ÄKTA design

Optional Configurations
ÄKTA basic

User Manual

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Amersham Biosciences

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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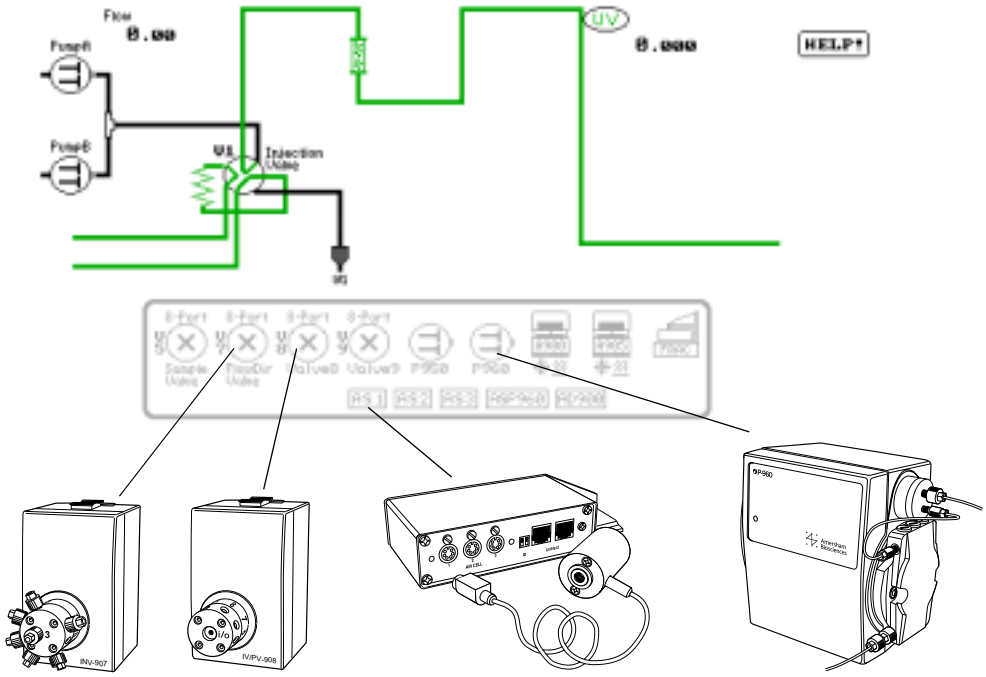
Contents

1 Introduction

1.1 General

ÄKTA™basic standard system configurations can be changed to optional configurations. This flexibility in ÄKTAbasic™ 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™ control system in the same way as ÄKTAbasic 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 ÄKTAbasic system.

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

1.3 Summary of optional configurations

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

Optional configurations supported by ÄKTAbasic 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 column selection valves.
- Connecting buffer selection valve.
- Connecting outlet valve.
- Connecting a flow direction valve.
- Connecting a sample selection valve.
- Connecting sample pump P-960.
- Connecting up to 2 motorized multi-port optional function valves. The actual use is completely user-defined.
- 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 ÄKTAbasic 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 ÄKTAbasic 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 ÄKTAbasic enables:

- Automated multiple sample injections from 1 µl up 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 ÄKTAbasic 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

- Two PV-908 rotary valves used in conjunction to switch between multiple columns. The valves are controlled synchronously by one common instruction.
- One PV-908 rotary valve used to divert between waste and seven positions; to collect flow-through, or to collect seven large fractions.
- One IV-908 rotary valve for buffer selection. Used to switch between different buffer solutions.
- 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 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.

1.3.6 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 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.

1.3.7 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.

1.3.8 AD-900

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

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

1.3.9 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 ÄKTAbasic is switched off before installing the optional components. The mains power to ÄKTAbasic 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 17 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 ÄKTAbasic optional equipment is installed correctly.

2.3 Pre-requisites

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

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

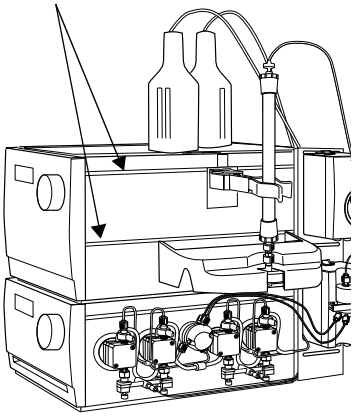
CAUTION! Make sure ÄKTAbasic is switched off before installing the optional components. The mains power to ÄKTAbasic must be switched OFF before disconnecting or connecting the UniNet-1 and UniNet-2 cables.

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.

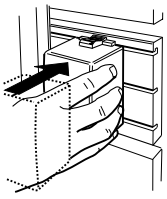
Note: Make sure that there is enough room on the side of the system for installing the desired components

Mounting rails for optional components (at opposite side too)

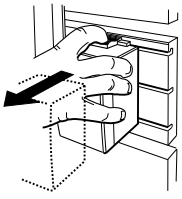


When selecting mounting location for the optional equipment it is important to choose a location which minimize the length of the capillaries used to connect the optional components to the rest of the system.

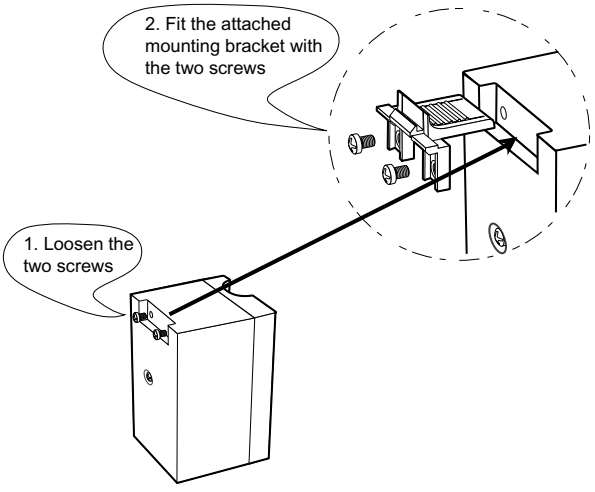
Many components that are attached to the mounting rails uses a snap-in bracket. The bracket is supplied separately with the component and needs to be fitted as shown below before the component can be attached.



Inserting components

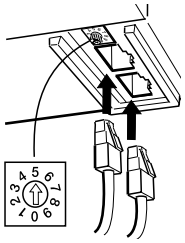


Removing components



Capillaries are connected using unions as specified in chapter 19 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:

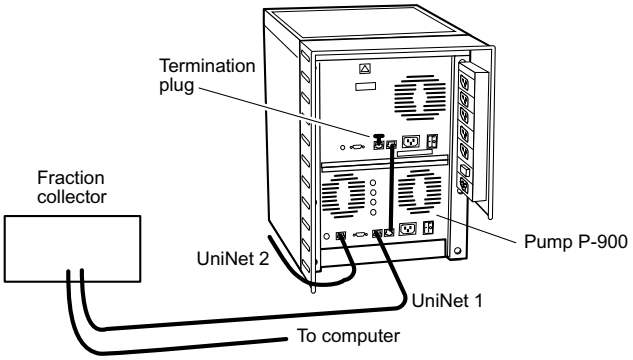
Valve function	*ID code*	*Valve type*	*Instruction in UNICORN*
Sample injection	1	INV-907	InjectionValve
Column selection	2	PV-908	ColumnPosition (combined instruction controlling both valves)
Column selection	3	PV-908	
Outlet fractionation	4	PV-908	OutletValve, OutletFractions (two instructions used 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.

* 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 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 ÄKTAbasic optional configuration system:

1 Switch on ÄKTAbasic with the mains switch located at the front left on the system base.

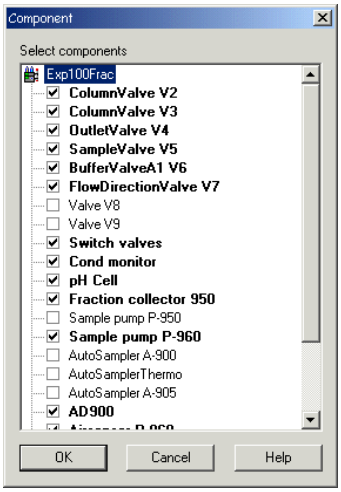
2 Check that the computer and printer are switched on.

3 Log in (see *ÄKTAbasic User Manual)*.

4 Make sure you have the correct strategy installed in your system, see 18 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 click **Edit**. Click **Component...**



7 From the **Component list**, select the optional component(s) you have installed by checking the box(es).

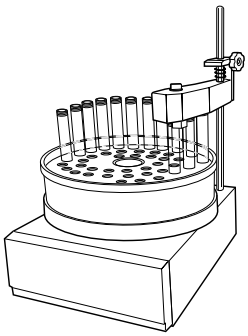
8 Click **OK** twice and then **Close**.

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 ÄKTAbasic. 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.

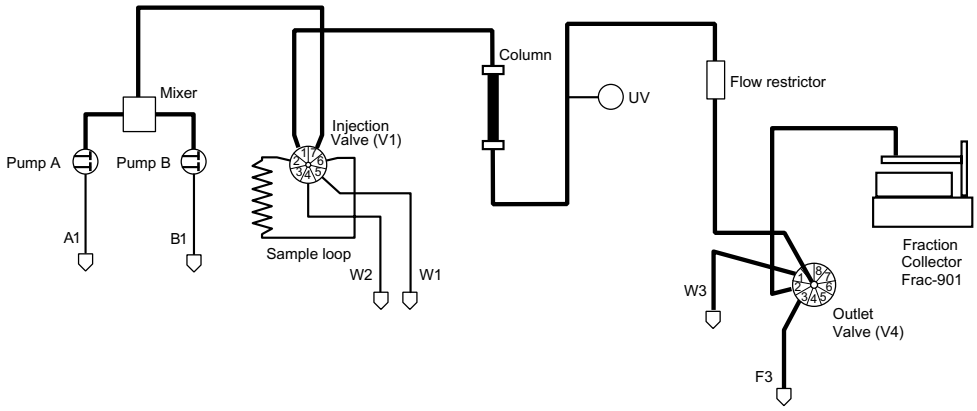
Frac-901 allows fixed volume fractionation and/or automatic peak fractionation. The latter function is based on peak detection using slope or level sensing. Fraction marks and fraction numbers make it easy to identify fractions and peaks. Fast tube change minimises spills between tubes, eliminating it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

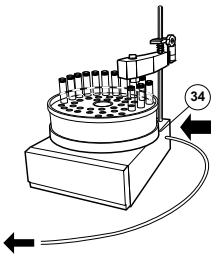
Drop synchronisation eliminates sample loss during tube change.

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.

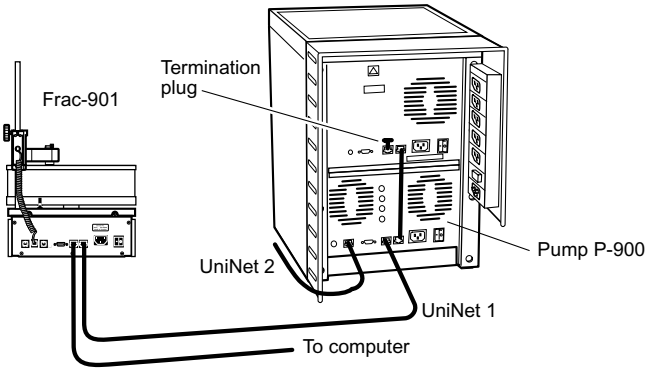




3 Connect port 2 of the outlet valve to the tube holder on the delivery arm on Frac-901. Follow the instructions given in the *Frac-900/901 User Manual*.

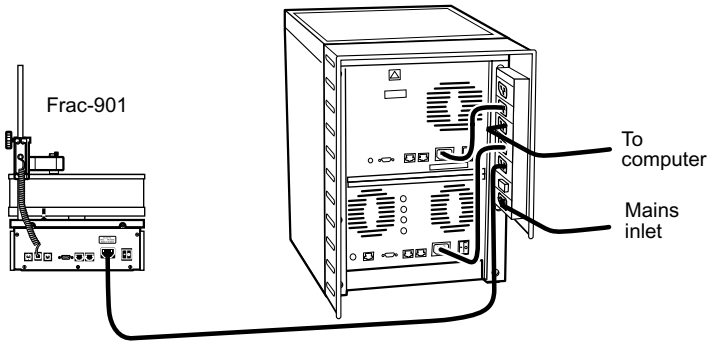
4 Extend the drain tubing from Frac-901 (34) to a suitable waste bottle.

5 Modify the UniNet-1 data communication chain by:



- 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 ÄKTAbasic.



3.3 Operation

3.3.1 Preparing for operation

1 Start your ÄKTAbasic 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 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.

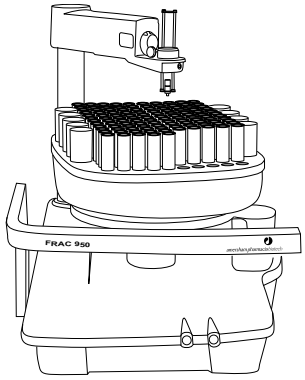
  When using the UV curve level or the AD-900 signal level, the level for starting and finishing the fractionation, fraction size and minimum peak width (if not specified a column) should be specified.

  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.

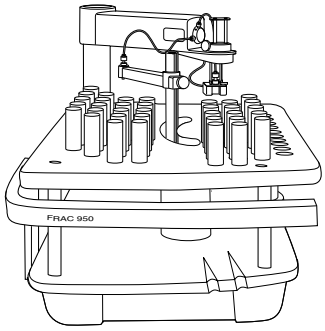
Note: When using Frac-901 in ÄKTAbasic 10 systems, we recommend a maximum flow rate of 3.5 ml/min with the i.d. 0.25 mm tubing kit. At higher flow rates, the drops will turn to a continuous liquid stream.

4 Fraction collector Frac-950

4.1 Generals



Standard mode



Prep mode

Frac-950 can be used for both small scale (standard mode) and preparative scale (prep mode) purifications with ÄKTAbasic. 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

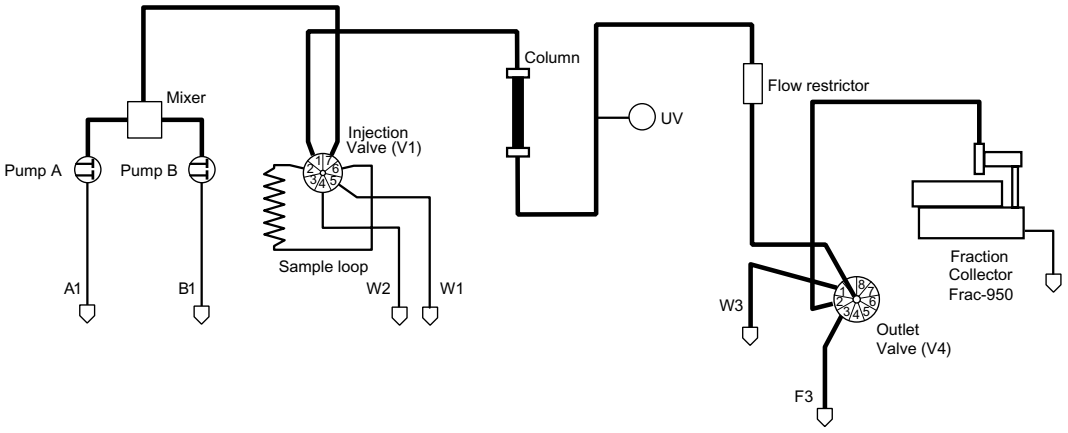
CAUTION! Make sure ÄKTAbasic is switched off before installing the optional components. The mains power to ÄKTAbasic must be switched OFF before disconnecting or connecting the UniNet-1 and UniNet-2 cables.

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 ÄKTAbasic.

3 Locate the capillary kit included with Frac-950 and select if you want to include the accumulator in Frac-950 or not.

4 Connect port 2 on the outlet valve in ÄKTAbasic to Frac-950 as described in the *Frac-950 User Manual*.



5 Connect the included waste tubing to the waste outlet on Frac-950 and route it to a suitable waste container.

6 Connect the UniNet-1 cables to Frac-950.

7 Connect the mains cable to Frac-950.

8 Check that all capillary and electrical connections are carried out correctly.

4.3 Operation

4.3.1 Preparing for operation

1 Start your ÄKTAbasic 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.

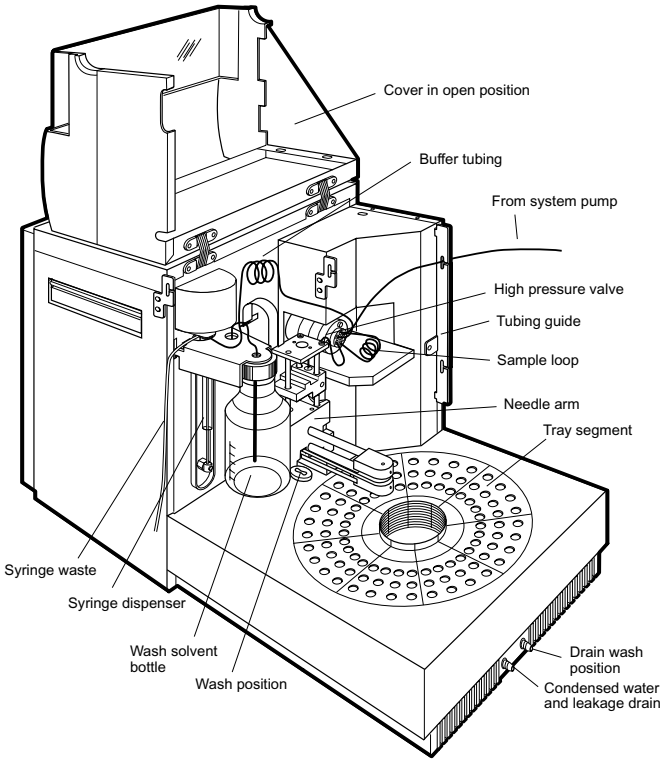
  When using the UV curve slope, the slope value for starting and finishing the fractionation, fraction size and minimum peak width (if not specified a column) should be specified.

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.

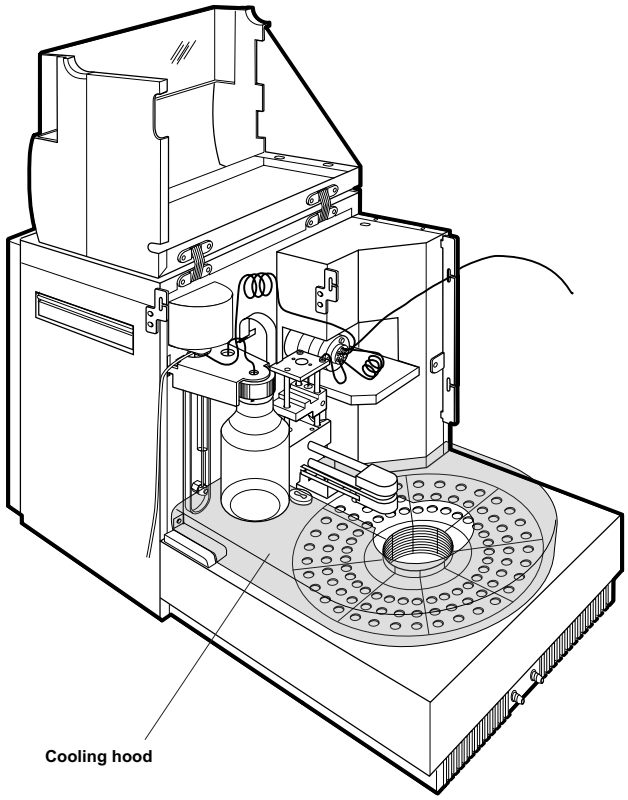
- **µl pick-up**
  After aspiration from the vial, the sample volume is transported into the loop with transport liquid (mobile phase) from another vial. This eliminates sample loss.

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.

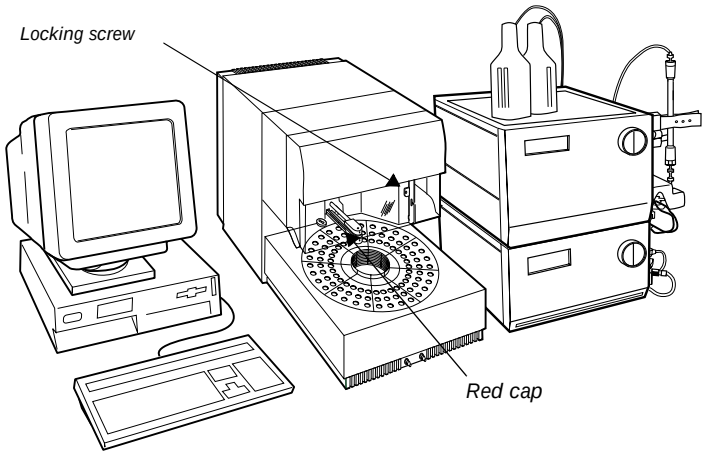


5.3 Installation

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~, 50/60 Hz, preferably taken from the ÄKTAbasic base platform.

The recommended position is to the left of the separation equipment of ÄKTAbasic, 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.

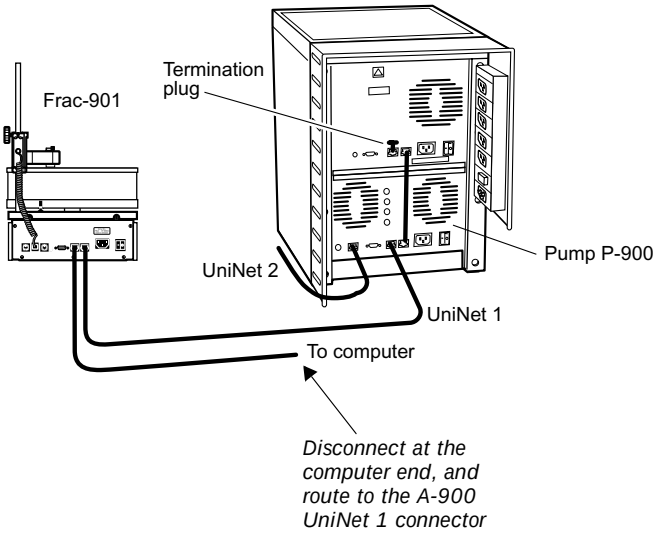
WARNING! For continued protection against risk of fire, replace only with fuses of specified type and rating. See Technical specifications in the A-900 User Manual for fuse data.

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

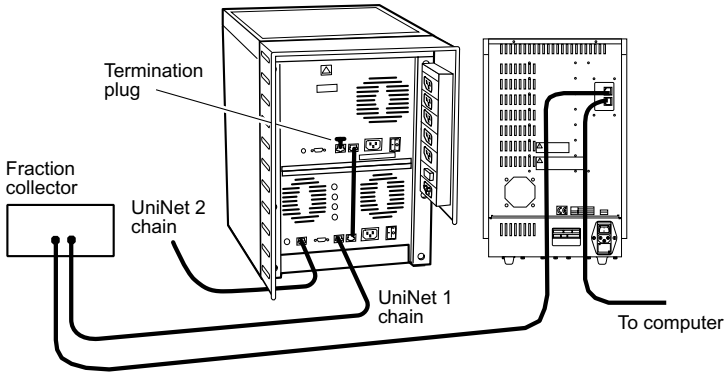
1 Make sure that the ÄKTAbasic chromatography system is switched off.

2 Disconnect the UniNet-1 cable running between P-900 and the computer, in the computer end.

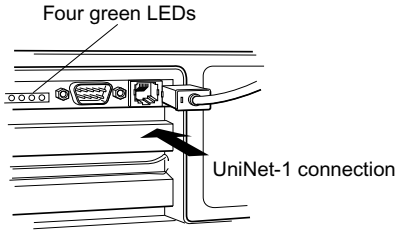
Note: If fraction collector Frac-901 or Frac-950 is used, disconnect the UniNet-1 cable running between Frac-901/ Frac-950 and the computer.



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:

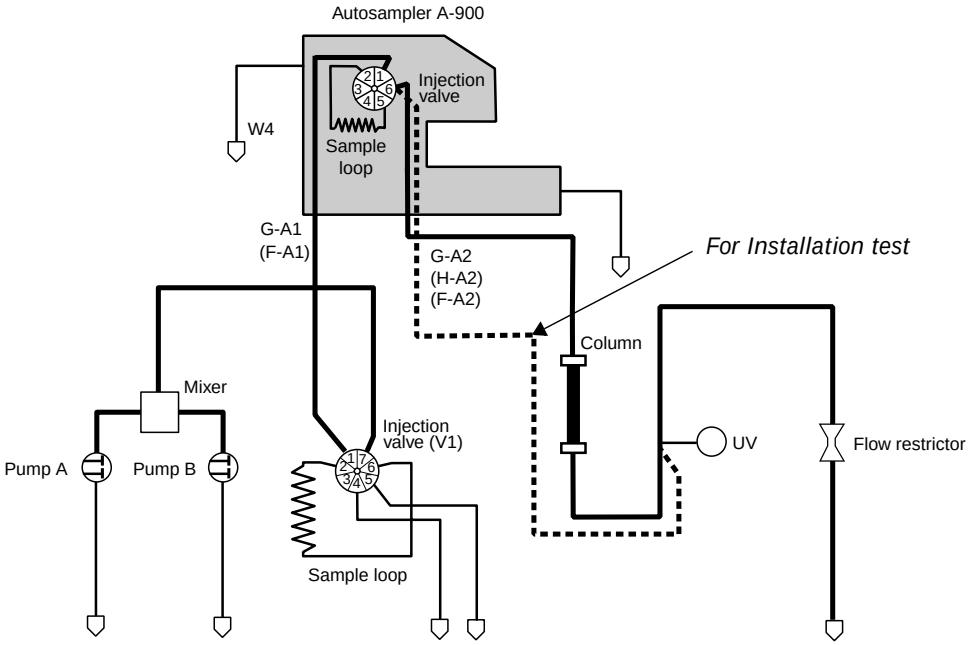
- For 110-120 VAC, use two 5 AT-fuses (slow).
- For 220-240 VAC, use two 2.5 AT-fuses (slow).

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

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 ÄKTAbasic separation unit.

5.3.3 Capillary connections

The capillaries to be connected between A-900 and the ÄKTAbasic 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 ÄKTAbasic flow path.



1 Remove the capillary between the injection valve V1 and the column top inlet.

2 Select the capillaries matching the tubing kit to be used in ÄKTAbasic 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 tubings.

Note: See ÄKTAbasic User Manual for general recommendations on selecting column and tubing kit.

3 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 ÄKTAbasic injection valve V1.

4 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 UV flow cell inlet.

Note: This connection (the dotted line) is only temporary. When the installation test is successfully performed, G-A2/ H-A2/ F-A2) is connected to the top inlet of the column, and the capillary connecting the column outlet and the UV flow cell inlet is refitted.

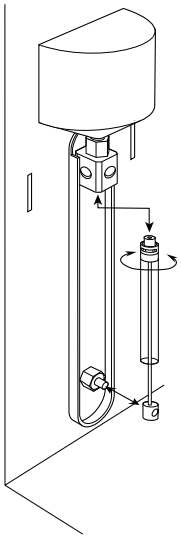
5 Check additional internal and waste tubing connections on A-900 according to the A-900 User Manual instructions.

5.4 General preparation

1 Start your ÄKTAbasic 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 µl), syringe volume (250 µl) and needle height (2 mm) are selected.



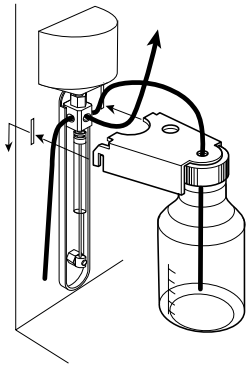
4 Manually, run the **SyringeEnd** instruction under **System Control:Manual:Flowpath:AutosamplerControl.**

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.).

5.5 Installation test

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

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

5.5.1 Preparation

1 Verify that the Pump P-900 is primed and purged according to the instructions in the *ÄKTAbasic User Manual*.

2 Prepare a flask containing 500 ml of 0.4% acetone in water solution.

3 Fill two standard vials with this solution and seal them properly with the correct type of cap or septum.

4 Put one vial in position 1, and the other vial in position 2 in a standard tray.

5 Immerse the inlet tubing B into the rest of the 0.4% acetone solution.

6 Immerse the inlet tubing A into a flask containing distilled water.

5.5.2 Starting the test

1 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.

2 Click the **Instant Run** button  .

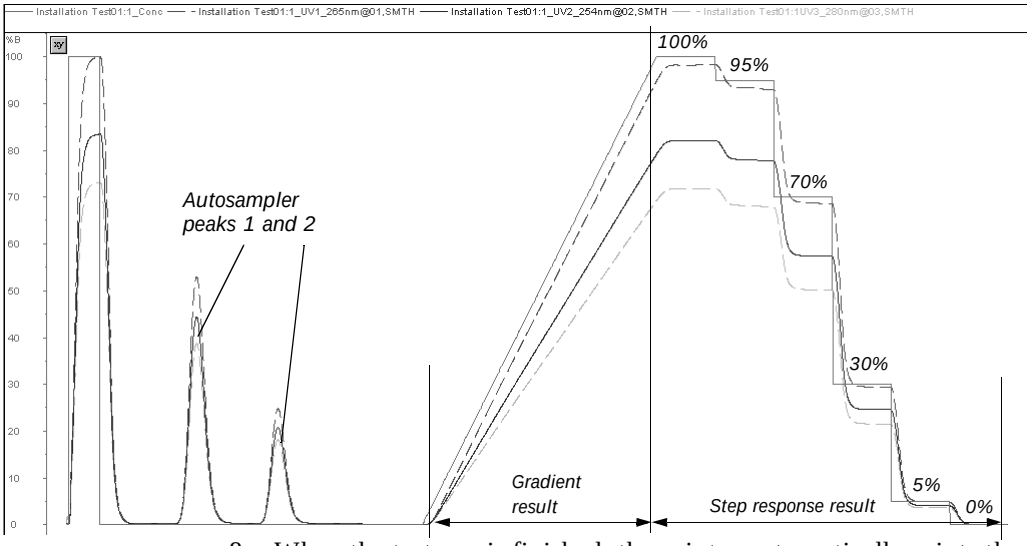
3 In the **InstantRun** window, select system and click **Run**.

4 In the Method Wizard, select **Installation_Test** in the **Main Selection** menu. Click **Run**.

5 In **Evaluation Procedures**, select **InstTest_Basic10_A90X**. Click **Next** twice.

6 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 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 

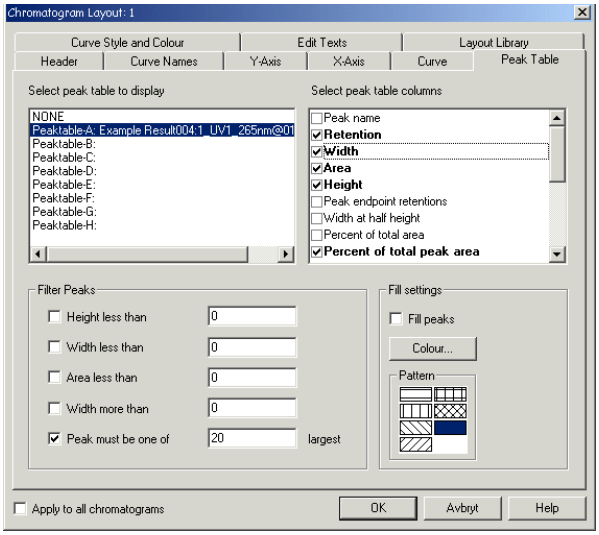
in the results panel and then double-click on the **Wizard Generated 001** icon to open the result file.

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 GeneratedOO1:1_UV1_265nm@01,SMTH**
- **Wizard GeneratedOO1:1_UV2_254nm@02,SMTH**
- **Wizard GeneratedOO1:1_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**.

7 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 GeneratedOO1:1_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 GeneratedOO1:1_UV2_254nm@02,SMTH**
- **Wizard GeneratedOO1:1_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 GeneratedOO1:1_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 GeneratedOO1:1_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

1 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)	*2 Value read 100%B 0% 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 **System 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 **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

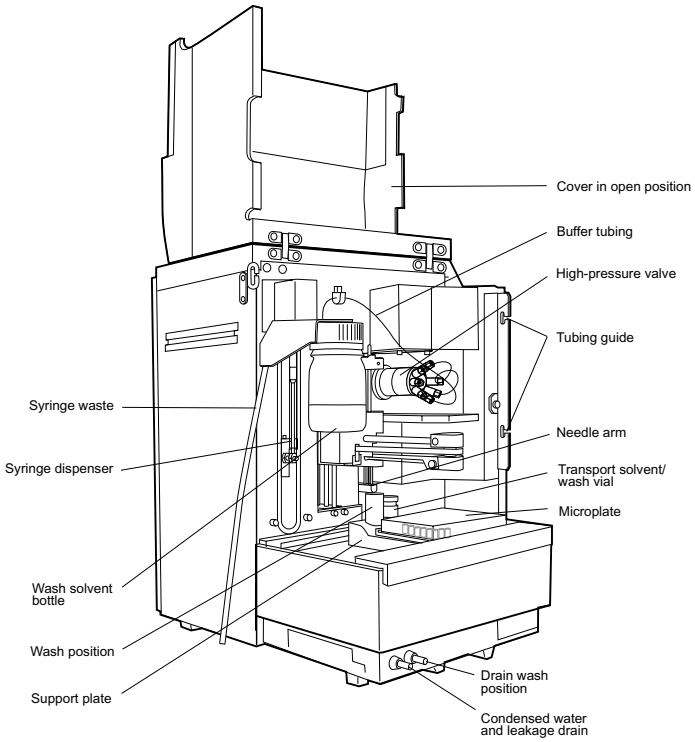
The **Warning_AutoSampler** parameter is used to set warning temperatures when using the cooling function.

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 1.0 µl.

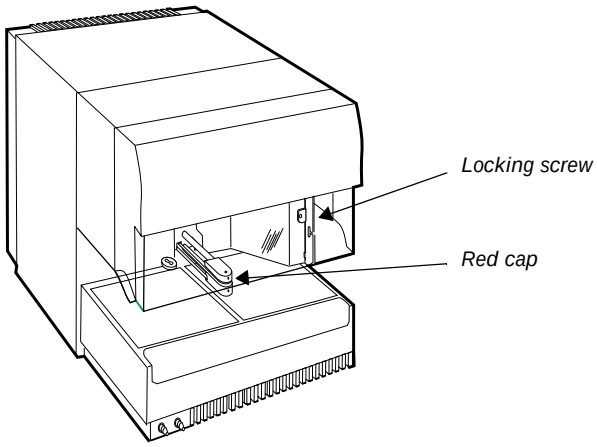


6.2 Installation

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 ÄKTAbasic base platform.

The recommended position is to the left of the separation equipment of ÄKTAbasic, 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.

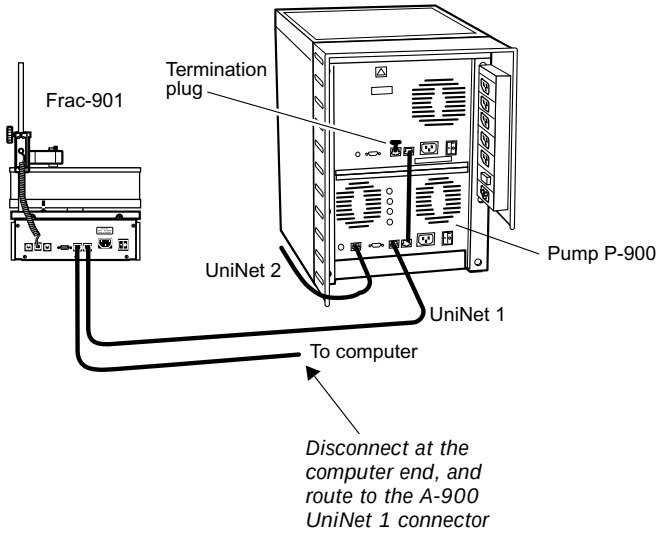
WARNING! For continued protection against risk of fire, replace only with fuses of specified type and rating. See Technical specifications in the A-905 User Manual for fuse data.

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

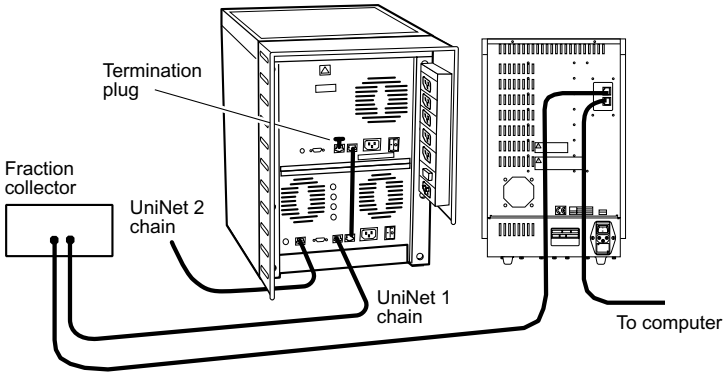
1 Make sure that the ÄKTAbasic chromatography system is switched off.

2 Disconnect the UniNet-1 cable running between P-900 and the computer, in the computer end.

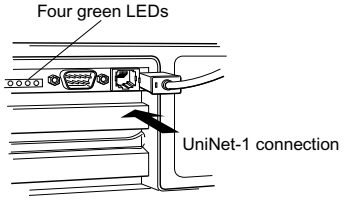
Note: If fraction collector Frac-901 or Frac-950 is used, disconnect the UniNet-1 cable running between Frac-901/ Frac-950 and the computer.



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).

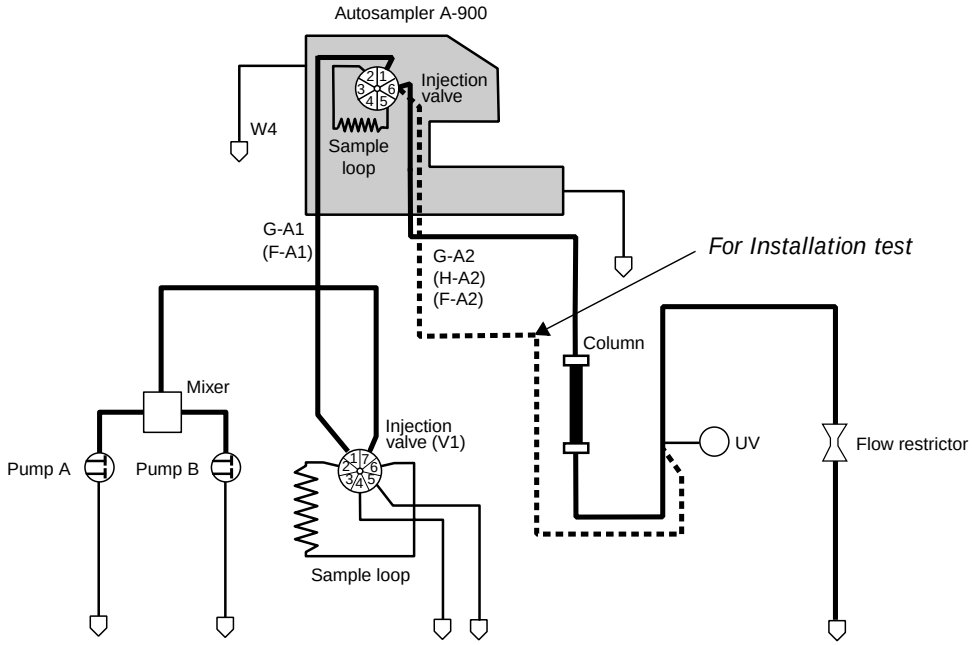
CAUTION! DO NOT switch on any of the equipment yet!

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 ÄKTAbasic separation unit.

6.2.3 Capillary connections

The capillaries to be connected between A-905 and the ÄKTAbasic 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 ÄKTAbasic flow path.



1 Remove the capillary between the injection valve V1 and the column top inlet.

2 Select the capillaries matching the tubing kit to be used in ÄKTAbasic 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 tubings.

Note: See ÄKTAbasic User Manual for general recommendations on selecting column and tubing kit.

3 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 ÄKTAbasic injection valve V1.

4 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 UV flow cell inlet.

Note: This connection (the dotted line) is only temporary. When the installation test is successfully performed, G-A2/ H-A2/ F-A2) is connected to the top inlet of the column, and the capillary connecting the column outlet and the UV flow cell inlet is refitted.

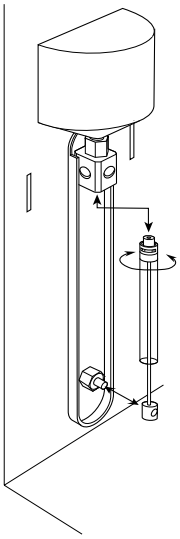
5 Check additional internal and waste tubing connections on A-905 according to the A-905 User Manual instructions.

6.3 General preparation

1 Start your ÄKTAbasic 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.



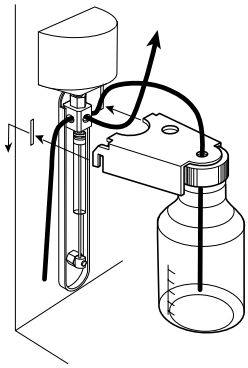
4 Run the **AutosamplerControl_905:SyringeEnd_905** command in **Manual:Flowpath.**

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-900

Refer to the *Autosampler A-905 for ÄKTA User Manual*.

6.4 Installation test

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

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

6.4.1 Preparation

1 Verify that the Pump P-900 is primed and purged according to the instructions in the *ÄKTAbasic User Manual.*

2 Prepare a flask containing 500 ml of 0.4% acetone in distilled water.

3 Prepare a flask containing 500 ml of distilled water.

4 Fill A1 and A2 in a 96-well low microplate with the 0.4% acetone solution.

5 Seal the microplate with a foil or a cap mat.

6 Immerse the inlet tubing B into the rest of the 0.4% acetone solution.

7 Immerse the inlet tubing A into the distilled water flask.

6.4.2 Starting the test

1 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.

2 Click the **Instant Run** button  .

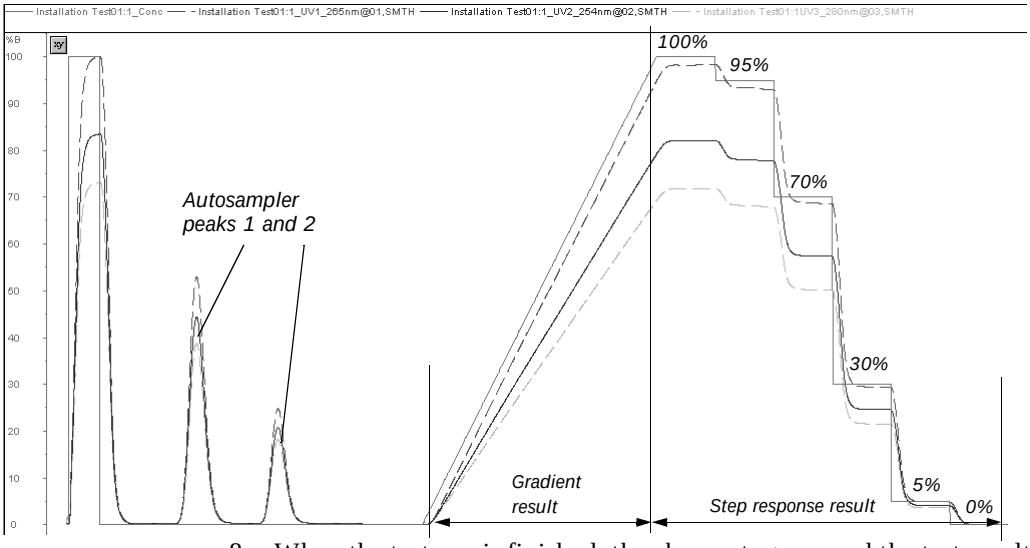
3 In the **InstantRun** window, select system and click **Run**.

4 In the **Method Wizard**, select **Installation_Test** in the **Main Selection** menu. Click **Run**.

5 In **Evaluation Procedures**, select **InstTest_Basic10_A90X**. Click **Next** twice.

6 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 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.

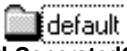
- If the gradient test result is OK, the print-out says "Gradient linearity accepted".
- If the step response test result is OK, the print-out says "Step response accepted".
- If the UV response test result is OK, the print-out says "UV response accepted".
- If the Autosampler peaks test result is OK,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

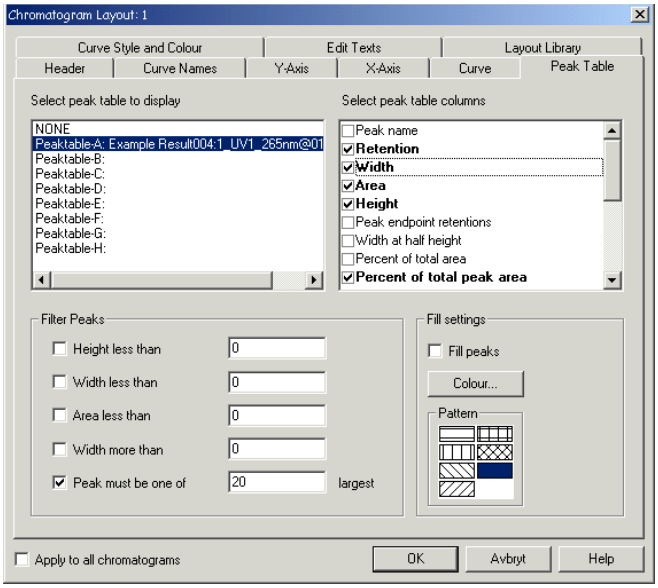
in the results panel and then double-click on the **Wizard GeneratedOO1** icon to open the result file.

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

4 Click the **Curves** tab and select the following curves:

- **Wizard GeneratedOO1:1_UV1_265nm@01,SMTH**
- **Wizard GeneratedOO1:1_UV2_254nm@02,SMTH**
- **Wizard GeneratedOO1:1_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**.

7 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 GeneratedOO1:1_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 GeneratedOO1:1_UV2_254nm@02,SMTH**
- **Wizard GeneratedOO1:1_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 GeneratedOO1:1_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 GeneratedOO1:1_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

1 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)	*2 Value read 100%B*	*0% 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 1.0 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.

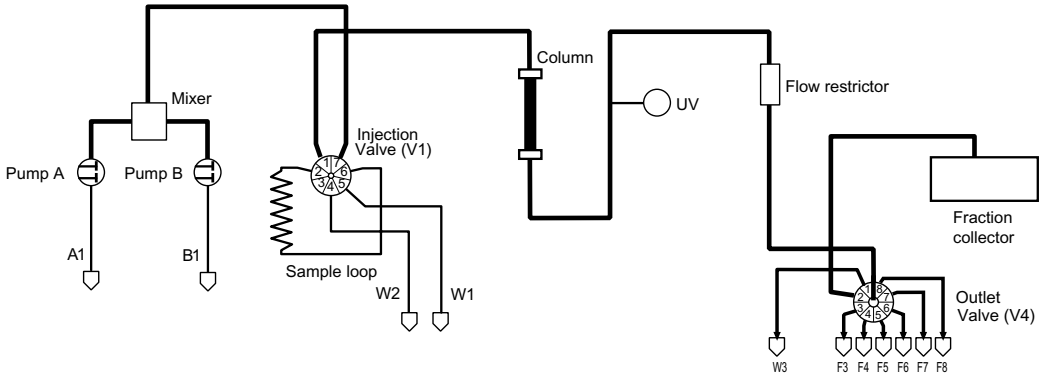
- **µl pick-up**
  After aspiration from the vial, the sample volume is transported into the loop with transport liquid (mobile phase) from another vial. This eliminates sample loss.

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 Outlet valve

7.1 Installation



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

1 Mark the PV-908 valve with **4**. Check that the ID code switch is set to **4**.

2 Mount the bracket and attach the valve to a mounting rail.

3 Cut and mount the new capillaries. Use a capillary with the correct i.d. as required by your system.

4 Use the attached UniNet-2 cable 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 connected to the injection valve.

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

7.2 Operation

7.2.1 Preparing for operation

1 Start your ÄKTAbasic optional configuration system as described in section 2.6 Preparation for use. Make sure that **OutletValve V4** is selected in the **Component** list.

2 The outlet valve is automatically recognized by UNICORN at system start-up. Check that the valve added is indicated in the flow scheme in UNICORN.

3 Verify that the outlet valve is functioning properly by issuing manual commands from UNICORN as follows:

- In **System Control**, select **Manual:FlowPath**.
- Select instruction **OutletValve.**
- Set the outlet valve to position **F4**.
- Manually, run Pump P-900 with distilled water at a flow rate of approximately 2 ml/min and check that water is coming out from port 4 of the outlet valve.

7.2.2 Using the outlet valve for fractionation

To use the outlet valve in a method, select the desired outlet valve options in the **Wash Out Unbound Sample** and/or **Elution Fractionation** dialogs when creating the method in the Method Wizard .

Two different fractionation instructions are available to use with the outlet valve:

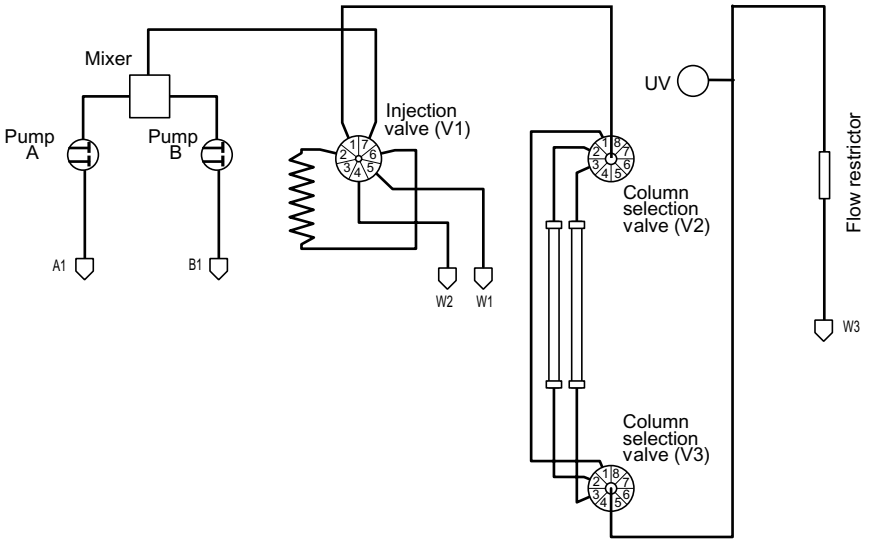
- **OutletValve**
  This instruction parameter turns the outlet valve to one of the eight positions available. This makes the instruction suitable for controlling flow-through fractionation before elution starts. The instruction is designated **V4_Outlet** in the run data window.

- **OutletFractions**
  This instruction has three parameters, **How Many?**, **Volume** and **Start at**. This makes the instruction suitable for controlling large volume fractionation (min. 5 ml) during elution. Both instructions are independent of using Fraction collector Frac-901 or Frac-950.

Note: Installing an outlet valve in the flow path affects the system delay volume. See section *17 Configuration dependent delay volume for instructions on how to re-calculate and change the delay volume.*

8 Column selection valves

8.1 Installation



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

1 Mark the two PV-908 valves with **2** and **3**. Check that the ID code switch is set to **2** and **3** respectively.

2 Mount the brackets and attach the two valves to a system mounting rail.

3 Cut and mount the required PEEK capillaries. Use a capillary with the correct i.d. as required by your system.
Mount one capillary between port 1 on column valve 2 and port 1 column valve 3. This is the column bypass position used as default position for the valves in UNICORN.

4 Use the attached UniNet-2 cables to connect the valves to the UniNet-2 communication link. The valves can be connected anywhere between the system pump P-900 and the termination plug connected to the injection valve.

5 Check that all capillary and electrical connections are carried out correctly.

8.2 Operation

Preparing for operation

1 Start your ÄKTAbasic optional configuration system as described in 2.6 Preparation for use. Make sure that **ColumnValve V2** and **ColumnValve V3** are selected in the **Component** list.

2 The column selection valves are automatically recognised by UNICORN at system start-up. Check that the valves added are indicated in Flow scheme in UNICORN.

3 Verify that the column selection valves are functioning properly by issuing manual commands from UNICORN as follows:

- In **System Control**, select **Manual:FlowPath**.
- Select instruction **ColumnPosition**.
- Set **ColumnPosition** to **Position4**.
- Manually, run the pump P-900 with distilled water at a flow rate of approximately 2 ml/min and check that water is coming out from port 4 of column valve 2.
- Connect a capillary between port 4 of column valve 2 and port 4 of column valve 3 and check that water is coming out from the center port of column valve 3.

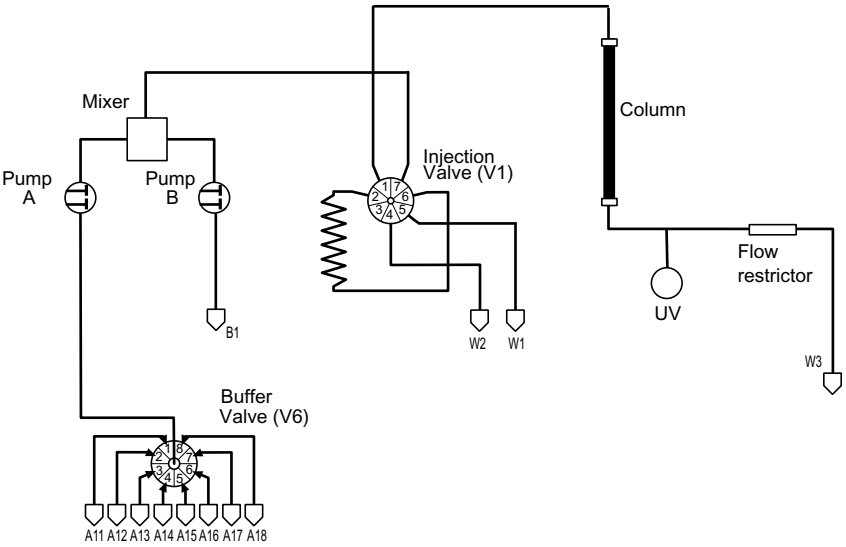
Using the column selection function in a run

To use the column selection valves in a method, select the desired valve option in the Method Wizard when creating the new method.

The column selection valves are designated **ColumnValves** in the flow scheme and **V2_ColPos** and **V3_ColPos** in the run data window.

9 Buffer selection valves

9.1 Installation



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

1 Mark the IV-908 valve with **A**. Check that the ID code switch is set to **6**.

2 Mount the brackets and attach the valve to the system rack.

3 Cut and mount the required teflon tubings (i.d. 1.6 mm in ÄKTAbasic 10 and i.d. 2.9 mm in ÄKTAbasic 100).

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 connected to the outlet valve.

5 Check that all capillary and electrical connections are carried out correctly.

9.2 Operation

9.2.1 Preparing for operation

1 Start your ÄKTAbasic optional configuration system as described in 2.6 Preparation for use. Make sure that **BufferValveA1 V6** is selected in the **Component** list.

2 The buffer selection valve is automatically recognised by UNICORN at system start-up. Check that the valve added are indicated in the Flow scheme in UNICORN.

3 Verify that BufferValve A1 is functioning properly by issuing manual commands from UNICORN as follows:

- In **System Control,** select **Manual:FlowPath**.
- Select instruction **BufferValveA1**.
- Set **BufferValveA1** to position **A14**.
- Manually, run pump P-900 with distilled water at a flow rate of approximately 2 ml/min and check that water is drawn through port 4 in BufferValve A.

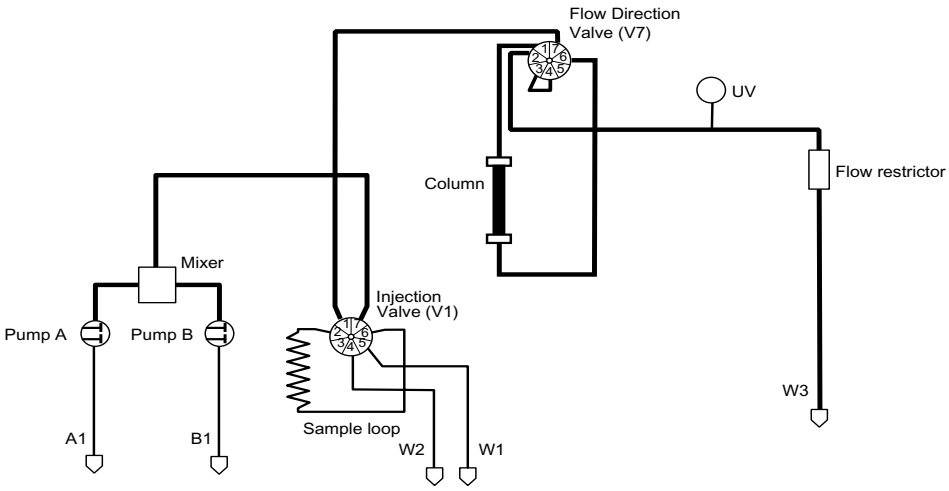
Using the buffer selection function in a run

To use the buffer selection valve in a method, select the desired valve options in the Method Wizard when creating the method.

The buffer valve is designated **BufferValveA1** in the flow scheme. In the run data window, it is designated **V6_BuffA1**.

10 Flow direction valve

10.1 Installation



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

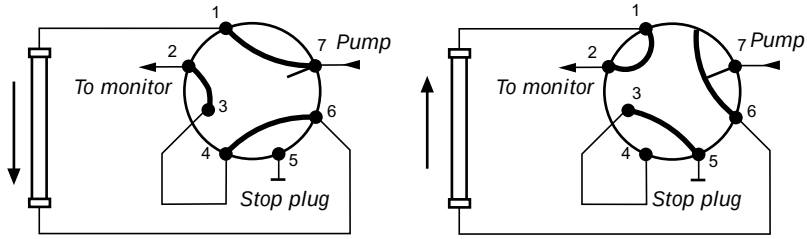
1 Mark the INV-907 valve with **V7**. Check that the ID code switch is set to **7**.

2 Attach the valve to a system mounting rail. 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.

10.2 Operation

10.2.1 Preparing for operation

1 Start your ÄKTAbasic 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.

10.2.2 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.

11 General function valve

11.1 Installation

To install any optional valve, proceed as follows:

CAUTION! Make sure ÄKTAbasic is switched off before installing the optional components. The mains power to ÄKTAbasic 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.

11.2 Operation

11.2.1 Preparing for operation

1 Start your ÄKTAbasic 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).

11.2.2 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.

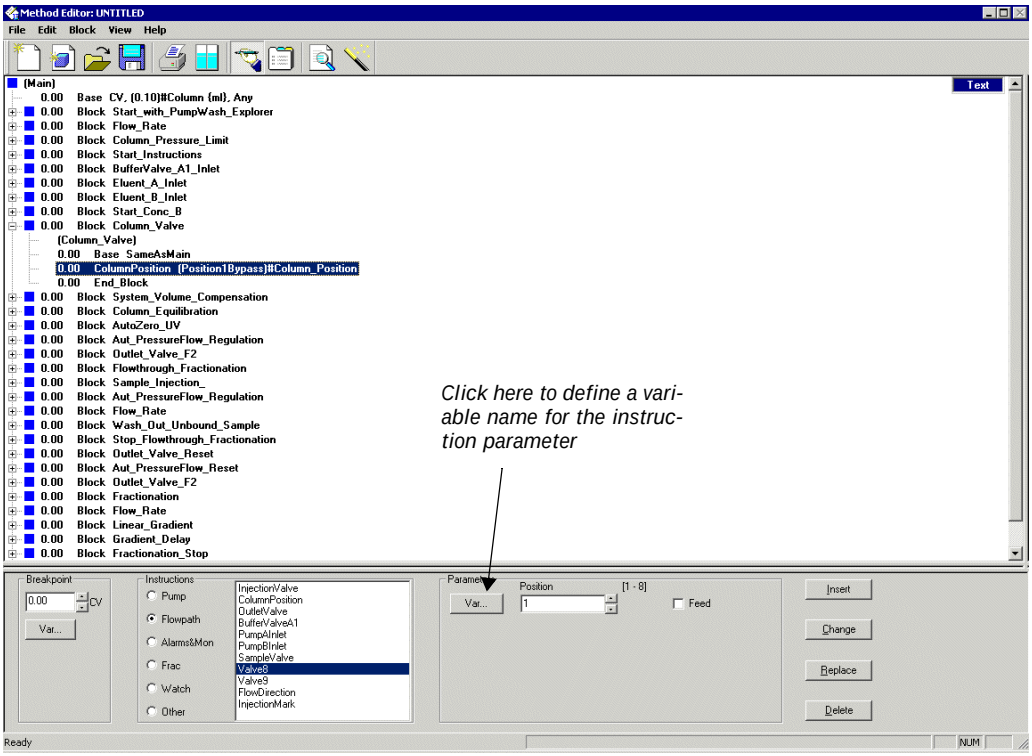
To use a valve in a method:

1 Create a new method by selecting **File:Method Wizard** in the **Method Editor.**

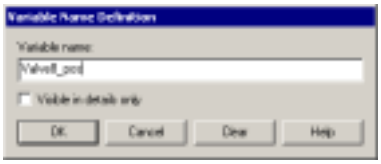
2 Select **View:Text instructions** to display the text instruction editor. Double-click on the instruction block where you want to add the general function valve instruction. Highlight the instruction below which you want to insert the valve instruction.

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.

7 Return to the **Variables** page by selecting **View:Run setup** to set the variable **Valvex_pos** to the desired position.

8 Save the method.

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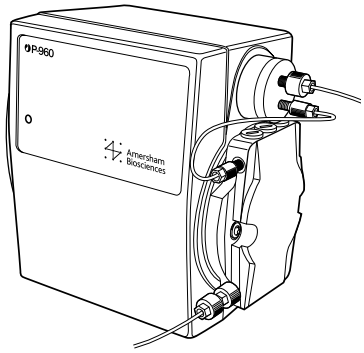
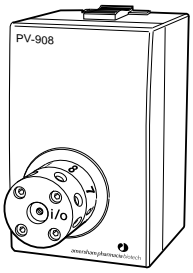
12 Sample pump P-960 and sample valve V5

12.1 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.

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

- Loading a sample loop.
- Direct loading onto the column
- 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 ÄKTAbasic 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

12.2 Installing the components

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

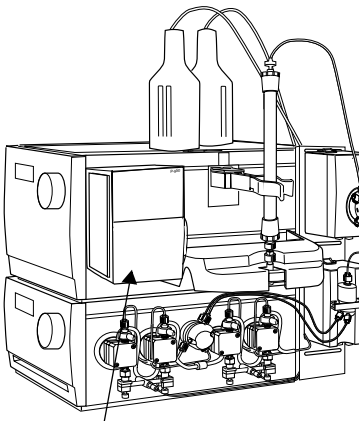
This section describes how to install the components in the system. To connect the capillaries, see the section describing that specific sample application method—*Loading a sample loop, Direct loading onto the column* or *Loading a Superloop*.

12.2.1 Installing Pump P-960

1 Make sure that the ÄKTAbasic chromatography system is switched off.

2 Install Pump P-960 in the separation unit of ÄKTAbasic.

- Locate the fixing screws and the clamp supplied with the pump.
- Fit the pump using the clamp and the screws.

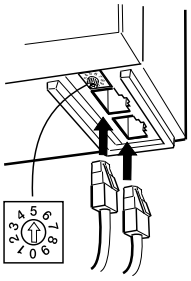


Pump P-950

12.2.2 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.



12.2.3 Installing UniNet-2 cables

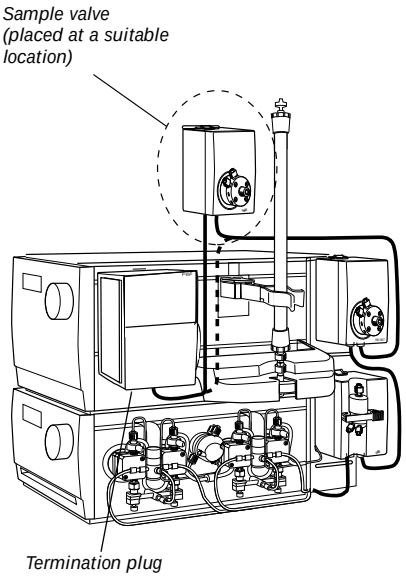
Connecting P-960 and the sample valve V5

1 Remove the termination plug from injection valve V1.

2 Connect a new cable (supplied) between the injection valve V1 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 injection valve V1.

2 Connect a new cable (supplied) between V1 and Pump P-960 (the leftmost socket).

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

12.2.4 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 12-1). 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

* Use a 10 cm long tubing between the flow restrictor and the port.

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

Table 12-1. Position of the flow restrictor

12.3 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.

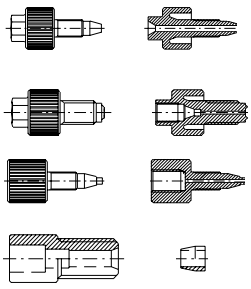
Note: The flow rate should not exceed 0.5 ml/min when filling the sample loop. Otherwise, cavitation might occur due to the resistance in the flow path.

12.3.1 Connecting the capillaries

1 Make sure that the sample pump and the sample valve (if included) are properly installed according to section 12.2 Installing the components.

2 Cut the capillaries to suitable lengths and connect them using connectors and ferrules as indicated in the table and in the figures. The lengths in the table are maximum lengths.

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



Fingertight connector 1/16"

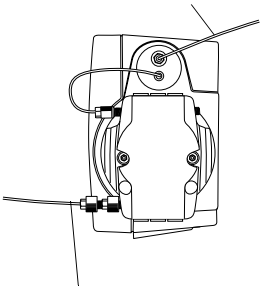
Union fingertight female/ M6 male

Union 5/16" female/ HPLC male

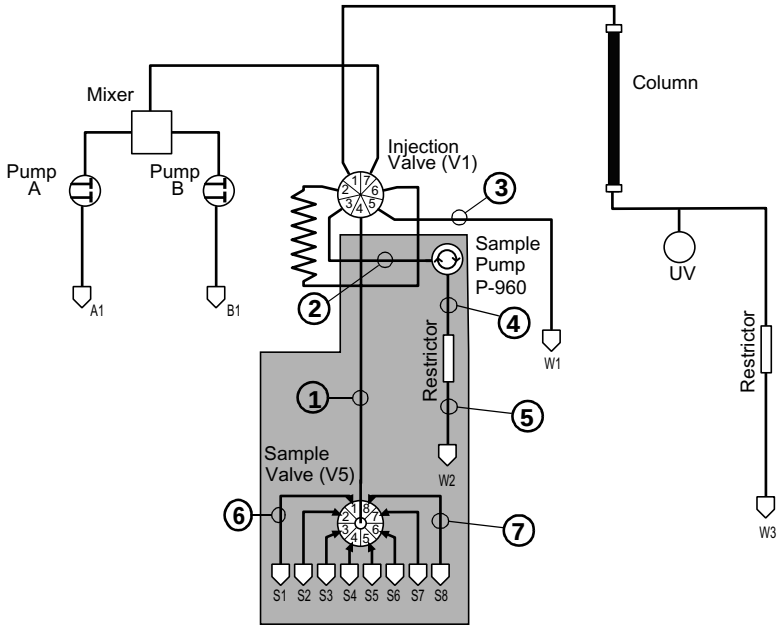
Tubing connector for 1/8" o.d. tubing with ferrule for 1/8" o.d. tubing

Pump P-960

Outlet connection



Inlet connection



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 50 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 10 cm	Fingertight connector 1/16", 18-1112-55	Sample pump, outlet
	Fingertight connector 1/16", 18-1112-55	Flow restrictor, inlet port
5 Tefzel i.d. 1.0 mm x 120 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, inlet 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 the non-used ports in the sample valve.

12.3.2 Preparing for operation

1 Start your ÄKTAbasic optional configuration system as described in 2.6 Preparation for use. Make sure that **Sample pump P-960** and (if required) **Sample Valve 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 12.3.1 Connecting the capillaries.

12.3.3 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 12.3.5.

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.

12.3.4 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 12.3.5 Removing air by using a purge tubing.

12.3.5 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:

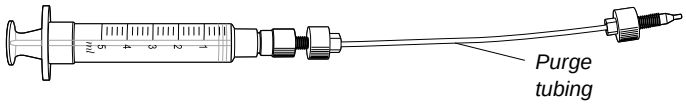
1 Put the chosen sample inlet tubings from port 1–7 in sample valve V5 into the sample tubes.

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 12.3.4 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 12.3.4.

8 Gently move the sample inlet tubing to the sample vessel.

12.3.6 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.

12.3.7 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.

12.4 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.

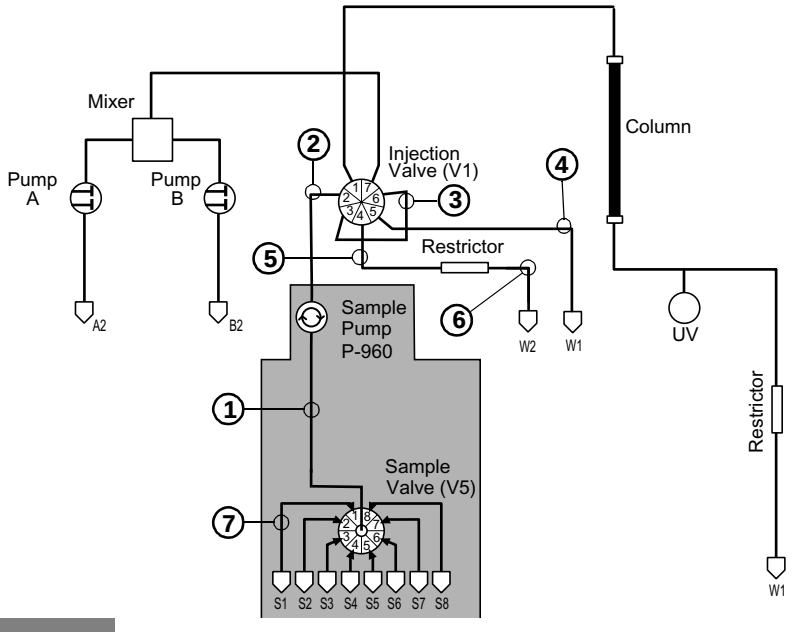
We particularly recommend decreasing the flow rate of the system pump during equilibration, before sample application, and before switching the sample pump in-line. The sample pump will not start if the back-pressure exceeds 2.0 MPa.

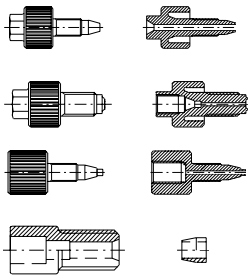
12.4.1 Connecting the capillaries

1 Make sure that the components are properly installed according to section 12.2 Installing the components.

2 Cut the capillaries to suitable lengths and connect them using connectors and ferrules as indicated in the table and in the figures. Those lengths are maximum values.

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





Fingertight connector 1/16"

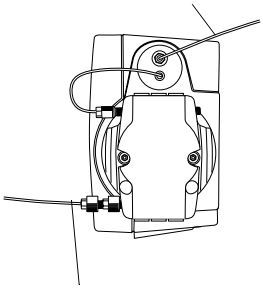
Union fingertight female/ M6 male

Union 5/16" female/ HPLC male

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

Pump P-960

Outlet connection



Inlet connection

Capillary (max. length)	***Connector***	***Connection points***
1 Tefzel i.d. 1.0 mm x 50 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.

12.4.2 Preparing for operation

1 Start your ÄKTAbasic optional configuration system as described in 2.6 Preparation for use. Make sure that **Sample pump P-960** and (if required) **Sample Valve 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 12.4.1 Connecting the capillaries.

12.4.3 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 12.4.5.

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.

12.4.4 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 12.4.5 Removing air by using a purge tubing.

12.4.5 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:

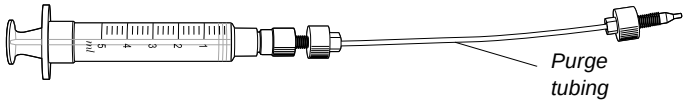
1 Put the chosen sample inlet tubings from port 1–7 in sample valve V5 into the sample tubes.

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 12.4.4 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 12.4.4 Removing air by running the sample pump.

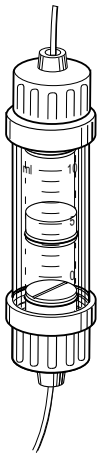
8 Gently move the sample inlet tubing to the sample vessel.

12.4.6 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 propertiess 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.



12.5 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.

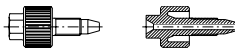
When loading a Superloop, a sample valve is usually not used.

12.5.1 Connecting the capillaries

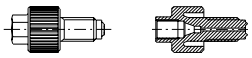
1 Make sure that the sample pump is properly installed according to section 12.2 Installing the components.

2 Cut the capillaries to suitable lengths and connect them using connectors and ferrules as indicated in the table and in the figures. The lengths are maximum values.

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



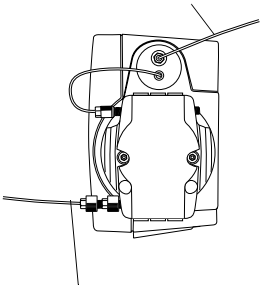
Fingertight connector 1/16"



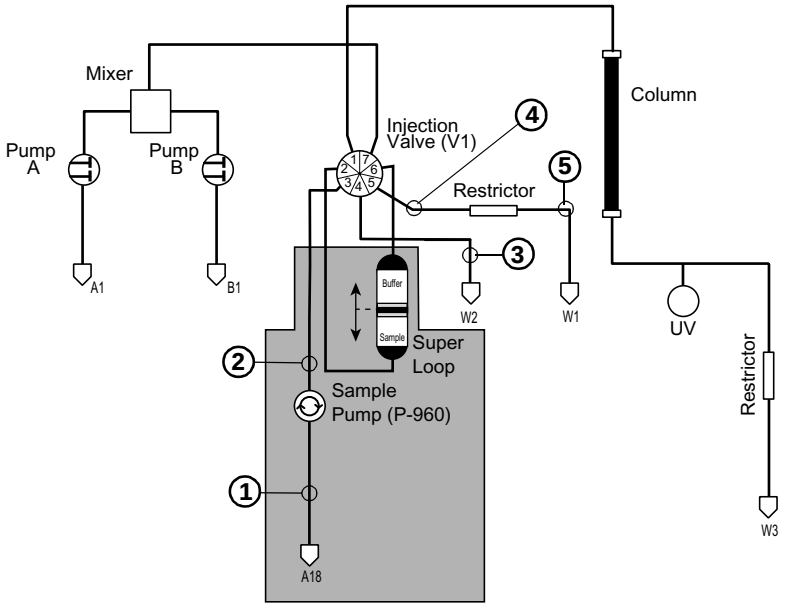
Union fingertight female/ M6 male

Pump P-960

Outlet connection



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

12.5.2 Preparing for operation

1 Start your ÄKTAbasic optional configuration system as described in 2.6 Preparation for use. Make sure that **Sample pump P-960** is selected in the **Component** list.

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 12.5.1 Connecting the capillaries.

12.5.3 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 12.5.5.

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.

12.5.4 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 12.3.5 Removing air by using a purge tubing.

12.5.5 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.

7 Flush the sample inlet tubing with buffer to remove any trapped air bubbles according to the procedure in section 12.3.4 Removing air by running the sample pump.

8 Gently move the sample inlet tubing to the sample vessel.

12.5.6 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 properties are designated **SampleFlow_960** and **P960_Press** in the flow scheme and run data windows.

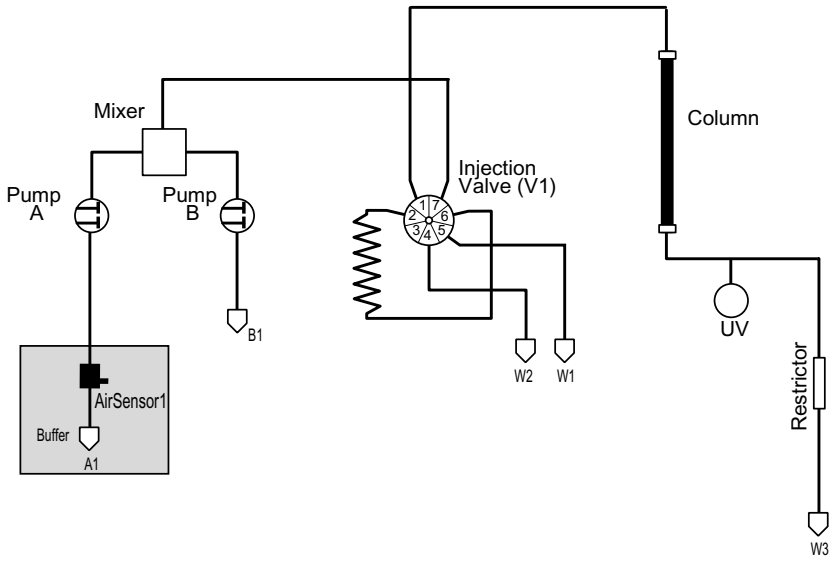
WARNING! Make sure that the upper pressure limit of the system is lower than the maximum allowed pressure of the Superloop.

13 Air sensors

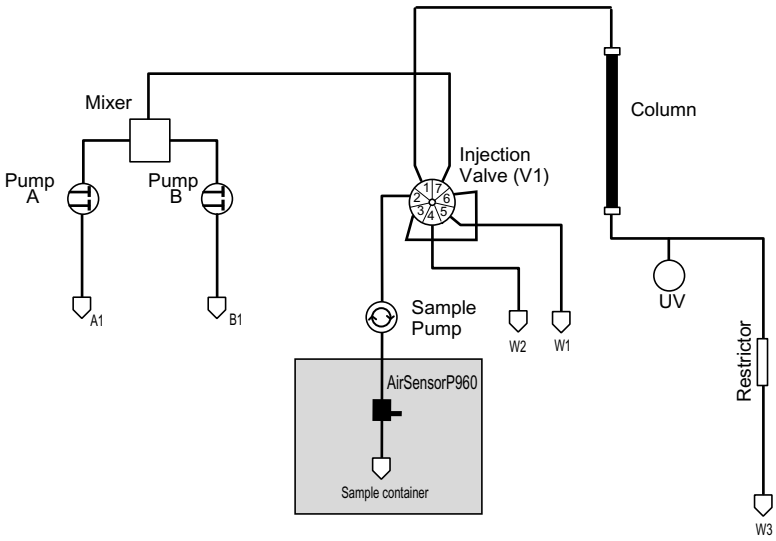
13.1 General

Air sensors Air-912, Air-912N, Air-925 and Air-925N can be used in the ÄKTAbasic 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 and Air-925N).

The examples below show two possible locations. Air sensors can be fitted in other locations in the ÄKTAbasic 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.

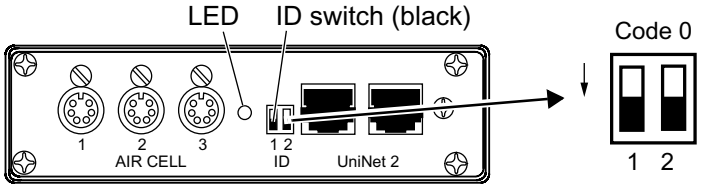
13.2 Installation

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

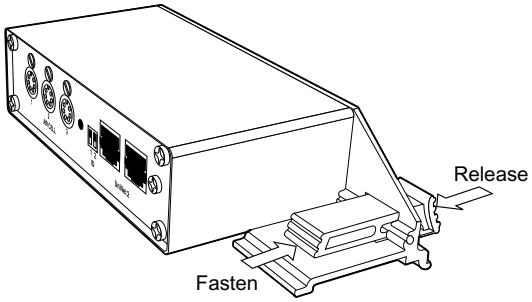
13.2.1 Fitting the Air-900 control box

1 Make sure that the ÄKTAbasic 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.

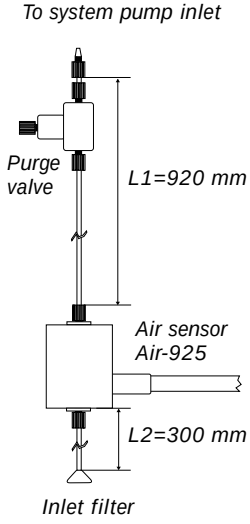


13.2.2 Connecting to the UniNet-2 communication network

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.

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

1 Remove the capillary from the switch valve inlet A1.



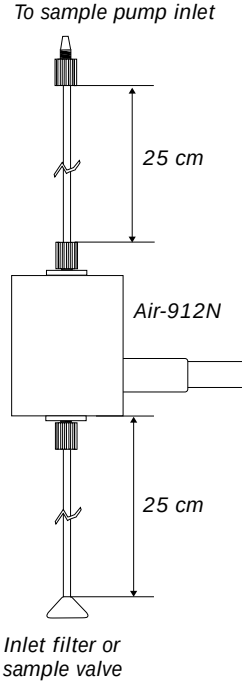
2 Cut new Teflon capillaries as shown in the figure and attach the purge valve and the air cell using the connectors and ferrules supplied.

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.

13.2.4 Air sensor Air-912N (AirSensorP960) in the sample inlet flowpath

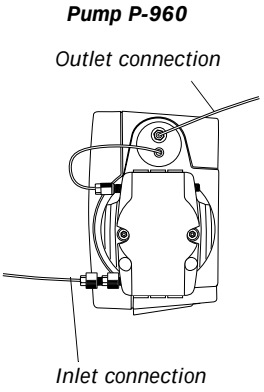
Air sensor Air-912N kit contains the necessary items required for this application. Air sensor Air-912N can only be connected to Pump P-960.

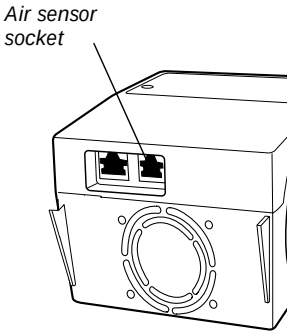


1 Remove the sample inlet capillary between the sample valve V5 (or sample) and the sample pump inlet connector.

2 Cut the i.d. 1.0 mm tubing supplied and connect to Air-912N using the fingertight connectors and union supplied.

3 Connect the air sensor cable to the socket underneath Pump P-960.



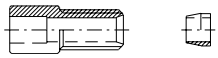


Note: Make sure that no air bubbles are trapped in the sample pump flow path before using the pump (refer to section 12 Sample pump P-960 and sample valve V5).

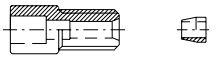
13.2.5 Capillary connectors

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

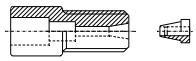
Airsensor Air-925 and Air-925N



Connector for 3/16" o.d. tubing, no. 18-1112-49, with ferrule, 18-1112-48

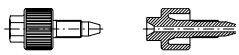


Connector for 1/8" o.d. tubing, no. 18-1121-17, with ferrule, 18-1121-18



Connector for 1/16" o.d. tubing, no. 18-1127-07, with ferrule, 18-1127-06

Airsensor Air-912 and Air-912N



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

13.3 Operation

13.3.1 Preparing for operation

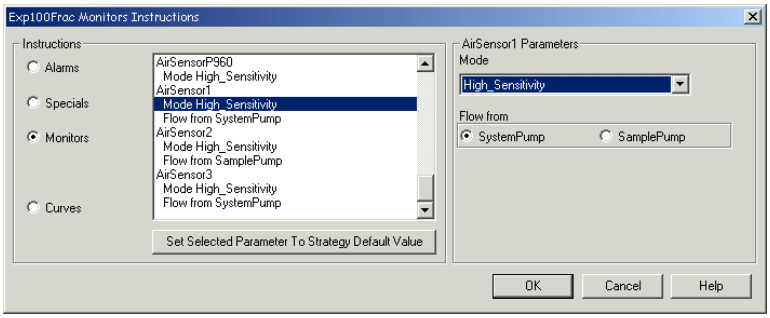
1 Start your ÄKTAbasic optional configuration system as described in 2.6 Preparation for use. Make sure that **Airsensor 1** (and/or **2** and/or **3**) is selected in the **Components** list.

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 you use the high sensitivity setting as standard.

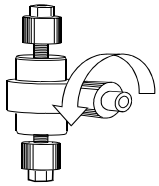
6 Check that the connected air cell(s) are indicated in the flow scheme in UNICORN.

7 Verify that the air cells are functioning properly by issuing manual commands from UNICORN as follows.

13.3.2 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 *13.3.4 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.

13.3.3 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.**

7 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.

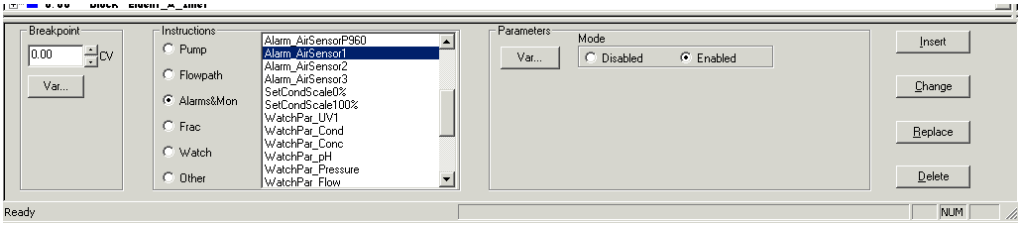
10 Set up instructions as described below to monitor the AirSensorP960 signal to suit your needs.

13.3.4 Setting up instructions for the air sensor function

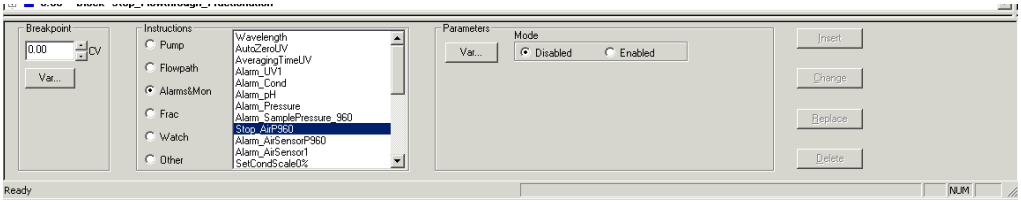
For AirSensor1-3, the presence of air in the flow path is detected by the Air-900 control box, which reports its status to UNICORN. For AirSensorP960, air is detected by the control software in Pump P-960.

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.

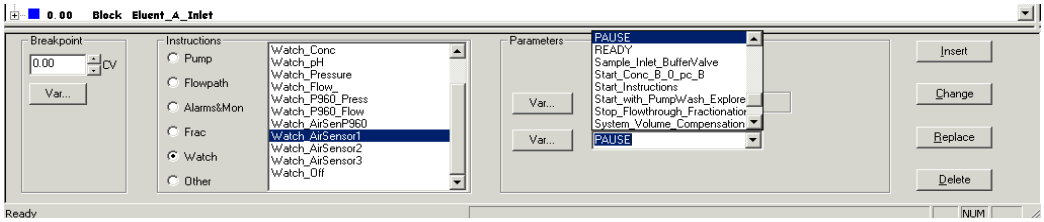
Alarm_AirSensor1-3 and Alarm_AirSensorP960
Enables/disables alarm generation for the air cells. An active alarm sets the chromatography system to **Pause** mode. Alarms can be activated/deactivated in **System:Settings** or inserted as instructions in a method. They can also be activated/deactivated 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.



13.3.5 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.

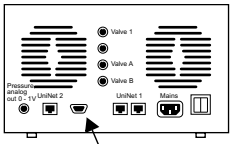
2 To enable the air sensor function, check the **Inject all Sample** box.

3 Type the maximum allowed sample volume in the **Maximal Injection Volume (0-20000 ml)** field.

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

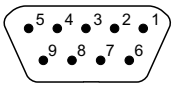
14.1 Installation

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



REMOTE connector on rear of P-901/903

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.

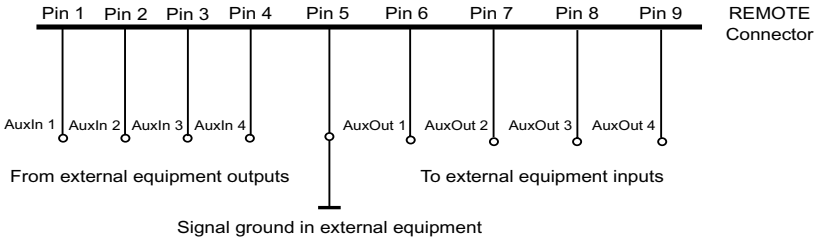


Pin	*Signal*	*Function*
1-4	AuxIn 1-4	Active state can be selected to 0 or 1 in UNICORN. Min. duration=50 ms. Max. frequency=1 Hz.
5	0V	Signal ground
6-9	AuxOut 1-4	Active state can be selected to 0 or 1 in UNICORN (5 V TTL signals).

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.

P-900
Digital signal interface



14.2 Operation

14.2.1 Preparing for operation

Start your ÄKTAbasic 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.**

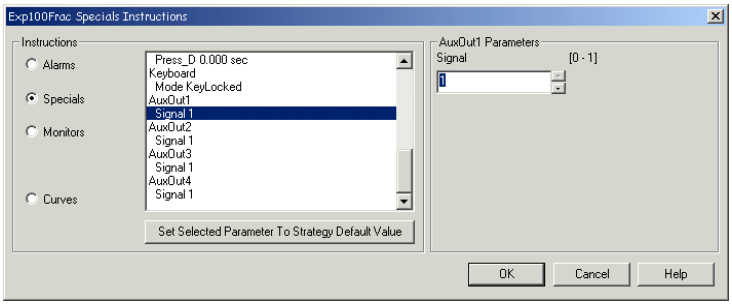
14.2.2 Instructions for signal handling

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.

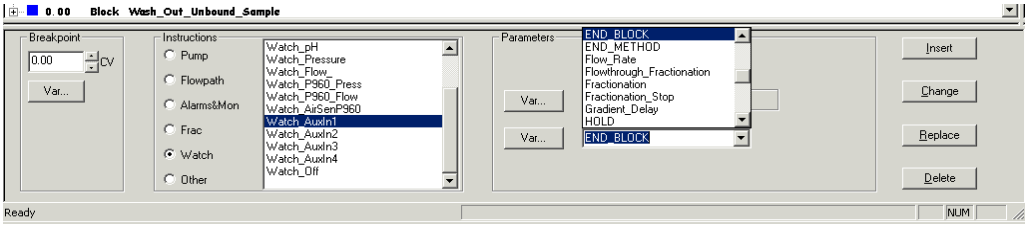
5 For **AuxOut1 Parameters/Signal**, set this value (0/1) as default. This ensures that the external equipment is reset to inactive mode when the method is completed.

6 Repeat this for all the output signals you want to use.

Watch_AuxIn1-4

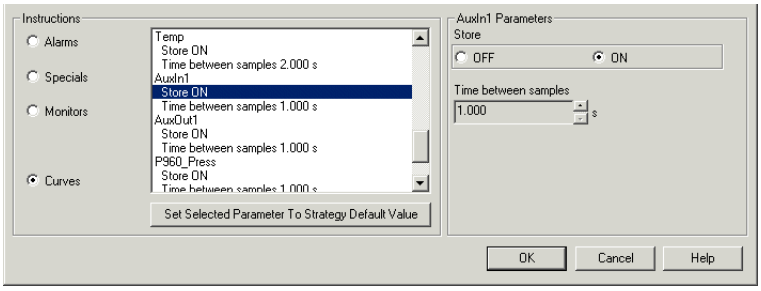
These instructions set up the initial status of the input signals. The status of the signals (1/0) can be shown in **Run Data** pane in UNICORN.

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. Pre-selected 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**.

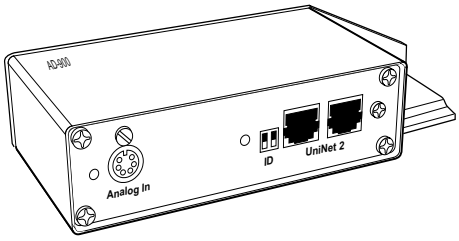


The parameter **Time between samples** is used to set the scan cycle for curve updating. The shortest possible interval is 1 s.

15 AD-900

15.1 General

AD-900 is an A/D converter for connecting an external instrument to ÄKTAbasic 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.

15.2 Installation

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

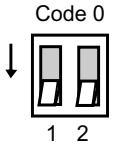
1 Connect the Mini-DIN cable connector to socket **Analog In** at AD-900.

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

2 Remove the protection cover from the wires marked 1 and 2 at the other end of the Mini-DIN cable.

3 Connect wire 1 (brown) to the external device high output.

4 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.

15.3 Operation

15.3.1 Preparing for operation

1 Start your ÄKTAbasic 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.

15.3.2 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:

1 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.

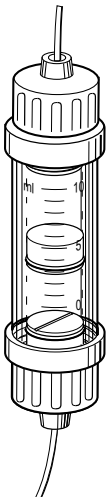
2 Click on the **Alarm&Mon** radio button.

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.

16 Superloop

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



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

Superloop is an accessory available in three sizes:

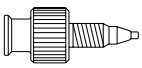
Volume	*Max. allowed column pressure*	*Code no.*
10 ml	4 MPa	18-1113-81
50 ml	4 MPa	18-1113-82
150 ml	2 MPa	18-1023-85

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

Normally, Superloop is filled manually with a syringe. However, Superloop can also be filled automatically, refer to section 12.5 Loading a Superloop on page 98.

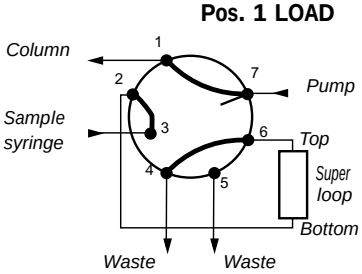
16.1 Preparation

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.

16.2 Filling Superloop

Filling Superloop is achieved as follows:

1 Set the injection valve to position INJECT.

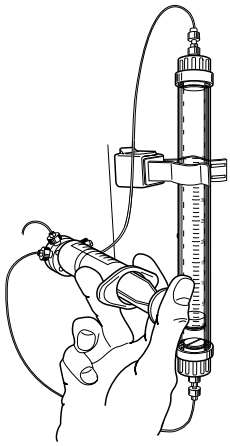
2 Start Pump P-900 and let it run until the movable seal has reached the bottom of Superloop.

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.

17 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**.

17.1 Standard configuration

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

ÄKTAbasic 10

- Including Frac-901: 62 µl
- Including Frac-950:

Tubing kit	*Including accumulator with tubing*	*No accumulator*
Standard mode	213 µl	62 µl
Prep mode	731 µl	Not applicable

ÄKTAbasic 100

- Including Frac-901: 374 µl
- Including Frac-950:

Tubing kit	*Including accumulator with tubing*	*No accumulator*
Standard mode	525 µl	374 µl
Prep mode	950 µl	Not applicable

17.2 Optional configuration

To calculate the volume of a tubing:

Volume of 200 mm of i.d. 0.25 mm tubing =

200/100 x 4.9 = 9.8 µl (4.9 µl = volume of 100 mm tubing

17.3 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
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

18 Strategies and Method Wizard

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

System designation	*Optional components installed*
XT	Autosampler

18.1 Strategies overview

ÄKTAbasic system	*Strategy name*[*]
Basic 100 with Frac-950	B100F400
Basic 10 with Frac-950	B10F400
Basic 100 with Frac-901	B100_400
Basic 10 with Frac-901	B10_400

[*] The last three digits in the strategy name is the version number.

18.2 Method Wizard

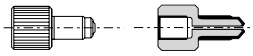
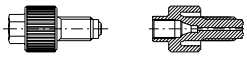
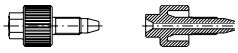
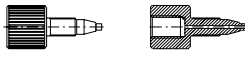
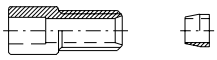
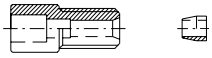
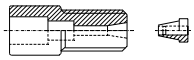
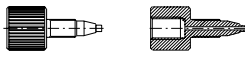
Method Wizard Configuration file: version 2.0

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

19 Accessories and consumables

19.1 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

19.2 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	A	18-6083-00
18 mm + 30 mm tube rack, complete with bowl, tube support and tube holder	1	A	18-6083-11
12 mm tube rack, complete with bowl, tube support and tube holder	1	A	18-6083-12
Microtiter plate + 30 mm tube rack, complete with bowl, tube support and tube holder	1	A	18-6083-13
30 mm tube rack (standard mode), complete with bowl, tube support and tube holder	1	A	18-6083-14
30 mm tube rack (prep mode), complete	1	A	18-6083-15
250 ml bottles rack, complete	1	A	18-6083-16
Funnel to flask kit, complete with tubing, funnels, tubing guide and extension legs	1	A	18-6083-17
Dispenser arm kit, complete	1	A	18-6083-18
Fraction Collector Frac-901			
Fraction Collector Frac-901, complete with 18 mm tube rack	1	A	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	A	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	A	18-8522-01
Flow diversion valve, FV-903, including mounting bracket	1	A	18-1114-50

Item	*Quantity/ pack*	*A/C**	*Code no.*
Autosampler A-900			
Autosampler A-900	1	A	18-1116-61
Autosampler A-900 with Cooling	1	A	18-1144-61
Tray segment set 1.5 ml vial (o.d. 12 mm)	1	A	18-1119-90
Tray segment set 0.5 ml vial (o.d. 7 mm)	1	A	18-1120-48
Sample needle, stainless steel	1	A	18-1148-15
Wash solvent bottle 250 ml	1	A	18-1120-33
Autosampler A-905 for ÄKTA			
Autosampler A-905 for ÄKTA complete	1	A	18-1175-93
Sample needle, fused silica, 5 µl	1	A	18-1158-68
PEEK loop 20 µl	1	A	18-1160-49
Common autosampler accessories			
ÄKTAdesign XT upgrade kit	1	A	18-6083-19
ÄKTA XT Tubing kit	1	A	18-1122-92
Sample needle, PEEK, 15 µl	1	A	18-1119-99
Buffer tubing 500 µl	1	A	18-1120-32
Buffer tubing 2000 µl	1	A	18-1120-31
Syringe 100 µl	1	A	18-1120-34
Syringe 250 µl	1	A	18-1120-35
Syringe 500 µl	1	A	18-1120-36
Syringe 1000 µl	1	A	18-1120-37
Luer lock connection, female	1	A	18-1120-38
PEEK loop 100 µl	1	A	18-1120-40
Syringe waste tubing	1	A	18-1120-44
Syringe tubing	1	A	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	A	18-1108-40
Valve IV-908 including one UniNet cable	1	A	18-1108-42
Valve PV-908 including one UniNet cable	1	A	18-1108-41
pH measurement			
pH electrode, round tip, including flow cell and holder	1	C	18-1134-84
pH electrode, round tip	1	C	18-1111-26
pH flow cell, round tip, including dummy electrode	1	A	18-1112-92
Dummy electrode, round tip	1	A	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	A	18-1175-86
Air sensor			
Air sensors			
Air-912 (1.2 mm)	1	A	18-1121-23
Air-925 (2.5 mm)	1	A	18-1121-24
Air sensor A-912N kit including air sensor, holder for ÄKTAexplorer, connectors and tubing	1	A	18-1175-84
Air-925N	1	A	18-1174-16
Air- 900 control box including pump tubing and one UniNet 2 cable	1	A	18-1121-22
Purge valve	1	A	18-1126-33

Item	*Quantity/ pack*	*A/C**	*Code no.*
AD-900			
AD-900 Analog/Digital converter	1	A	18-1148-62
Superloops			
Superloop 10 ml, 50 ml			
Superloop 10 ml, complete	1	A	18-1113-81
Superloop 50 ml, complete	1	A	18-1113-82
Tubing kit for Superloop (10 ml)	1	A	18-1113-83
Tubing kit for Superloop (50 ml)	1	A	18-1113-84
Superloop 150 ml			
Superloop 150 ml, complete	1	A	18-1023-85
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	A	18-1128-64
Communication cable P-920–P-50	1	A	18-1129-60

*) A = accessory, C = consumable

Index

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

1 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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