector 1/16", 18-1112-55	Injection valve, port 5
5 Tefzel i.d. 1.0 mm x 10 cm	Fingertight connector 1/16", 18-1112-55	Injection valve, port 4
	Fingertight connector 1/16", 18-1112-55	Flow restrictor, inlet
6 Tefzel i.d. 1.0 mm x 120 cm	Fingertight connector 1/16", 18-1112-55	Flow restrictor, outlet
7 Teflon i.d. 1.6 mm x 130 cm*	Union 5/16" female/ HPLC male, 18-1142-08. Tubing connector for 1/8" o.d. tubing, 18-1121-17 with ferrule for 1/8" o.d. tubing, 18-1121-18.	Sample valve, ports 1–8*

^{*} Only if you have a sample valve in your configuration.

Note! Connect stop plugs to non-used ports in the sample valve.

Note! If low sample flows are used (≤10 ml/min), sample inlet tubings connected to ports 1–7 can be replaced with Tefzel i.d. 1.0 mm tubing together with finger-tight connectors. The sample inlet tubing should be adjusted to a suitable length.

10.4.2 Preparing for operation

- Start your ÄKTAexplorer optional configuration system as described in 2.6 Preparation for use. Make sure that Sample pump P-960 and (if required) SampleValve V5 are selected in the Component list.
- 2 Check that the sample pump and the sample valve (if included) are indicated in the flow scheme window.
- 3 Make sure the system is connected as previously described in section 10.4.1 Connecting the capillaries.

10.4.3 Testing the pump and sample valve

Verify that the sample pump is functioning properly by issuing manual commands from UNICORN as follows:

- 1 Fill a container with distilled water and submerge the sample inlet capillary S1 (or, for example, S7 if the sample valve is included) in the container.
- 2 Set the injection valve to position WASTE.
- 3 Remove air from the sample flow path using the purge tubing according to section 10.4.5.
- 4 In System Control select Manual: Flowpath.
- 5 If included, select the instruction SampleValve in the list and select S7.
- 6 Click on Execute.
- 7 Select Manual:Pump.
- 8 Select the instruction SampleFlow_960 in the instruction list.
- 9 Set Flowrate to 1 ml/min and click Execute.
- 10 Check that the sample pump is delivering flow through port 4 (W2) in the injection valve.
- 11 Stop the pump by clicking End in the System Control toolbar.

10.4.4 Removing air by running the sample pump

If there are only small amounts of air in the sample flow path, remove the air as follows:

- 1 Immerse the end of the sample inlet tubing in a suitable buffer (inlet S8, if using sample valve V5).
- 2 If using sample valve V5, set the valve to port 8 (buffer inlet).
- 3 Set the injection valve to WASTE.
- 4 Run the pump at 40 ml/min for 1 min.

If the pump fails pumping liquid, remove the air using a purge tubing according to section 10.4.5 Removing air by using a purge tubing.

10.4.5 Removing air by using a purge tubing

If there is plenty of air in the sample inlet tubings or if the sample pump is dry, use a purge tubing to fill the tubings and the pump.

Procedure for system including sample valve V5

This procedure describes how to first fill the sample inlet tubings and then the buffer inlet tubing.

To fill the sample inlet tubings in port 1–7:

- 1 Put the chosen sample inlet tubings from port 1–7 in sample valve V5 into the sample tubes.
- 2 Immerse the tubing from port 8 in V5 in a buffer vessel.
- 3 Set the valve to any of the chosen sample inlet ports 1-7.

Note: The sample inlet tubings should be filled before filling the buffer tubing connected to port 8.

4 Disconnect the connector fitted to injection valve V1, port 3. Connect the purge tubing to the same port.



- 5 Set the injection valve V1 to LOAD.
- 6 Draw sample with the syringe until the liquid level has passed through the sample valve.

7 Switch sample valve V5 to the next sample inlet tubing to be filled.

Note: If the syringe needs to be emptied, switch the sample valve V5 before removing the syringe to prevent sample from flowing back to the vessel.

8 Repeat step 6 and 7 for the remaining sample inlet tubings.

To fill the buffer inlet tubing in port 8:

- 1 Set the sample valve V5 to port 8.
- 2 Draw buffer with the syringe until the liquid level has passed through the sample pump.

The check valves in the sample pump will prevent the liquid from being withdrawn when removing the purge tubing.

- 3 Disconnect the purge tubing.
- 4 Fit the original connector to the port.
- 5 Flush the sample flow path tubing with buffer to remove any trapped air bubbles and rinse the pump according to the procedure in section 10.4.4 Removing air by running the sample pump.

Procedure for system not including sample valve V5

- 1 Put the sample inlet tubing in a buffer vessel.
- 2 Disconnect the connector fitted to injection valve V1, port 3. Connect the purge tubing to the same port.
- 3 Set the injection valve V1 to LOAD.
- 4 Draw buffer with the syringe until the liquid level has passed through the sample pump.

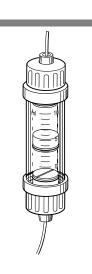
The check valves in the sample pump will prevent the liquid from flowing back when removing the purge tubing.

- 5 Disconnect the purge tubing.
- 6 Fit the original connector to the port.
- 7 Flush the sample inlet tubing with buffer to remove any trapped air bubbles according to the procedure in section 10.4.4 Removing air by running the sample pump.
- 8 Gently move the sample inlet tubing to the sample vessel.

10.4.6 Preparing a method for direct loading onto column
To use the sample pump for direct loading onto the column, select
Sample Pump Direct Loading P-960 as sample application technique in the
Method Wizard when creating the new method.

The sample pump properties are designated P960_Flow and P960_Press in the flow scheme and run data windows.

Note: Make sure that any air trapped in the sample flow path is removed before use.



10.5 Loading a Superloop

The sample pump can be used to fill a Superloop. The Superloop allows introduction of larger sample volumes into a pressurized fluid system. The sample is pushed into the Superloop by the sample pump.

Superloop consists of a movable seal in a glass tube. The seal divides the tube into two separate chambers. Depending on the flow direction, the seal moves towards either end piece of the glass tube.

Superloop is available in three sizes (10, 50, 150 ml) allowing application of 1–10, 1–50 and 1–150 ml samples respectively.

When loading a Superloop, a sample valve is usually not used.

10.5.1 Connecting the capillaries

- 1 Make sure that the sample pump is properly installed according to section 10.2 Installing the components.
- 2 Connect all capillaries using connectors and ferrules as indicated in the table and in the figures.
- 3 Check that all capillary and electrical connections have been carried out correctly.





Fingertight connector 1/16"

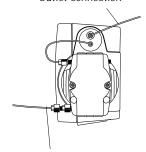




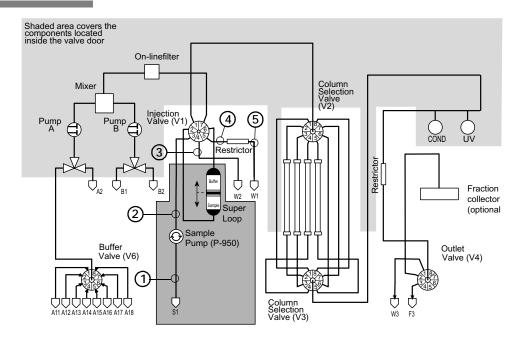
Union fingertight female/ M6 male







Inlet connection



Capillary (max. length)	Connector	Connection points
1 Tefzel i.d. 1.0 mm x 130 cm (Sample inlet tubing)	Fingertight connector 1/16", 18-1112-55 Union fingertight female/ M6 male, 18-1112-57	Sample pump, inlet
2 Tefzel i.d. 1.0 mm x 50 cm	Fingertight connector 1/16", 18-1112-55	Sample pump, outlet
	Fingertight connector 1/16", 18-1112-55	Injection valve, port 3
3 Tefzel i.d. 1.0 mm x 130 cm	Fingertight connector 1/16", 18-1112-55	Injection valve, port 4
4 Tefzel i.d. 1.0 mm x 10 cm	Fingertight connector 1/16", 18-1112-55	Injection valve, port 5
	Fingertight connector 1/16", 18-1112-55	Flow restrictor, inlet
5 Tefzel i.d. 1.0 mm x 120 cm	Fingertight connector 1/16", 18-1112-55	Flow restrictor, outlet

10.5.2 Preparing for operation

- 1 Start your ÄKTAexplorer optional configuration system as described in 2.6 Preparation for use.
- 2 Check that the sample pump is indicated in the flow scheme window.
- 3 Make sure the system is connected as previously described in section 10.5.1 Connecting the capillaries.

10.5.3 Testing the sample pump

Verify that the sample pump is functioning properly by issuing manual commands from UNICORN as follows:

- 1 Fill a container with distilled water and submerge the sample inlet capillary S1.
- 2 Set the injection valve to position INJECT.
- 3 Remove air from the sample flow path using the purge tubing according to section 10.5.5.
- 4 In System Control select Manual:Pump.
- 5 Select the instruction SampleFlow_960 in the instruction list.
- 6 Set Flowrate to 5 ml/min and click on Execute.
- 7 Check that the sample pump is delivering flow.
- 8 Stop the pump by clicking End in the System Control toolbar.

10.5.4 Removing air by running the sample pump

If there are only small amounts of air in the sample flow path, remove the air as follows:

- 1 Immerse the end of the sample inlet tubing in a suitable buffer.
- 2 Set the injection valve to INJECT.



WARNING! OVER-PRESSURE. The injection valve must be set to position INJECT in the "Loading a Superloop" configuration. If not, the Superloop might rupture due to over-pressure when running the sample pump, resulting in injury.

3 Run the pump at 40 ml/min for 1 min.

If the pump fails pumping liquid, remove the air using a purge tubing according to section 10.5.5 Removing air by using a purge tubing.

10.5.5 Removing air by using a purge tubing

If there is plenty of air in the sample inlet tubings or if the sample pump is dry, use a purge tubing to fill the tubings and the pump.

- 1 Put the sample inlet tubing in a buffer vessel.
- 2 Disconnect the connector fitted to the injection valve, port 2. Connect the purge tubing to the same port.
- 3 Set the injection valve to LOAD.
- 4 Draw buffer with the syringe until the liquid level has passed through the sample pump.

The check valves in the sample pump will prevent the liquid from flowing back when removing the purge tubing.

- 5 Disconnect the purge tubing.
- 6 Fit the original connector to the port.
- Flush the sample inlet tubing with buffer to remove any trapped air bubbles according to the procedure in section 10.5.4 Removing air by running the sample pump.
- 8 Gently move the sample inlet tubing to the sample vessel.

10.5.6 Preparing a method for loading a Superloop

To use the sample pump for loading a Superloop, select Sample Pump Loop Filling P-960 as sample application technique in the Method Wizard when creating the new method.

Note: Do NOT select the S8 - Prerinse Sample Loop (2 ml) check box!

The sample pump instructions are designated SampleFlow_960 and P960_Press in the flow scheme and run data windows.



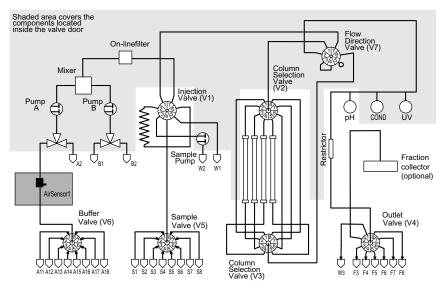
WARNING! Make sure that the upper pressure limit of the system is lower than the maximum allowed pressure of the Superloop.

11 Air sensors

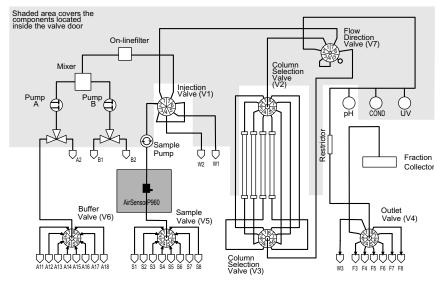
11.1 General

Air sensors Air-912, Air-912N (standard in Explorer 100 Air), Air-925 and Air-925N can be used in the ÄKTAexplorer for detecting air in the system flow path or sample flow path. Up to four air sensors can be used with the instruction names AirSensor1, AirSensor2, AirSensor3, and AirSensorP960 (Air-912N or Air-925N).

The examples below show two possible locations. Air sensors can be fitted in other locations in the ÄKTAexplorer system flow path. The installation procedure is similar to the ones described in the following sections.



AirSensor1 in the inlet flow of Pump P-900 for checking the buffer supply.



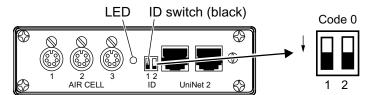
AirSensorP960 in the sample inlet flow when applying sample directly onto the column with a sample pump.

11.2 Installation

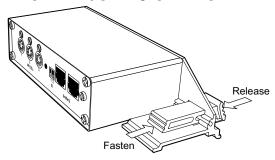
CAUTION! Make sure ÄKTAexplorer is switched off before installing the optional components. The mains power to ÄKTAexplorer must be switched OFF before disconnecting or connecting the UniNet-2 cables.

11.2.1 Fitting the Air-900 control box

- 1 Make sure that the ÄKTAexplorer chromatography system is switched off.
- 2 Check that the ID switches on the connector panel of the Air-900 control box are set to code 0 (both switches in ON position).



3 Attach the Air-900 control box vertically to a suitable mounting slot. Lock in position by pushing up the snap lock.



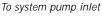
11.2.2 Connecting to the UniNet-2 communication network
Use the attached UniNet-2 cable to connect the Air-900 control box to
the UniNet-2 communication link. It can be connected anywhere
between the system pump P-900 and the termination plug.

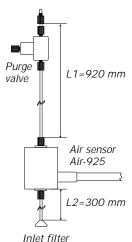
11.2.3 Air sensor Air-925 (AirSensor1) in the system pump inlet

- 1 Remove the capillary from the switch valve inlet A1.
- 2 Cut new Teflon capillaries as shown in the figure and attach the purge valve and the air cell using the connectors and ferrules supplied.

Note: The length of L1 is dependent on which flow, sensitivity and type of instruction (alarm or watch) is used in UNICORN. The optimal length of L1 must be tested in the user configuration.

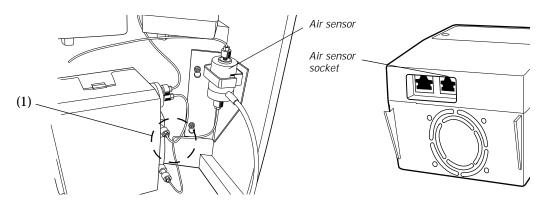
3 Route the signal cable from the air cell to the Air cell 1 connector on the Air-900 control box and plug it in.





11.2.4 Air sensor Air-912N (AirSensorP960) in the sample inlet flowpath Air sensor Air-912N kit contains the necessary items required for this application. Air sensor Air-912N can only be connected to Pump P-960.

1 Fasten the bracket inside the ÄKTAexplorer door using the sample holder screws.



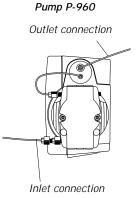
- 2 Connect the air sensor cable to the socket underneath Pump P-960.
- and 4 Cu 25 cm cor fing sup No

25 cm

To sample pump inlet

Inlet filter or sample valve

- Remove the sample inlet capillary between the sample valve V5 (or sample) and the sample pump inlet connector.
- 4 Cut the i.d. 1.0 mm tubing supplied and connect to Air-912N using the fingertight connectors and union supplied.
 - Note: Route the tubing to the sample pump through the pre-bent tubing loop (see (1) in the figure above).
 - Note: Make sure that no air bubbles are trapped in the sample pump flow path before using the pump (refer to section 10 Sample pump P-960 and sample valve V5).



11.2.5 Capillary connectors

The following capillary connectors are recommended for connecting the liquid tubings to the air sensors:

Airsensor Air-925 and Air-925N



Airsensor Air-912 and Air-912N



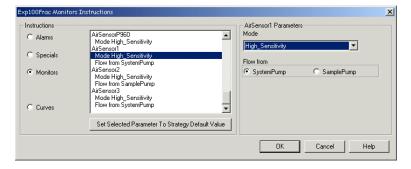


Fingertight connector for 1/16" o.d. tubing, no. 18-1112-55

11.3 Operation

11.3.1 Preparing for operation

- 1 Start your ÄKTAexplorer optional configuration system as described in 2.6 Preparation for use. Make sure that Airsensor 1 (and/or 2 and/or 3) is selected in the Components list.
- 2 In System Control, select System: Settings.
- 3 Click the Monitors radio button.
- 4 In the Instruction field, select, e.g., AirSensor1.
- 5 In the Parameters field, select High_Sensitivity for the air cell(s) to be used. Select the flow source (for AirSensor1–3), i.e. SystemPump if the air cell is located in the main system flow path, or SamplePump if the air cell is located in the sample injection flow path.



Note: The sensitivity of the air cells can be selected at three levels; high, medium or low sensitivity. We recommend using the high sensitivity setting as standard.

- 6 Check that the connected air cell(s) are indicated in the flow scheme in UNICORN.
- 7 Verify that the air cells are functioning properly by issuing manual commands from UNICORN as follows.

- 11.3.2 Testing AirSensor1 located in the main system flow path
- 1 Confirm that the air sensor AS1 is shown in the flow scheme.
- 2 Fill a sample container with distilled water and submerge the inlet filter of the A1 tubing. Purge the air cell using a syringe and the purge valve mounted on the inlet capillary. The purge valve is opened to the syringe by turning the syringe connector a 1/2 turn counter clockwise.



- 3 In System Control select Manual:Pump.
- 4 Select the instruction Flow in the instruction list.
- 5 Set Flowrate to 5 ml/min and click Execute.
- 6 While the system pump is running, check that the AS1 symbol in the flow diagram is indicated with white colour.
- 7 Lift the inlet filter out of the flask and check that the white indication changes to red when air enters the AS1 flow sensor.
- 8 Submerge the inlet filter and check that the red indication changes back to white again. Stop the system pump by clicking End in the System Control toolbar.
- 9 Set up instructions as described in *11.3.4 Setting up instructions for the air sensor function*, to monitor the AirSensor1 signal to suit your needs.

Note: AirSensor2 and AirSensor3 are tested in a similar way.

- 11.3.3 Testing AirSensorP960 located in the sample flow path
- 1 Make sure that the sample pump is functioning properly.
- 2 Confirm that air sensor ASP960 is shown in the flow scheme.
- 3 Fill a sample container with distilled water and submerge the sample tubing.
- 4 In System Control select Manual: Pump in UNICORN.
- 5 Select the instruction SampleFlow_960 in the instruction list.
- 6 Set Flowrate to 5 ml/min and click Execute.
- While the sample pump is running, check that the ASP960 symbol in the flow diagram is indicated with white colour.

- 8 Lift the sample tubing inlet out of the flask and check that the white indication changes to red when air enters the ASP960 flow sensor.
- 9 Submerge the sample tubing inlet and check that the red indication changes back to white again. Stop the pump by clicking End in the System Control toolbar.
- 10 Set up instructions as described below to monitor the AirSensorP960 signal to suit your needs.

11.3.4 Setting up instructions for the air sensor function

For AirSensor1-3, the presence of air in the flow path is detected by the Air-900 control box, which reports its status to UNICORN. For AirSensorP960, air is detected by the control software in Pump P-960.

The desired control action, based on the status report, is governed by instructions in the method used. Instructions for handling the air sensors must be inserted manually in a method.

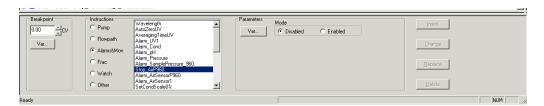
Alarm AirSensor1-3 and Alarm AirSensorP960

Enables/disables alarm generation for the air cells. An active alarm sets the chromatography system to Pause mode. Alarms can be activated/deactivated in System:Settings or inserted as instructions in a method. They can also be activated/deactivated manually in Manual:Alarms&Mon.



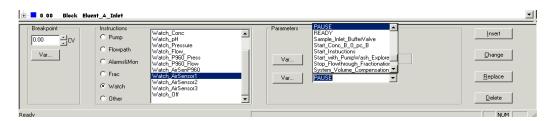
Stop AirP960

When the instruction is enabled, Pump P-960 will stop when air is detected. The method execution then continues immediately with the next instruction, i.e. the system will not be set to Pause mode. The instruction can be enabled/disabled in System:Settings or inserted as instructions in a method. It can also be enabled/disabled manually in Manual:Alarms&Mon.



Watch_AirSensor1-3 and Watch_AirSensorP960

Monitors the air sensor status signals from the air cells. Performs a user-defined action if the test condition is fulfilled. Monitoring is cancelled automatically once the condition has been fulfilled. The user-defined action can be, for example, PAUSE or a call to a block.



11.3.5 Preparing a method for using air sensors

The following two examples show how to use air sensors in a method.

Example 1

In this example, AirSensor1 is used when applying sample directly onto the column using the system pump.

- 1 In the Method Wizard, select System Pump Direct Loading as sample application technique.
- 2 To enable the air sensor function, check the Support for Air Sensor 1 box.

Example 2

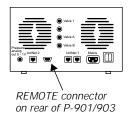
In this example, AirSensorP960 is used together with sample pump P-960 in a configuration designed to accomplish direct sample loading onto the column and checking the sample supply.

- 1 In the Method Wizard, select Sample Pump Direct Loading P-960 as sample application technique.
- 2 To enable the air sensor function, check the Inject all Sample box.
- 3 Type the maximum allowed sample volume in the Maximal Injection Volume (0-20000 ml) field.

12 Auxiliary equipment controlled via Pump P-901/903 REMOTE connector

12.1 Installation

CAUTION! Make sure ÄKTAexplorer is switched off before installing the optional components.



Auxiliary equipment using digital input/output signals can be connected to the 9-pole D-SUB female REMOTE connector on the rear panel of Pump P-900 (5 V TTL signals only). Pins 1–4 handle digital input signals, and pins 6–9 handle digital output signals. Pin 5 is signal ground.

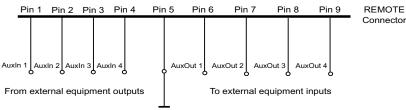


Pin	Signal	Function
1-4	AuxIn 1-4	Active state can be selected to 0 or 1 in UNICORN. Min. duration=50 ms. Max. frequency=1 Hz.
5	OV	Signal ground
6-9	AuxOut 1-4	Active state can be selected to 0 or 1 in UNICORN (5 V TTL signals).

Use a shielded cable with a 9-pin D-SUB male connector for the P-900 REMOTE connector. The other end of the cable connects to the auxiliary equipment and must be adapted to its relevant connector.

The connection example below shows the principle for connecting input/output signals.





Signal ground in external equipment

12.2 Operation

12.2.1 Preparing for operation

Start your ÄKTAexplorer optional configuration system as described in 2.6 Preparation for use. Make sure that Auxiliary Equipment is selected in the Components list.

Four digital input and four digital output signals can be handled via the P-900 REMOTE connector. The input signals are designated AuxIn1-4 and the output signals are designated AuxOut1-4.

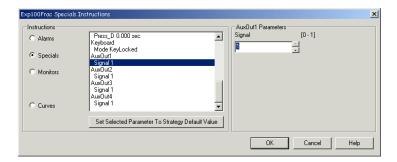
12.2.2 Instructions for signal handling

To set up signal handling in a method, the following parameters and instructions must be inserted manually in a method.

AuxOut1-4

To set up and use the output signals, do as follows:

1 In System Control, select System:Settings.

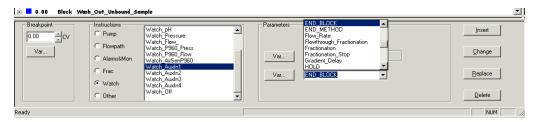


- 2 Click on the Specials radio button.
- 3 In the Instruction field, select the signal you want to use, e.g., AuxOut1.
- 4 Check in the external equipment User Manual which logical value, 0 or 1, that corresponds to inactive mode.
- 5 For AuxOut1 Parameters/Signal, set this value (0/1) as default. This ensures that the external equipment is reset to inactive mode when the method is completed.
- 6 Repeat this for all the output signals you want to use.

Watch AuxIn1-4

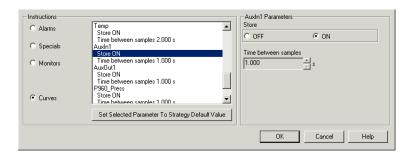
These instructions set up the initial status of the input signals. The status of the signals (1/0) can be shown in Run Data pane in UNICORN.

The instructions also monitor the signal status for the input signals and perform user-defined actions if test conditions are fulfilled. Monitoring is cancelled automatically once a condition has been fulfilled. Preselected action can be, for example, call to a block.



Curve representation of input/output signals

The status for signals AuxIn1 and AuxOut1 can be stored as curves in the chromatogram. This is selected in System:Settings by setting Curves:Store ON.



The parameter Time between samples is used to set the scan cycle for curve updating. The shortest possible interval is 1 s.

AD-900 13

13.1 General

AD-900 is an A/D converter for connecting an external instrument to ÄKTAexplorer system. The module has one high resolution analog input for monitoring e.g. pressure, UV monitor signals or other signals available as voltage outputs.



AD-900 communicates with the UNICORN control system via UniNet-2.

13.2 Installation

CAUTION! Make sure ÄKTAexplorer is switched off before installing the optional components. The mains power to ÄKTAexplorer must be switched OFF before disconnecting or connecting the UniNet-1 and UniNet-2 cables.

1 Connect the Mini-DIN cable connector to socket Analog In at AD-900.

The length of the analog cable must not exceed 3 m. Note:

- Remove the protection cover from the wires marked 1 and 2 at the other end of the Mini-DIN cable.
- Connect wire 1 (brown) to the external device high output.
- Connect wire 2 (red) to the external device low output.
- 5 Set the ID switches as shown in the figure.



6 Connect the module with two UniNet cables as a part of the UniNet 2 chain. One UniNet cable is supplied with the module. The valve can be connected anywhere between the system pump P-920 and the termination plug connected to the injection valve.

Note: The power on-LED on AD-900 flashes for a few seconds when connecting the UniNet cable. The light gets steady when the communication with UNICORN is established.

- 7 Attach the module to the system rack.
- 8 Check that all electrical connections have been made correctly.

13.3 Operation

13.3.1 Preparing for operation

- 1 Start your ÄKTAexplorer optional configuration system as described in section 2.6 Preparation for use. Make sure that AD900 is selected in the Components list.
- 2 Check that AD-900 is indicated in the flow scheme in UNICORN. A steady light from the green power on-LED on AD-900 indicates that the communication is established.
- 3 Verify that the module is functioning by running the system pump manually and checking that a signal is shown in the Run Data pane.

13.3.2 Using AD-900 for monitoring an external signal The AD-900 instructions are designated AD900 in the flow scheme. In the run data window, they are designated AD900.

To use AD-900 for peak fractionation in a method instead of using the UV measurement in Monitor UV-900:

- 1 In the Method Wizard, select a fractionation option that includes Peak Fractionation.
- 2 In the Peak Fractionation dialog, select AD900 from the Peak Identification menu.
- 3 Type suitable values in AD900 Level, Peak Fractionation Volume and, if no column is selected, Minimum Peak Width.
- 4 Save the method when finished.

Note: When adding the instruction in an existing method in the Method Editor, insert it before the fractionation.

The settings of the AD-900 filter can be changed in System: Specials. Autozero of the AD-900 can be performed in Manual: Alarms & Mon.

To set specific filter properties in an existing method:

In the Method Editor, select View:Text Instructions to display the text instructions editor. Double-click on the first block in the method where the AD-900 instructions should be inserted. Highlight an instruction. The instruction will be inserted below this instruction.

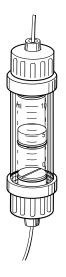
Note: Make sure that the instruction is inserted before the sample injection.

- 2 Click on the Alarm&Mon radio button.
- 3 From the Instructions list, select AD900Filter. Choose Type and Length and click Insert.
- 4 From the Instructions list, select AutoZeroAD900 and click Insert. The defined instruction is inserted in the method.

14 Superloop







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

Superloop is an accessory available in three sizes:

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

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

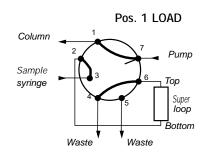
Normally, Superloop is filled manually with a syringe. However, Superloop can also be filled automatically, refer to section 10.5 Loading a Superloop on page 96.

14.1 Preparation

Prepare the injection valve and connect Superloop as follows:



- 1 Connect the supplied Union Luer female/1/16" male to port 3 of the injection valve.
- 2 Ensure that tubing for the waste is connected to port 4 of the injection valve.



- 3 Ensure that Superloop is filled with liquid (see separate Superloop Instruction).
- 4 Mount Superloop in a column holder as close to the injection valve as possible.
- 5 Connect the bottom tubing to injection valve port 2.
- 6 Connect the top tubing to injection valve port 6.
- 7 Make sure all connections are fingertight.

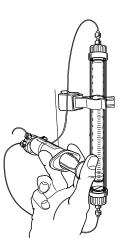
14.2 Filling Superloop

Filling Superloop is achieved as follows:

- 1 Set the injection valve to position INJECT.
- 2 Start Pump P-900 and let it run until the movable seal has reached the bottom of Superloop.
- 3 Stop Pump P-900 and set the injection valve to position LOAD.
- 4 Load a large volume syringe with sample.
- 5 Gently load the syringe contents into Superloop through port 3.
- 6 Leave the syringe in position. The loaded sample can be injected all at once or in several smaller volumes, down to 1 ml portions.
- 7 In the Method Wizard, select Manual as sample application technique. The volume to inject is set by parameter Empty Loop with.
- 8 The sample is applied to the column when the injection valve is set to position INJECT. When the required volume has been injected, set the valve to LOAD.



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



15 Configuration dependent delay volume

The system configuration delay volume must be recalculated and altered when standard configuration after the UV cell is changed to optional configuration, or vice versa. This is set in parameter System:Settings:Specials:FracParameters:DelayVol.

15.1 Standard configuration

The standard configuration of an ÄKTAexplorer system has the following delay volumes:

ÄKTAexplorer 10

Including Frac-901:

Tubing kit	Delay volume		
0.25 mm i.d. tubing kit	105 μΙ		
0.50 mm i.d. tubing kit	301 μΙ		

• Including Frac-950:

Tubing kit	Including accumulator with tubing	No accumulator	
0.25 mm i.d. tubing kit			
Standard mode	256 μΙ	105 µl	
Prep mode	773 µI	Not applicable	
0.50 mm i.d. tubing kit			
Standard mode	451 µl	301 µl	
Prep mode	969 μΙ	Not applicable	

ÄKTAexplorer 100 (includes the pH flow cell) • Including Frac-901:

Tubing kit	Delay volume		
0.50 mm i.d. tubing kit	419 µl		
0.75 mm i.d. tubing kit	773 µl		
1.0 mm i.d. tubing kit	1267 μΙ		

Including Frac-950:

Tubing kit	Including accumulator with tubing	No accumulator	
0.50 mm i.d. tubing kit			
Standard mode	570 μΙ	419 µl	
Prep mode	1087 μΙ	Not applicable	
0.75 mm i.d. tubing kit			
Standard mode	924 µl	773 µl	
Prep mode	1441 μΙ	Not applicable	
1.0 mm i.d. tubing kit			
Standard mode	1418 μΙ	1267 µl	
Prep mode	1935 μΙ	Not applicable	

15.2 Optional configuration

Calculate the contribution from each new component and capillary, according to the table below, and add this additional volume to the default value.

The example below shows a calculation for including a pH flow cell in an ÄKTAexplorer 10 system with Frac-901.

Capillary volume + cell volume = $110/100 \times 19.6 + 100 \mu l = 122 \mu l$.

The length of the additional i.d. 0.5 capillary is 110 mm, the volume of 100 mm tubing is 19.6 μ l, and the volume of the pH flow cell is 100 μ l.

The new delay volume value is $301 + 122 = 423 \mu l$.

15.3 Component and capillary volumes

The volumes are theoretical values.

Component/capillary	Volume (μl)
Valve PV-908	7
Valve IV-908	27
Valve INV-907	9
Valve FV-903	113
Flow cell UV-900, 2 mm	1
Flow cell UV-900, 10 mm	4
Conductivity flow cell	24
pH flow cell	100
Accumulator (Frac-950)	40
Union 1/16" male/ 1/16" male (0.25 mm i.d.)	1.8
Union 1/16" male/ 1/16" male (0.5 mm i.d.)	7
Flow restrictor FR-902	5
Capillary i.d. 0.25 x 100 mm	4.9
Capillary i.d. 0.50 x 100 mm	19.6
Capillary i.d. 0.75 x 100 mm	44.2
Capillary i.d. 1.0 x 100 mm	78.5

16 Strategies and Method Wizard

The table below lists the optional ÄKTAexplorer system configurations, and the additional components that are required.

System designation	Optional components installed
Air	Air sensors with control box (no control box required for AirSensorP960)
S	Sample pump and sample valve
XT	Autosampler

16.1 Strategies overview

ÄKTAexplorer system	Strategy name*
Explorer 100 with Frac-950	E100F400
Explorer 100 Air with Frac-950	ECAF400
Explorer 10 with Frac-950	E10F400
Explorer 10S with Frac-950	E10SF400
Explorer 100 with Frac-901	E100_400
Explorer 100 Air with Frac-901	ECA_400
Explorer 10 with Frac-901	E10_400
Explorer 10S with Frac-901	E10S_400

^{*} The last three digits in the strategy name is the version number.

16.2 Method Wizard

Method Wizard Configuration file: version 2.0

The following components are supported by the Method Wizard:

- Fraction collector Frac-901
- Fraction collector Frac-950
- Autosampler A-900
- Autosampler A-905
- Flow direction valve
- Column valve
- Outlet valve
- Buffer valve A1
- Sample pump P-960
- Sample pump P-950
- Sample valve
- Superloop (partly)
- Air sensor 1–3
- Air sensor P-960
- AD-900
- pH monitoring
- Conductivity monitoring

17 Accessories and consumables

17.1 Connections, chromatography - standard selection

Description and design	Connector cat. no.	Ferrule cat. no.	Connections	Tubing o.d	Material/ colour	Pressure limit [bar]
5/16" female/M6 male	18-1127-76		UNF 5/16-32 / M6		PEEK black	100
Fingertight female/M6 male	18-1112-57		UNF 10-32 /M6		PEEK black	100
Fingertight connector 1/16" male	18-1112-55		UNF 10-32	1.6 mm (1/16")	PEEK black	250
Fingertight male/M6 female	18-1112-58		UNF 10-32 / M6		PEEK black	100
Tubing connector for 3/16" o.d. tubing	18-1112-49	18-1112-48	UNF 5/16-24	4.8 mm (3/16")	PEEK black	70
Tubing connector for 1/8" o.d. tubing	18-1121-17	18-1121-18	UNF 5/16-24	3.2 mm (1/8")	PEEK black	70
Tubing connector for 1/16" o.d. tubing	18-1127-07	18-1127-06	UNF 5/16-24	1.6 mm (1/16")	PEEK black	70
Union 5/16" female/ HPLC male	18-1142-08		UNF 5/16-32 / UNF 10-32		PEEK black	70

17.2 Optional components and consumables

Item	Quantity/ pack	A/C*	Code no.
Fraction Collector Frac-950			
Fraction collector Frac-950 complete with 18 mm + 30 mm tube rack	1	А	18-6083-00
18 mm + 30 mm tube rack, complete with bowl, tube support and tube holder	1	А	18-6083-11
12 mm tube rack, complete with bowl, tube support and tube holder	1	А	18-6083-12
Microtiter plate + 30 mm tube rack, complete with bowl, tube support and tube holder	1	А	18-6083-13
30 mm tube rack (standard mode), complete with bowl, tube support and tube holder	1	А	18-6083-14
30 mm tube rack (prep mode), complete	1	Α	18-6083-15
250 ml bottles rack, complete	1	А	18-6083-16
Funnel to flask kit, complete with tubing, funnels, tubing guide and extension legs	1	Α	18-6083-17
Dispenser arm kit, complete	1	А	18-6083-18
Fraction Collector Frac-901			
Fraction Collector Frac-901, complete with 18 mm tube rack	1	А	18-1118-97
Tube racks, complete with bowl, tube support, holder and guide: 12 mm 18 mm 30 mm	1 1 1	A A A	19-8684-03 18-3050-03 18-1124-67
Tube support	1	Α	18-3054-02
Tube holder and guide: 12 mm 18 mm 30 mm	1 1 1	A A A	19-7242-02 19-8689-02 18-1124-68
Eppendorf tube holder for 12 mm rack	100	Α	18-8522-01
Flow diversion valve, FV-903, including mounting bracket	1	А	18-1114-50

Item	Quantity/ pack	A/C*	Code no.
Autosampler A-900			
Autosampler A-900	1	Α	18-1116-61
Autosampler A-900 with Cooling	1	Α	18-1144-61
Tray segment set 1.5 ml vial (o.d. 12 mm)	1	Α	18-1119-90
Tray segment set 0.5 ml vial (o.d. 7 mm)	1	Α	18-1120-48
Sample needle, stainless steel	1	Α	18-1148-15
Wash solvent bottle 250 ml	1	Α	18-1120-33
Autosampler A-905 for ÄKTA			
Autosampler A-905 for ÄKTA complete	1	Α	18-1175-93
Sample needle, fused silica, 5 µl	1	Α	18-1158-68
PEEK loop 20 μl	1	А	18-1160-49
Common autosampler accessories			
ÄKTAdesign XT upgrade kit	1	Α	18-6083-19
ÄKTA XT Tubing kit	1	Α	18-1122-92
Sample needle, PEEK, 15 µl	1	Α	18-1119-99
Buffer tubing 500 μl	1	Α	18-1120-32
Buffer tubing 2000 μl	1	Α	18-1120-31
Syringe 100 μI	1	А	18-1120-34
Syringe 250 µI	1	А	18-1120-35
Syringe 500 µl	1	А	18-1120-36
Syringe 1000 μI	1	А	18-1120-37
Luer lock connection, female	1	А	18-1120-38
PEEK loop 100 μl	1	А	18-1120-40
Syringe waste tubing	1	Α	18-1120-44
Syringe tubing	1	Α	18-1120-45

Item	Quantity/ pack	A/C*	Code no.
Valves			
Valve INV-907 including one UniNet cable (fill port, needle and syringe holder are not included)	1	А	18-1108-40
Valve IV-908 including one UniNet cable	1	Α	18-1108-42
Valve PV-908 including one UniNet cable	1	А	18-1108-41
pH measurement			
pH electrode, round tip, including flow cell and holder	1	С	18-1134-84
pH electrode, round tip	1	С	18-1111-26
pH flow cell, round tip, including dummy electrode	1	Α	18-1112-92
Dummy electrode, round tip	1	А	18-1111-03
Pump P-960			
Pump P-960 kit, including Pump P-960, UniNet cable 0.7 m holders for ÄKTAexplorer, ÄKTApurifier™, ÄKTAbasic™, and ÄKTAFPLC™, flow restrictor, purge kit, unions, connectors and tubing	1	A	18-6727-00
Sample valve kit, including Valve PV-908, UniNet cable 0.7 m unions, connectors and tubing	1	А	18-1175-86
Air sensor			
Air sensors Air-912 (1.2 mm) Air-925 (2.5 mm) Air sensor A-912N kit including air sensor, holder for ÄKTAexplorer, connectors and tubing Air-925N	1 1 1	A A A	18-1121-23 18-1121-24 18-1175-84
Air- 900 control box including pump tubing and one UniNet 2 cable	1	А	18-1121-22
Purge valve	1	А	18-1126-33

Item	Quantity/ pack	A/C*	Code no.
AD-900			
AD-900 Analog/Digital converter	1	Α	18-1148-62
Superloops			
Superloop 10 ml, 50 ml			
Superloop 10 ml, complete	1	Α	18-1113-81
Superloop 50 ml, complete	1	А	18-1113-82
Tubing kit for Superloop (10 ml)	1	А	18-1113-83
Tubing kit for Superloop (50 ml)	1	А	18-1113-84
Superloop 150 ml			
Superloop 150 ml, complete	1	А	18-1023-85
Tubing			
PEEK, blue, i.d. 0.25 mm, o.d. 1/16"	2 m		18-1120-95
PEEK, orange, i.d. 0.50 mm, o.d. 1/16"	2 m		18-1113-68
PEEK, green, i.d. 0.75 mm, o.d. 1/16"	2 m		18-1112-53
PEEK, brown, i.d. 1.0 mm, o.d. 1/16"	2 m		18-1115-83
Teflon, i.d. 1.6 mm, o.d. 1/8"	3 m		18-1121-16
Teflon, i.d. 2.9 mm, o.d. 3/16"	3 m		18-1112-47
Tefzel, i.d. 0.25 mm, o.d. 1/16"	2 m		18-1121-36
Tefzel, i.d. 0.50 mm, o.d. 1/16"	2 m		18-1120-96
Tefzel, i.d. 0.75 mm, o.d. 1/16"	2 m		18-1119-74
Tefzel, i.d. 1.0 mm, o.d. 1/16"	3 m		18-1142-38
Miscellaneous			
Extra rack panel, ÄKTAFPLC	1	А	18-1128-64
Communication cable P-920-P-50	1	А	18-1129-60

^{*)} A = accessory, C = consumable

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Short instructions

The following short instructions are checklists for users who are fully familiar with the safety precautions and operating instructions described in this manual. The instructions assume that optional equipment is installed according to the installation instructions.

Converting to optional configurations

- Wash the ÄKTAdesign flow path with distilled water.
- 2 Switch off ÄKTAdesign.
- 3 Prepare the component(s).
- 4 Attach the components to ÄKTAdesign.
- 5 Prepare the necessary capillaries and connectors.
- 6 Mount the capillaries and connectors.
- 7 Connect the components to the UniNet chain and if required to the mains power supply.
- 8 Check all connections.
- 9 Start up ÄKTAdesign.
- 10 Select the component(s) in System Setup.
- 11 Test the function of the optional equipment manually.
- 12 Create a new method with the Method Wizard and make the appropriate selections to support the optional configuration.
 - If the component is not supported by the Method Wizard, insert the required instructions in their proper positions in a new or modified method
- 13 Set up parameters to suit your needs.
- 14 Perform a test run with distilled water to verify the optional configuration in the new or modified method.
- 15 Correct any faults and store the method.

Reverting to standard configuration

- 1 Flush ÄKTAdesign with distilled water.
- 2 Disconnect the system in System Control.
- 3 Switch off ÄKTAdesign.
- 4 Disconnect equipment to be removed from the UniNet chain and reconnect the UniNet chain for the standard configuration.
- 5 Remove capillaries and reconnect the system flow path to the standard configuration.
- 6 Check all connections.
- 7 Start up ÄKTAdesign.
- 8 Select a method supporting the standard configuration.
- 9 Set up parameters to suit your needs.
- 10 Perform a test run with distilled water to verify the standard configuration in the selected method.

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