ÄKTA*design*

Autosampler A-900

User Manual

18-1117-50

amersham pharmacia biotech

Important user information

All users must read this entire manual to fully understand the safe use of Autosampler A-900.

WARNING!

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

Caution!

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

Note

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

Declaration of conformity

Safety standards

This product meets the requirements of the Low Voltage Directive 73/23/EEC through the harmonized standard EN 61 010-1:1993 + A2:1995.

EMC standards

This product meets the requirements of the EMC Directive 89/336/EEC through the harmonized standard EN 61 326-1:1997 + A1:1998.

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

- used as a stand-alone unit, or
- connected to other CE-marked Amersham Pharmacia Biotech instruments, or
- connected to other products recommended or described in this manual, and
- used in the same state as it was delivered from Amersham Pharmacia Biotech except for alterations described in this manual.

WARNING!

This is a Class A product. In a domestic environment this product may cause radio interference in which case the user may be required to take adequate measures.

Terms and Conditions of Sale

All goods and services are sold subject to the terms and conditions of sale of the company within the Amersham Pharmacia Biotech group which supplies them. A copy of these terms and conditions is available on request.

Should you have any comments on this product, we will be pleased to receive them at:

Amersham Pharmacia Biotech AB
SE-751 84 Uppsala
Sweden

Trademarks

ÄKTA and UNICORN are trademarks of Amersham Pharmacia Biotech Limited or its subsidiaries.

Amersham is a trademark of Nycomed Amersham plc. Pharmacia and Drop Design are trademarks of Pharmacia & Upjohn Inc.

Windows and Windows NT are registered trademarks of Microsoft Corporation.

Office Addresses

Amersham Pharmacia Biotech AB
SE-751 84 Uppsala
Sweden

Amersham Pharmacia Biotech UK Limited
Amersham Place
Little Chalfont
Buckinghamshire
England HP7 9NA

Amersham Pharmacia Biotech Inc.
800 Centennial Avenue
P.O. Box 1327
Piscataway, N.J. 08855
USA

Amersham Pharmacia Biotech Europe GmbH
Postfach 5480
D-79021 Freiburg
Germany

Amersham Pharmacia Biotech KK
Sanken Building
3-25-1 Hyakunincho, Shinjuku-ku
Tokyo 169–0073
Japan

Contents

About this manual

This manual comprises two parts; a practical part (sections 1–5) and a reference part (sections A-D). Sections 1–5 contain the necessary information for operating the instrument.

1 Introduction

1.1 General

Autosampler A-900 is an automated sampling injector for use, with ÄKTA™ design systems in liquid chromatography.

The A-900 features:

- Sample injection from a few microlitres to 1 ml.
- Three different methods for injection.
- Biocompatibility.
- Different sizes of sample vials.

Autosampler A-900 with Cooling is a separate autosampler that provides active cooling of the sample vials.

1.2 Safety

- The instrument is designed for indoor use only.
- Do not use in a dusty atmosphere or close to spraying water.
- Do not block the air inlet and outlet of the unit.

WARNING! Always disconnect the power supply before attempting to replace any item on the instrument.

WARNING! Do not operate the A-900 with the front cover open.

WARNING! The instrument must not be opened by the user. It contains high voltage circuits that can deliver a lethal electric shock.

WARNING! For continued protection against risk of fire, replace only with fuses of specified type and rating. See technical specifications for fuse data.

WARNING! The sample needle in stainless steel can be used for successful sample injection in Autosampler A-900 in most chromatographic applications. However, please mind that using steel is an exception to the Amersham Pharmacia Biotech tradition of having biocompatible material in all equipment coming in touch with biological samples. Please also mind that the use of salt-containing buffers with a pH lower than 3.5 might negatively affect the lifetime of the needle.

1.3 Safety symbols on the A-900

The A-900 contains the following safety symbol:

**SHARP NEEDLE
AND MOVING PARTS
KEEP HANDS CLEAR**

This sticker denotes the proximity of the sharp needle and the moving needle arm of the A-900, which, if not handled with care, could result in injury or damage or destruction of these parts.

2 Installation

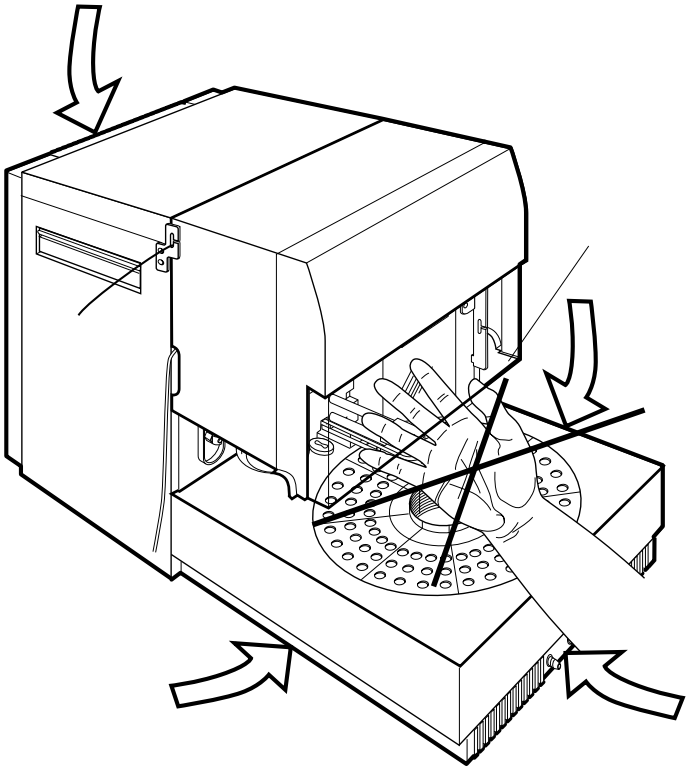
2.1 Unpacking

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

Keep all packing materials if onward transport of the instrument is expected.

Note: *Do NOT lift the instrument by the front cover!*

Lift the A-900 with both hands under the instrument or with one hand under the front and the other hand grasping the rear top (see figure below).



2.2 Locating the A-900

The 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 ÄKTA system base platform.

Be sure that none of the ventilation holes are blocked. Blockage of the ventilation holes can cause malfunctioning of the A–900 Autosampler or even damage the electronics.

Do not install the A–900 in areas subject to excessive dust, direct sunlight or shocks, and do not place it near a source of heat.

2.3 Power connections

Before connecting the mains cord to your ÄKTA separation unit, check that the voltage setting of the A-900 matches the local mains supply voltage and main fuses. Use only a supply appliance with protective grounding.

If the indicated voltage is not correct, select the proper voltage by removing, inverting, and then reinserting the voltage selector-cartridge. Check that the right fuses are installed. If not replace them with the fuses stated below:

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

WARNING! For continued protection against risk of fire, replace only with fuses of specified type and rating. See Technical specifications for fuse data.

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

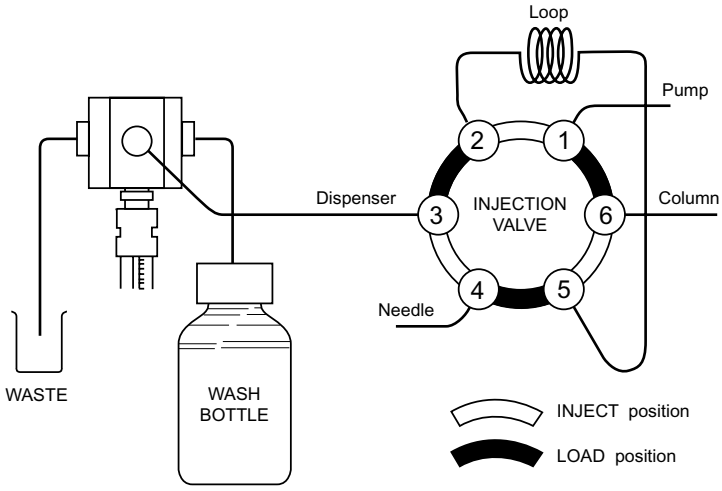
2.4 Fluid connections

The A-900 is factory installed with a 250 µl syringe, a 100 µl PEEK loop, a 500 µl buffer tubing and a PEEK sample needle.

A schematic presentation of all parts that have contact with fluid is shown below.

To get access to the fluid parts, it is necessary to open the front cover of the A-900, therefore remove the screw on the right-hand side of the cover. The table below lists the dimensions of the standard installed tubing.

For optimal performance of the A-900, a correct combination of syringe, loop and buffer tubing should be installed.



Tubing	*Material*	*Dimensions*
Standard sample needle tubing (Volume: 15 µl)	PEEK	135 mm x 0.5 mm OD x 0.25 mm ID
Buffer tubing from high pressure valve to syringe valve (Volume: 500 µl)	Tefzel	640 mm x 1/16" OD x 1.0 mm ID
Tubing, syringe valve to wash solvent bottle	Tefzel	300 mm x 1/16" OD x 1.0 mm ID
Tubing, syringe valve to waste solvent bottle	Tefzel	400 mm x 1/16" OD x 1.6 mm ID
100 µl PEEK loop	PEEK	1/16" OD x 0.5 mm ID
Tubing, general waste	Silicon	2 x 1.2 m, 10 mm OD x 7 mm ID

ÄKTA connections

To ensure reproducible injections, make the connections to your ÄKTA chromatography system according to the installation instructions, given in the *ÄKTAdesign Optional Configurations User Manual*.

The A-900 has been flushed with isopropanol: make sure that the mobile phase of your ÄKTA chromatography system is miscible with isopropanol. If not, start with an intermediate solvent as mobile phase (disconnect the column).

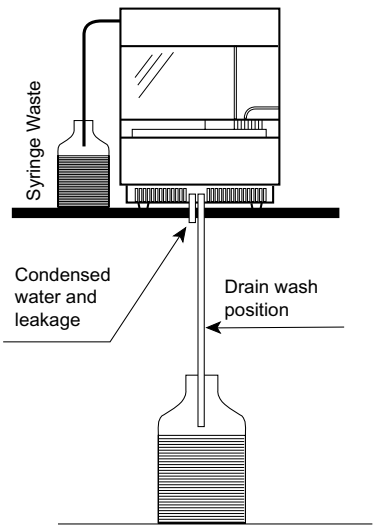
CAUTION! It is essential that the contents of the sample loop are injected in back flush onto the column. Therefore, do not exchange column and pump connections at the injection valve.

Waste tubing

General waste

Connect the supplied drain tubing to the right-hand drain hose connector of the A–900 and put the other end in a bottle placed on the floor. This drain removes all the liquid dispensed into the wash position.

Note: *Sample that is not injected is also removed through this tubing.*



Syringe waste

Place the syringe waste tubing in a small bottle below the A–900. If injection volumes no larger than the volume of the buffer tubing are programmed, the syringe waste will only be wash solvent.

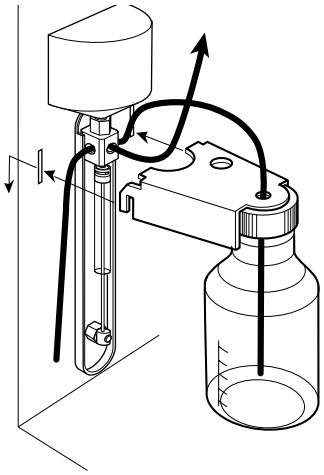
Leakage drain

All leakage solvents are drained through the left-hand hose connector.

CAUTION! Make sure that the drain and waste tubings are not twisted and obstructing the flow path.

Wash solvent

The A-900 has a built-in wash solvent reservoir of 250 ml.



To position the wash solvent bottle:

1 Fill the wash solvent bottle with the appropriate wash solvent.

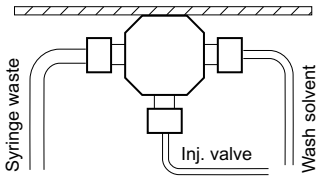
Note: *Use methanol or a mixture of one part isopropanol in four parts of water as wash solvent. Before using the wash solvent, degas the solvent with helium or an ultrasonic bath.*

2 Screw the bottle in the wash solvent holder and place the holder on the A-900.

3 Put the wash solvent tubing in the wash solvent bottle.

4 Fill the tubing using the **AutosamplerControl: SyringeEnd** and **SyringeHome** commands in **Manual:Flowpath**.

SyringeEnd aspirates 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 until the wash solvent tubing and the syringe are completely filled.

5 After the wash solvent tubing and the syringe are filled, use the **AutosamplerControl: InitialWash** command in **Manual:Flowpath** to perform a standard wash. All tubing connected to the syringe valve will be filled and flushed with wash solvent.

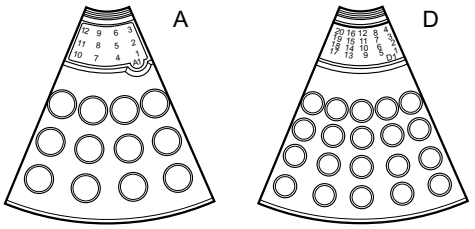
The wash solvent bottle is now ready for use.

If you have an application that requires more than 250 ml of wash solvent for a complete run, you can replace the wash solvent tubing by a longer tubing with one end flanged for the valve fitting and place a larger bottle outside the A-900. To fill this wash solvent tubing, repeat the previous fill procedure a few times.

2.5 Preparation of vials

Vial dimensions

The A-900 is very flexible in the use of vials and septa. Almost every type of vial with an OD of 12 or 7 mm can be used from micro vials (0.5 ml) to standard vials (1 ml). By using inserts, the vial volumes of standard vials can be reduced.



To hold all types of vials, the A-900 can be equipped with two types of tray segments, each for vials with a specific diameter (see figure).

Vials used should have the following dimensions:

Maximum vial height, including cap:		47 mm
Minimum vial height, including cap:		32 mm
Maximum vial diameter:		
	Standard vial	12 mm
	Micro vial	7 mm

Filling and sealing the vials

The standard vials, as well as the conical vials, can best be filled by a narrow–end pipette to allow air to escape when filling the vial.

Do not fill vials to the edge! If you do, sample will be forced into the air needle, risking extra cross-contamination of samples and fouling of the needle pair.

It is important that the seal is airtight. This maintains a pressure on the vial that prevents air bubbles and to stop evaporation of volatile samples.

CAUTION! Do not re-use a sample vial without replacing its cap or septum.

Note: *Check the seal after crimping. If the cap can be turned easily, the seal is not airtight (re-adjust hand crimper). The seal must not be deformed after crimping, since this may also deform the needle.*

Loading the sample tray

The tray segment types used must be entered in **System Settings**.

It is not necessary that all segments are available on the tray at the start of a run.

To place a segment on the tray, there must be an open position in the front half of the tray. The tray can be rotated if the A-900 is not running by using the **AutosamplerControl:RotateTray** command in **Manual:Flowpath**. Place the tray segment on the front half of the tray.

CAUTION! Do not place or remove a tray segment at the rear half of the A-900 tray. This damage the tray sensors.

3 Operation

3.1 General

- Switch on/off the A-900 using the mains switch located above the mains inlet on the rear panel.
- The A-900 is operated from UNICORN™ control system. Operating instructions for the A-900 from UNICORN control system is described in *ÄKTAdesign Optional Configurations User Manual*.
- When a 500 µl or 1000 µl syringe is used, the **AutosamplerSyringSpeed** command should be set to LOW in UNICORN **System Settings:Special.**
- If samples of high viscosity (5-10 cP) is used, the **AutosamplerSyringSpeed** command should be set to LOW, and the **Scaling** parameter to 0.2 in UNICORN **System Settings:Special.**
- Always run the A-900 with the front cover folded down.
- Never open the front cover unless the A-900 is in idle mode.

Autosampler A-900 with Cooling

Note: *Always keep the plastic cover over the sample vial rack when using the cooling option.*

To use the cooling option:

1 Start UNICORN™ by double-clicking on the UNICORN icon.

2 Log into the system according to your normal procedure. Make sure that the communication with the autosampler is established, i.e. that no error messages appears.

3 Click on **1. System Control** in the Toolbar at the bottom of the screen.

4 Select **System:Settings**.

5 Choose the **Special** button and then select **Autosampler_Temperature:TempOff**.

6 To enable cooling deselect the **OFF** check box.

7 Set the desired temperature (+4 – +40 °C).

8 Click on **OK**.

Autosampler A-900 now starts the cooling process.

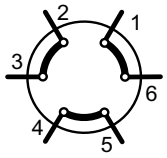
4 Maintenance

4.1 Injection valve

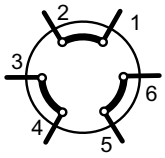
Description

The Injection valve is a two position, six port valve. The figure shows the flow diagram of the valve.

The six small circles represent the ports in the rotor seal. Rotating the valve 60° switches the valve from one position to another.



Shaft assembly rotated clockwise



Shaft assembly rotated counterclockwise

Specifications

- Maximum temperature is 50 °C.
- The valve withstands 34.5 MPa (5000 psi).
- Wetted surfaces are PEEK, alumina ceramic, and an inert polymer.

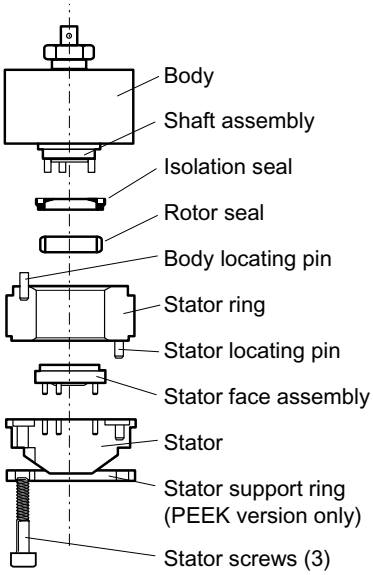
WARNING! Do not operate the A-900 with the front cover open.

CAUTION! Use only plastic ferrules in the PEEK stator ports. metal ferrules can cause irreparable damage to the plastic stator.

Maintenance

With normal use, the valve will give many tens of thousands of cycles of trouble-free operation. The main cause of early failure, which is seen as a leak in the valve, is abrasive particles in the sample and/or mobile phase scratching the rotor seal.

The procedure for changing the rotor seal, stator face assembly, and isolation seal is as follows. Refer to the figure and proceed:



1 Remove the three stator screws with a 9/64 inch hex key.

2 Remove the stator, stator face assembly and stator ring from the valve body.

3 Pull the rotor seal off the pins.

4 Remove the isolation seal.

5 Mount the new isolation seal with the spring side facing away from the rotor seal.

6 Mount the new rotor seal. The three pins on the shaft assembly fit into the mating holes in the rotor seal only one way. Mount the seal with the grooves facing the stator.

7 Replace the stator ring so that the body locating pin in the stator ring enters the mating hole in the body.

8 Put the new stator face assembly on the stator. The three pins on the assembly fit the mating holes in the stator only one way.

9 Mount the stator and stator face assembly on the valve so the stator locating pin in the stator ring enters the mating hole in the stator.

10 Replace the three stator screws. Tighten each an equal amount until the screws are tight.

Operating suggestions

CAUTION! Never operate the injection valve when it is dry. This will reduce the lifetime of the injection valve and probably cause malfunction.

CAUTION! Never leave the A-900 for more than one day with buffer solution in the tubings. Rinse the A-900 with distilled water prior to long stops.

Aqueous buffers or salt solutions

Prevent the formation of salt crystals in the valve by flushing out the flow passages and needle port with water after using salt solutions. Formation of salt crystals may otherwise damage the injection valve.

Calibrating sample loops

Sample loop sizes are designated by nominal values that can vary from the actual value by as much as 20%. This variation is due to the tolerance of the tubing ID. Since standards and unknowns are usually analysed with the same loop, knowledge of absolute loop volume is rarely needed. If the actual loop volume must be known, calibrate it in place on the valve, so that the flow passages in the valve (one in the rotor, two in the stator) are also taken into account.

The following PEEK sample loops for the injection valve are available from the valve manufacturer (Rheodyne):

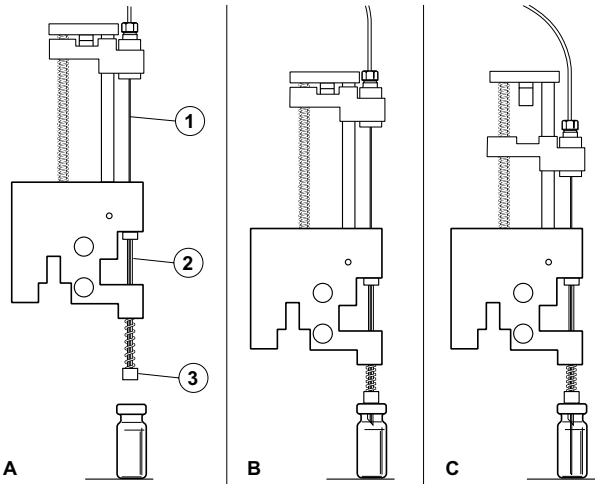
Loop µl	*Bore I.D.*	*O.D.*	*Manufacturers catalog no.*
6	0.007"	1/16"	9055-020
10	0.010"	1/16"	9055-021
20	0.010"	1/16"	9055-022
50	0.020"	1/16"	9055-023
100	0.020"	1/16"	9055-024
200	0.020"	1/16"	9055-025
500	0.030"	1/16"	9055-026
1000	0.030"	1/16"	9055-027

4.2 Needle and tubing

The A–900 has separate needles for puncturing and sampling; the air needle, which punctures the septum of the vial and provides the headspace pressure in the vial, and the sample needle for transporting the sample from the vial into the loop of the valve.

WARNING! The sample needle in stainless steel can be used for successful sample injection in Autosampler A-900 in most chromatographic applications. However, please mind that using steel is an exception to the Amersham Pharmacia Biotech tradition of having biocompatible material in all equipment coming in touch with biological samples. Please also mind that the use of salt-containing buffers with a pH lower than 3.5 might negatively affect the lifetime of the needle.

The sample needle enters a vial as follows:



A Initial position.

B First the air/prepuncturing needle (2, Figure A) is lowered by moving the complete needle arm downwards. The vial sensor strip (3, Figure A) detects if there is a vial and at the same time measures the vial height. If no vial is detected, the needle arm is moved upwards and the A–900 will stop. When the A–900 detects a vial, the lowering speed of the arm is decreased to provide just enough force to puncture the septum of the vial. After forcing the air/prepuncturing needle into the vial, air is applied to the vial. An air outlet nut reduces the pressure in the vial to approximately 0.5 bar. The pressure in the vial is a function of the diameter of the air outlet nut, the air needle and the sample needle.

C Finally the sample needle (1, Figure A) is lowered into the vial to the depth programmed in **System Settings.** Now the A–900 is ready for the first injection from this vial.

4.3 Syringe, sample loop and buffer tubing combinations

Four sizes of syringes are available for the dispenser: 100, 250, 500 and 1000 µl.

The 250 µl syringe is the standard syringe. When combined with the standard 500 µl buffer tubing and the standard 100 µl sample loop, the following injection volume range is available for the various injection modes:

- Flushed loop: 100 µl
- Partial loopfill: 1–50 µl
- µl pick-up: 1–25 µl

The maximum injection volumes are calculated as follows:

• Flushed loop:	injection volume =	loop volume
• Partial loopfill:	max. injection volume =	50% of loop volume
• µl pick-up:	max. injection volume =	(loop volume - 3 x needle volume) / 2 (standard needle volume is 15 µl)

Sample volume requirements:

Partial loopfill[1]:	injection volume + flush volume (using tapered or standard vial)
µl pick-up[1]:	injection volume (using tapered or standard vial)
Flushed loop[1]:	
loop volume < 100 µl:	min. 3 x loop volume + flush volume
loop volume 100–499 µl:	min. 2 x loop volume + flush volume
loop volume 500–1000 µl:	min. 1.5 x loop volume + flush volume

[1] The first injection from a vial requires additional sample volume. A tapered vial needs 4 µl extra volume and a standard vial needs 300 µl. These volumes must be added to the sample volumes given above.

Note that:

- Flushed loop gives maximum reproducibility, but not maximum accuracy, which depends on the loop used. Minimum sample loss = 230 µl (2 x loop overfill + flush volume for needle).
- Partial loopfill gives maximum accuracy and reproducibility better than 0.5% RSD for injection volumes > 5 µl.
  Minimum sample loss (flush volume) = 30 µl.
- 30 µl is the recommended minimum flush volume.
  35 µl is the recommended flush volume with an air segment.
  40 µl is the default value for flush volume.
- µl Pick-up offers zero sample loss and maximum accuracy (same as partial loop fill), but slightly less reproducibility. RSD is better than 1% for injection volumes > 5 µl.
- If, for some reason, an air segment is selected from the **InjectionulPickup** instruction in **Manual:Flowpath**, 5 µl of air is injected in the system together with the sample.

Injection volumes smaller than 5 µl

Partial loopfill

Install a 100 µl syringe to obtain maximum reproducibility and accuracy. Preferably install a 20 µl sample loop to avoid loss of accuracy due to expansion of the loop content when switching from inject to load position prior to sample loading. This is especially recommended when working with high pressures (15.0–25.0 MPa).

Note that the minimum sample loss in partial loopfill mode is 30 µl (recommended minimum flush volume) for the injection. For zero sample loss injections, use the µl-pick injection mode.

µl Pick-up

Install a 100 µl syringe for optimum accuracy and reproducibility.

Note: *To guarantee quantitative injections, do not install a smaller sample loop than 100 µl!*

Injection volumes up to 200 µl

With the standard 250 µl syringe, standard needle with tubing (15 µl) and standard 500 µl buffer tubing, but with a 200 µl sample loop, the maximum injection volumes are:

Flushed loop:	200 µl	(sample loss remains 230 µl since loops > 100 µl need 2 x loop volumes plus 30 µl flushed volume)
Partial loopfill:	100 µl	
µl pick-up:	77 µl	

CAUTION! Smaller volumes than 5 µl may be injected, but reproducibility and accuracy may not be < 0.5% for partial loopfill or < 1% for µl pick-up.

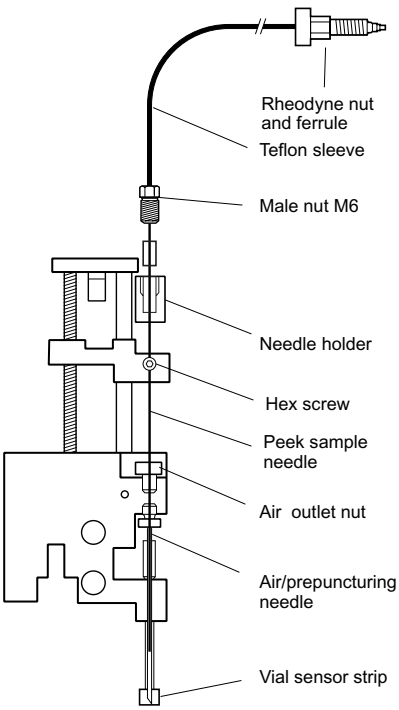
For volumes larger than 200 µl

Install the 2000 µl buffer tubing. Install the appropriate sample loop size and the appropriate syringe: Syringe volume > 2 x injection volume. Injection volumes larger than 500 µl are possible, but the sample may contaminate the syringe. Program sufficient wash after use!

Buffer tubing:

Syringe	*Buffer tubing*	*Loop volume*
100 µl	500 µl buffer tubing	5-20 µl
250 µl	500 µl buffer tubing	20-200 µl
500 µl	2000 µl buffer tubing	200-500 µl
1000 µ	2000 µl buffer tubing	500-1000 µl

4.4 Replacing the sample needle



To replace the sample needle, proceed as follows:

1 Use the **AutosamplerControl: NeedleFront** command in **Manual:Flowpath** to move the needle to the front.

2 Loosen the needle connection nut (Male nut M6) that fixes the sample needle.

CAUTION! Do not loosen or otherwise unscrew the Hex screw!

3 Loosen the Rheodyne Rheflex nut that connects the tubing to the injection valve.

4 Remove the sample needle by pulling it out of its fitting.

5 Put in the new needle assembly.

6 Connect the loose end of needle connection tubing to port 4 of the injection valve (use a ferrule made of PEEK).

CAUTION! Do not tighten excessively; it may block the tubings.

7 Check the sample needle height. The default value for the needle height is 2 mm.

8 To check the needle height, place a vial in tray segment A1, position 1. Remove tray segment A2. Do an injection and check the needle height in the vial.

9 If a tapered vial is used, an alternative way of checking the needle height can be useful; Do a large injection until air is injected, then check the sample level and adjust the needle height.

10 The needle height is adjusted in **System:Settings:Specials:Needlehght** in UNICORN.

11 Wash to clean the new needle by issuing the **AutosamplerControl: InitialWash** command in **Manual:Flowpath.**

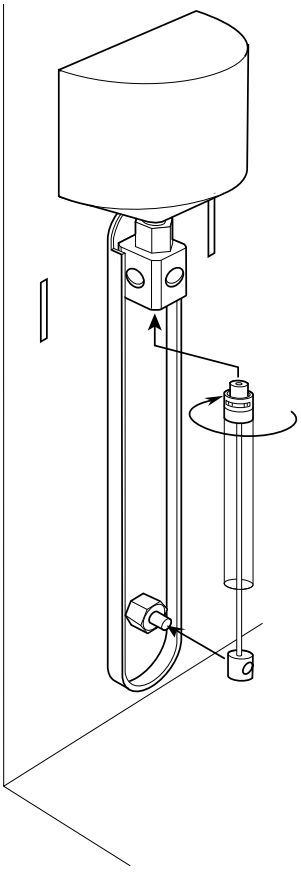
4.5 Syringe

The A–900 is factory equipped with a 250 µl syringe, but can also be equipped with a 100 µl, 500 µl or 1000 µl syringe.

To install another syringe, proceed as follows:

Note: *Do not disconnect the power supply of the A–900. It is needed to move the syringe.*

Note: *After the syringe change, the syringe must be in HOME position.*



1 Move the syringe to the end position by issuing the **AutosamplerControl:SyringeEnd** command in **Manual:Flowpath**.

2 Unscrew the syringe from the syringe valve. Make sure that the Luer connector in the valve remains in place.

3 Disconnect the plunger from the syringe drive.

4 Fill the new syringe with wash solvent. Remove most air bubbles from the syringe.

5 Connect the plunger of the filled syringe to the syringe drive and connect the syringe with the Luer connector at the syringe valve.

6 Screw the syringe firmly into the Luer connector.

7 Remove the air from the syringe by issuing the **AutosamplerControl:SyringeHome** command **Manual:Flowpath** The syringe will move to its HOME position and dispense its contents to the syringe waste.

8 If some air remains in the syringe, issue the **AutosamplerControl:SyringeEnd** command in **Manual:Flowpath** again. The syringe is filled with wash solvent.

9 Issue the **AutosamplerControl:SyringeHome** command in **Manual:Flowpath** to dispense the wash solvent to waste. If there is still air in the syringe, repeat the previous action and tap gently on the syringe as the wash solvent is dispensed to the syringe waste.

The A-900 is now ready for use.

5 Trouble-shooting

WARNING! Always disconnect the power supply before attempting to replace any item on the instrument.

WARNING! The instrument must not be opened by the user. It contains high voltage circuits that can deliver a lethal electric shock.

CAUTION! Only spare parts approved or supplied by Amersham Pharmacia Biotech may be used for maintaining and servicing the instrument.

5.1 Faults and actions

If the suggested actions do not correct the fault, call Amersham Pharmacia Biotech.

Fault	***Action** (see procedures below)*
Bad reproducibility	
Injection valve switched dry	• Replace rotor seal.
Syringe valve coupling leaking	• Check and tighten coupling. Replace if necessary.
Little or no peak	• Syringe leaking problem. Replace syringe.
Peaks of different height	• Ferrule not tightened in injection valve. Tighten ferrule.
Air in the syringe.	• Check and tighten coupling. Remove the air by issuing the **AutosamplerControl:SyringeEnd** and **AutosamplerControl:SyringeHome** commands in **Manual:Flowpath**. If there is still air in the syringe, remove it according to section 4.5.
Injection valve switches to slowly	• Rotor seal worn. Replace rotor seal.
Syringe action failed, motor generates noise	• Syringe not tightened enough when replaced. Tighten syringe.
Sample needle bent. No injection performed	• Septa type wrong, septa deformed or needle depth set too low. Replace needle and readjust needle depth if necessary.
Error message during initial wash	• Syringe valve not properly switched. Check syringe installation. Refit if necessary.
Needle destroyed in injection using µl vial segments	• Segments badly oriented, or septa bent down. Replace needle.
Ghost peaks at high pressure	• Bad capillary connections. Replace connectors if necessary.

Fault	*Action (see procedures below)*
A-900 does not execute commands	• Syringe in END position. Order the syringe to HOME position using the **AutosamplerControl:SyringeHome** command in **Manual:Flowpath**.
No sample injected	• Syringe in END position. Order the syringe to HOME position using the **AutosamplerControl:SyringeHome** command in **Manual:Flowpath.**
	• All segments in vial tray are not of the same type. Change to same type.
	• Wrong vial position programmed. Program correct position
Ghost or unexpected peaks	• Washing not performed properly, due to: -Run out of washing liquid. -Washing liquid flows back in washing position because waste flask not located below A-900.

Reference information

A Description

A.1 General

A unique 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 methods for injection 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 resulting in no sample loss.

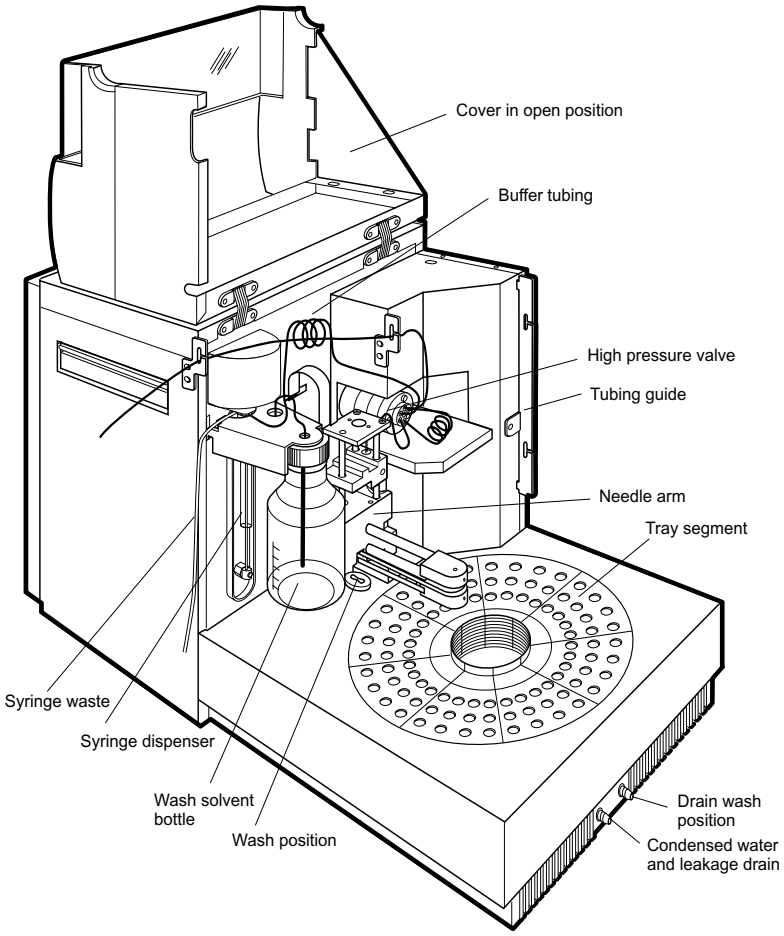
The A-900 uses a syringe to aspirate the sample from a vial into the sample loop. To prevent contamination of the syringe the A-900 is equipped with a buffer tubing between the syringe and the injection valve.

The wash solvent removes the sample from the buffer tubing and sample needle and also rinses both components.

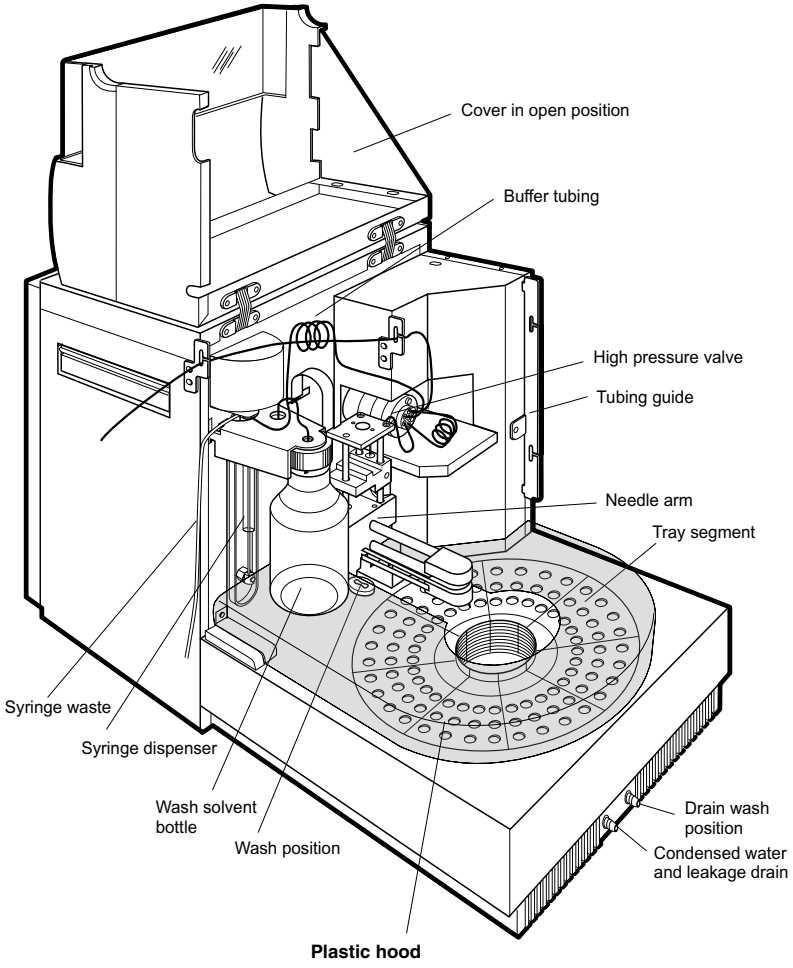
Autosampler A-900 with Cooling

Autosampler A-900 with Cooling is a separate autosampler that provides cooling of the sample vials. This is accomplished by a Peltier element located under the vial rack, a fan at the rear of the unit, and a plastic hood covering the vial rack. These additional components are pre-mounted at the factory.

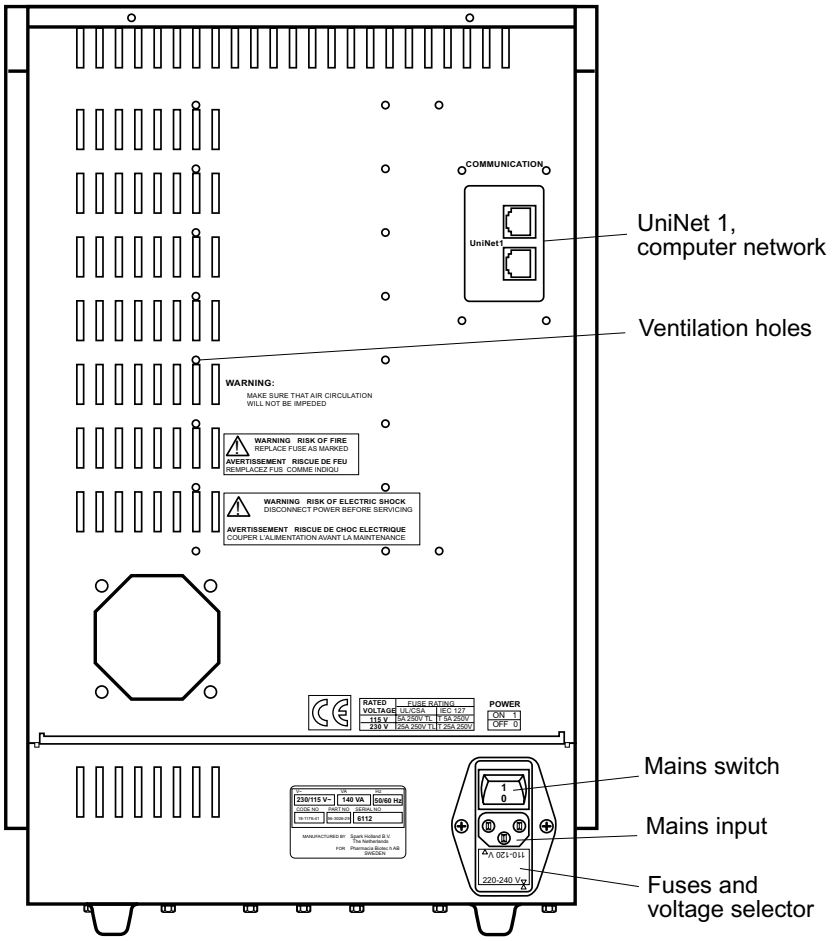
A.2 Autosampler A-900



A.3 Autosampler A-900 with Cooling

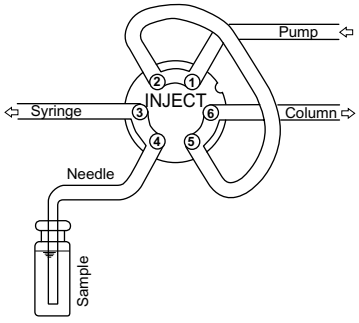


A.4 Rear view

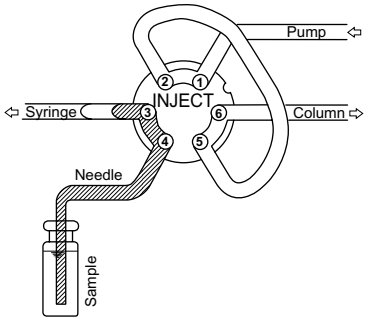

COMMUNICATION
UniNet1
UniNet 1,
computer network
Ventilation holes
WARNING:
MAKE SURE THAT AIR CIRCULATION
WILL NOT BE IMPEDED
WARNING RISK OF FIRE
REPLACE FUSE AS MARKED
AVERTISSEMENT RISCUE DE FEU
REMPLACEZ FUS COMME INDIQU
WARNING RISK OF ELECTRIC SHOCK
DISCONNECT POWER BEFORE SERVICING
AVERTISSEMENT RISCUE DE CHOC ELECTRIQUE
COUPER L'ALIMENTATION AVANT LA MAINTENANCE
RATED VOLTAGE
FUSE RATING
UL/CSA
IEC 127
115 V
5A 250V FL
T 5A 250V
230 V
2.5A 250V TL
T 2.5A 250V
POWER
ON 1
OFF 0
230/115 V~
140 VA
50/60 Hz
6112
Mains switch
Mains input
110-120 V
220-240 V
Fuses and
voltage selector

A.5 Flushed loop injections

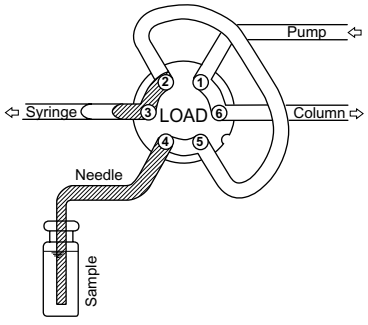
The switching sequence for a flushed loop injection is schematically shown in the following figures:



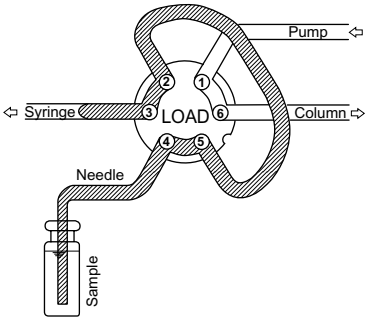
The Initial position: the injection valve is in the INJECT position. The sample needle and air needle have entered the vial. The head-space pressure, applied through the outer air needle, ensures that no air or vapour bubbles are formed during sample aspiration.



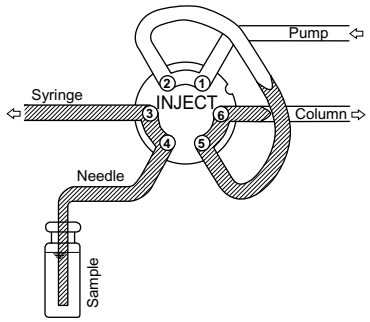
The syringe dispenser aspirates the “flush volume” from the sample vial to fill the sample lines with sample and remove wash solvent.



The injection valve switches to the LOAD position, placing a “sharp” sample front at the inlet of the sample loop.



For flushed loop injections, the sample loop is quantitatively filled by transporting two or more times the loop volume through the loop, depending on the volume of the loop.



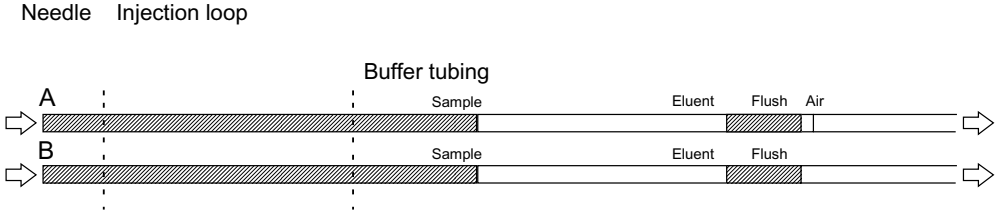
The injection valve switches to the INJECT position. The sample loop is now part of the ÄKTA mobile phase flow path. Sample is transported to the column.

If one injection is to be made from each vial, a wash routine has to be performed after every injection.

Air segment

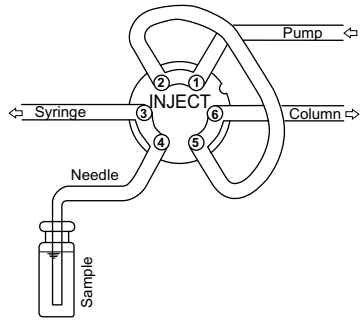
An air segment should be used to reduce the amount of flush volume. This air segment is at the front of the flush volume. It will not be injected and will therefore not influence the injection. The air segment is selected when the injection method is selected in **Manual:Flowpath.**

With a standard needle, the flush volumes must be: minimum 30 µl for injections with an air segment and 35 µl for injections without. If the samples are highly viscous, it may be necessary to program larger flush volumes and reduce the syringe speed to optimize performance.

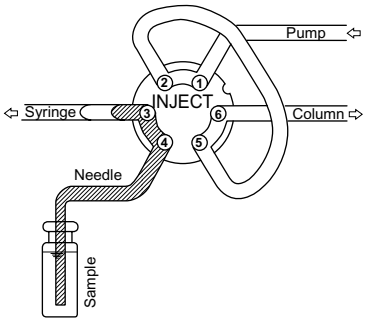


A.6 Partial loopfill injections

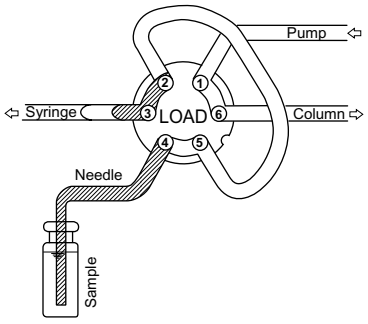
The switching sequence for a partial loopfill injection is schematically shown in the following figures:



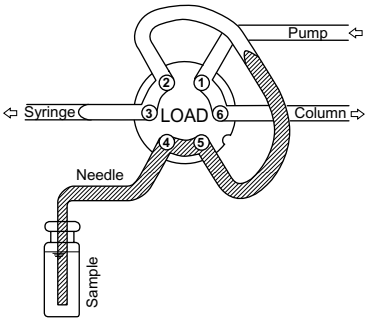
The Initial position: the injection valve is in the INJECT position. The sample needle and air needle have entered the vial. The head-space pressure, applied through the outer air needle, ensures that no air or vapour bubbles are formed during sample aspiration.



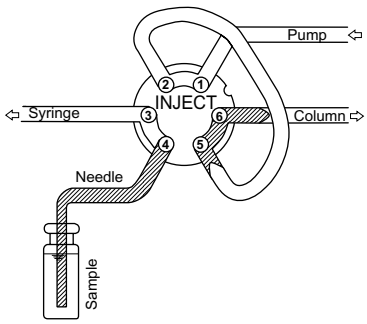
The syringe dispenser aspirates the “flush volume” from the sample vial to fill the sample lines with sample and remove wash solvent.



The injection valve switches to the LOAD position, placing a “sharp” sample front at the inlet of the sample loop.



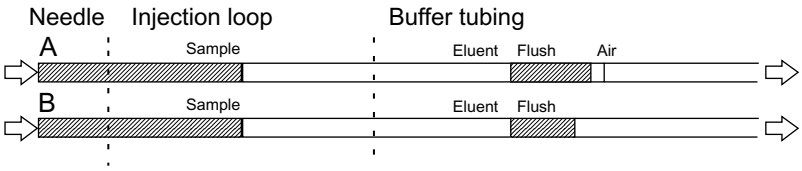
For partial loopfill injections, the sample loop is filled by transporting the programmed injection volume into the sample loop.



The injection valve switches to the INJECT position. The sample loop is now part of the ÄKTA mobile phase flow path. Sample is transported to the column.

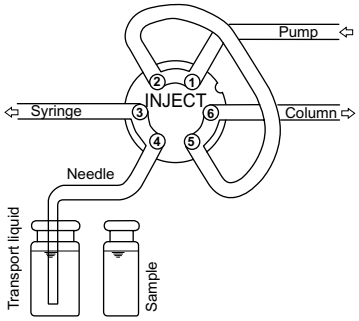
Air segment

An air segment should be used to reduce the amount of flush volume. This air segment is at the front of the flush volume and will not be injected. The air segment can be selected when the injection method is selected in **Manual:Flowpath.**

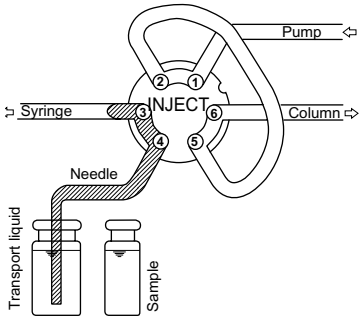


A.7 µl Pick-up injection

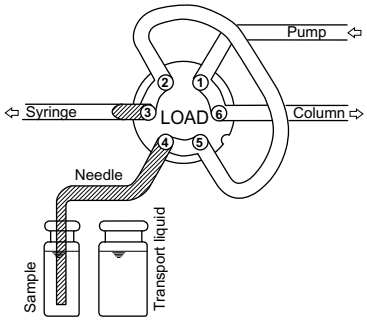
The switching sequence for a µl pick-up injection is schematically shown in the following figures:



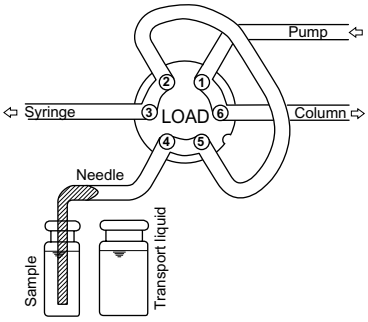
The Initial position: the injection valve is in the INJECT position. The sample needle has entered the vial of transport liquid (mobile phase used to avoid disturbing the chromatogram with an additional peak of the transport solvent).



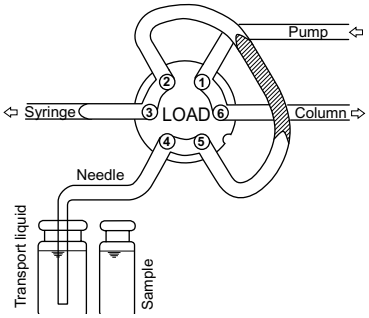
The syringe dispenser aspirates transport liquid from the transport vial to fill the sample line with transport liquid and remove wash solvents.



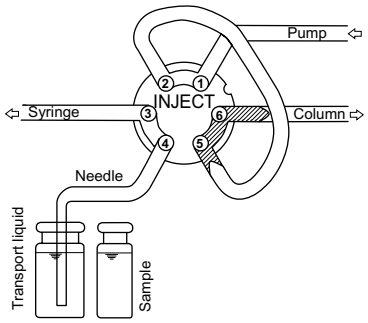
The needle moves from the transport vial to the sample vial. The injection valve switches to the LOAD position.



The programmed injection volume is aspirated from the sample vial.



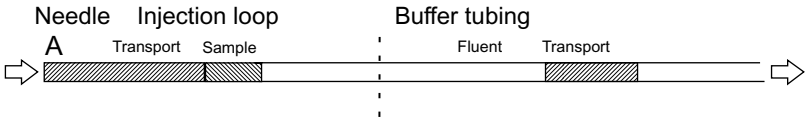
The sample needle moves back to the transport vial. The sample is quantitatively transported into the loop with 30 µl of transport liquid (mobile phase) from the transport vial.



The injection valve switches to the INJECT position. The sample loop is now part of the ÄKTA mobile phase flow path. Sample is transported to the column.

Air segment

Normally an air segment shall not be programmed for µl Pick-up injections, since it then is injected with the sample into the system.



Note: *For µl pick-up injections, there will be no air pressure (head-space pressure) on the sample vial. This avoids sample errors due to air expansion during vial exchange from the sample vial to the transport solvent vial.*

B Technical specifications

Sampling	
Sample capacity	Standard tray: 96 vials of 1.5 ml (Std), 12 vials per segment Micro vial tray: 160 vials of 0.5 ml, 20 vials per segment
Vial dimensions (cap included)	Maximum vial height: 47 mm Minimum vial height: 32 mm
Loop volume	5–1000 µl
Dispenser syringe	100 µl, 250 µl (standard), 500 µl or1000 µl syringe
Vial detection	Missing vial detection by vial sensor
Headspace pressure	Built-in compressor
Switching time injection valve	Electrically < 100 ms
Piercing precision needle	± 0.6 mm
Wash solvent	250 ml internal wash solvent bottle
Wetted parts	PEEK, Stainless steel, PTFE, glass, Tefzel, alumina ceramic and inert polymer
Analytical performances	
Reproducibility	RSD <= 0.3% for flushed loop injections RSD <= 0.5% for partial loopfill injections, injection volumes > 5 µl (5 µl sample, 100 µl sample loop, ≤ 2.5 MPa; 10 µl sample, 20 µl sample loop, ≤ 25 MPa) RSD <= 1.0% for µl pick-up injections, injection volumes >5 µl
Memory effect	< 0.1% with programmable needle wash
A-900 with Cooling	
Cooling capacity	> 11.5 °C below ambient temperature (T) for 16 °C <T<40 °C
Typical cooling times	34 min from 23 °C to 4 °C (at 45% relative humidity) 60 min from 32 °C to 4 °C (at 35% relative humidity)

Physical data

Dimensions (W x D x H)	280 x 540 x 440 mm
Weight	
A–900	22 kg
A–900 with Cooling	30 kg

Electrical data

Power supply voltage	100–240 VAC, 50-60 Hz
Power requirement	250 VA
Fuses	
For 100–120 VAC	Two 5.0 AT-fuses
For 220–240 VAC	(slow, _"x"_, UL/CSA) Two 2.5 AT-fuses (slow, 5 x 20 mm, IEC 127)

Communication

Serial communication port	UniNet-1

Standards

EMC Standards	This product meets the requirement of the EMC Directive 89/336/EEC through the harmonized standards EN 61 326-1:1997 + A1:1998.(emission and immunity). **Note**: The declaration of conformity is valid for the instrument when it is: • used in laboratory locations • used in the same state as it was delivered from Amersham Pharmacia Biotech except for alterations described in the user manual • used as "stand alone" unit or connected to other CE labelled Amersham Pharmacia Biotech instruments or other products as recommended.
Safety Standards	This product meets the requirement of the Low Voltage Directive (LVD) 73/23/EEC through the harmonized standard EN 61010-1:1993 + A1:1995.

Environment

Working condition	+4 to +40 °C
	20–95% relative humidity
Sound pressure level	<70 dB (A)
Installation category	Category II, according to IEC 1010
Pollution degree	Degree 2, according to IEC 1010

C Accessories and consumables

Item	Quant./pack	Code no.
Accessories		
Tray segment set 1.5 ml vial (O.D. 12 mm)	1	18-1119-90
Tray segment set 0.5 ml vial (O.D. 7 mm)	1	18-1120-48
Consumables		
Rheflex ferrule	10	18-1119-91
Rheflex nut	10	18-1119-92
Flange tube fitting 1/8"	5	18-1119-93
Flange tube fitting 1/16"	5	18-1119-94
Plunger replacement tip 100 µl	10	18-1119-96
Plunger replacement tip 500 µl	10	18-1119-97
Plunger replacement tip 250 µl	10	18-1119-98
PEEK sample needle	1	18-1119-99
Sample needle, stainless steel	1	18-1148-15
Buffer tubing 2000 µl	1	18-1120-31
Buffer tubing 500 µl	1	18-1120-32
Wash solvent bottle 250 ml	1	18-1120-33
Syringe 100 µl	1	18-1120-34
Syringe 250 µl	1	18-1120-35
Syringe 500 µl	1	18-1120-36
Syringe 1000 µl	1	18-1120-37
Luer lock connection, female	1	18-1120-38
PEEK loop 100 µl	1	18-1120-40
PEEK rotor (for Rheodyne 9740 valve)	1	18-1120-41
Syringe waste tubing	1	18-1120-44
Syringe tubing	1	18-1120-45
Wash vial	1	18-1120-46
Union nut air	1	18-1120-47

May 2001

TC information ab, Uppsala. Printed in Sweden by T. K. i Uppsala AB