ÄKTA design

ÄKTA basic

User Manual

18-1140-76

Amersham Biosciences

Important user information

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

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.

CE Certification

This product meets all requirements of applicable CE-directives. A copy of the corresponding Declaration of Conformity is available on request.

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

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

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Unless otherwise agreed in writing, all goods and services are sold subject to the terms and conditions of sale of the company within the Amersham Biosciences group which supplies them. A copy of these terms and conditions is available on request.

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About this manual

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

Installation, system description, system maintenance and trouble-shooting are also found in this manual.

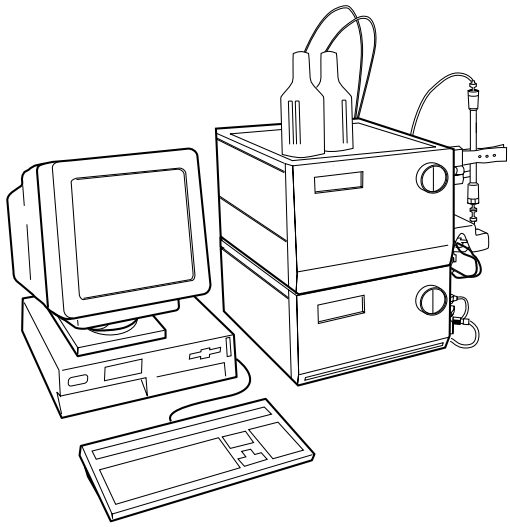
1 Introduction

1.1 General

ÄKTAbasic™ is an automated liquid chromatography system designed for method development and research applications. The system simplifies the transition from laboratory to full scale production. Scale-up to production is predictable and trouble-free.

ÄKTA™ basic features:

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

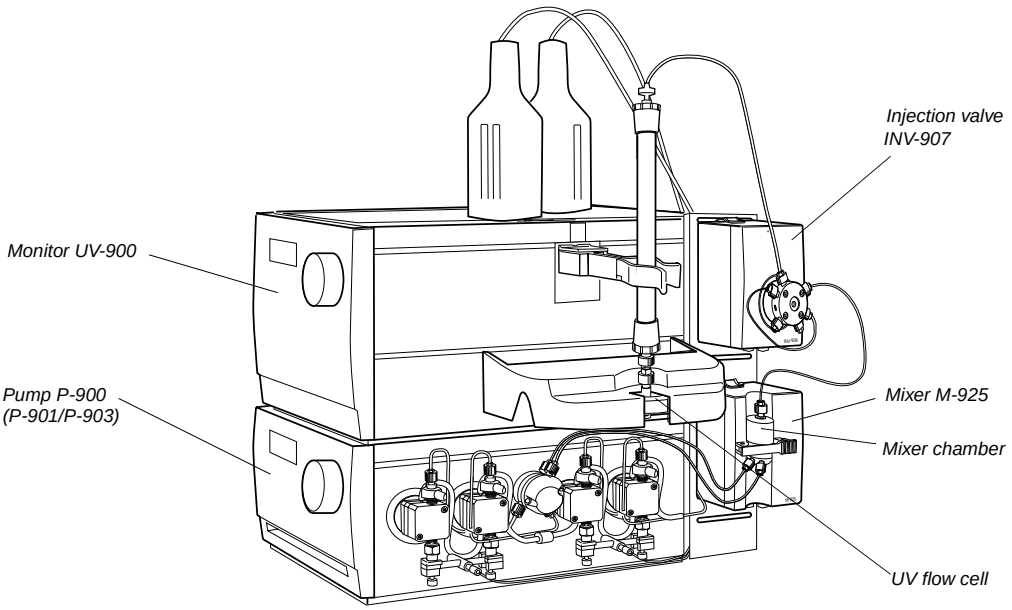


ÄKTAbasic consists of a compact separation unit and a personal computer running UNICORN™ control system version 4.12 or higher. Fraction collectors are available as accessories.

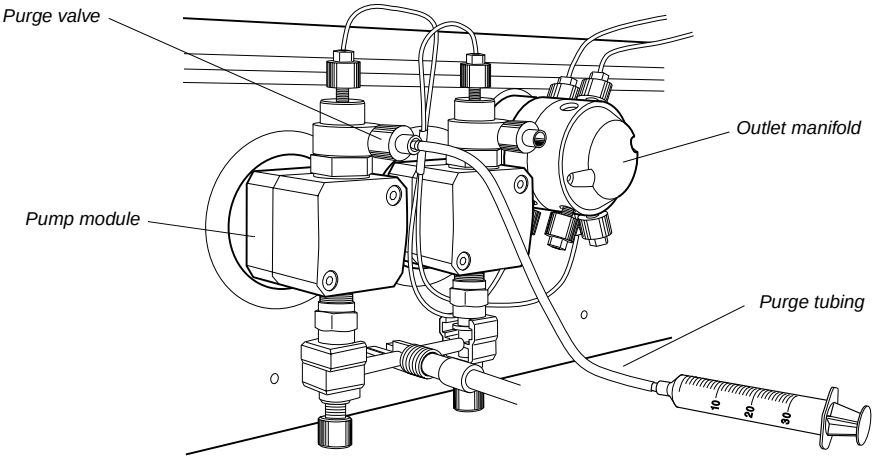
The chromatography system is described in detail in section 6.1 of *Reference information* in this manual. Brief descriptions of the individual components are given in section 6.2 of *Reference information*. Detailed information on the components can be found in

their respective User Manuals and Instructions. UNICORN control system is described in the separate UNICORN User Manuals.

The location of the modules and components of the separation unit is shown in the following illustration.



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



1.2 Safety

- The system is designed for indoor use only.
- Do not use in a dusty atmosphere or close to spraying water.

WARNING! The system must be connected to a grounded mains socket.

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

WARNING! Monitor UV-900 uses high intensity ultra-violet light. Do not disconnect the optical fibers while the lamp is ON.

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

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

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

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

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

WARNING! Use ONLY tubings supplied by Amersham Biosciences to ensure that the pressure specifications of the tubings are fulfilled.

WARNING! When using hazardous chemicals, make sure that the entire system has been flushed thoroughly with distilled water before service and maintenance.

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

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

WARNING! There must always be a sample loop or Superloop connected to ports 2 and 6 of the injection valve. This is to prevent liquid spraying out of the ports when switching the valve. This is especially dangerous if hazardous chemicals are used.
When using a sample pump for sample application directly onto the column, a tubing must be connected between ports 3 and 6 on the Injection valve.

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

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

2 Installation

2.1 General

ÄKTAbasic is assembled and fully tested before shipping.

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

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

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

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

2.1.1 Installation procedure overview

- Unpack ÄKTAbasic *12*
- Detach secured items and install items enclosed *13*
- Unpack and install Frac-901 or Frac-950 (optional) *15*
- Unpack and install the computer *15*
- Connect UniNet-1 data communication chain cabling *16*
- Connect mains power cabling *16*
- Complete the two first sections of the Installation record *17*
- Prepare ÄKTAbasic for the installation test *19*
- Run the installation test method *23*
- Evaluate the gradient *28*
- Evaluate the step response *28*
- Evaluate the UV response *28*
- Complete the Test record 30
- Complete the Registration form 31
- Complete the last section of the Installation record *18*
- Store photocopies of all records and forms in the System Logbook

2.2 Unpacking

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

2.3 Pre-requisites

WARNING! ÄKTAbasic must be connected to a grounded mains socket.

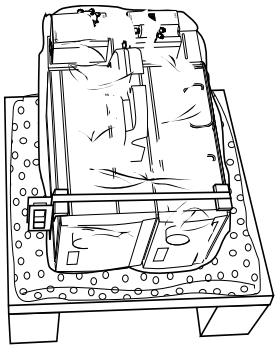
- Two people are recommended to lift ÄKTAbasic onto the workbench.
- To install ÄKTAbasic, a working area of about 200 x 80 cm is required.
- ÄKTAbasic requires 100-120/220-240 V~, 50-60 Hz electrical supply with safety grounding.
- Pliers are recommended for cutting plastic straps.
- A waste flask.
- The installation test requires the following solutions:
  - 1000 ml of distilled water for priming and purging the pump.
  - 500 ml of 0.4% acetone in distilled water.
  - 100 ml of 20% ethanol in distilled water.

2.4 Installation of ÄKTAbasic

Begin by creating a clean and dry working area of 200 x 80 cm that allows easy access. Then follow the step-by-step instructions below and check the Installation Record as you go along, see page 18.

Note: Some packing lists are included in the paper boxes.

1 After having removed the cardboard hood, check the contents against the attached packing list. Check also all included boxes. Store all the enclosed paper boxes and plastic bags in a convenient nearby place.



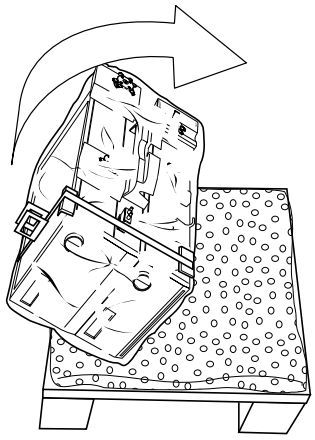
2 Raise the system to an upright position on the pallet.

3 Lift ÄKTAbasic onto the working area using the handle at the top front and the recess under the mounting plate at the top rear.

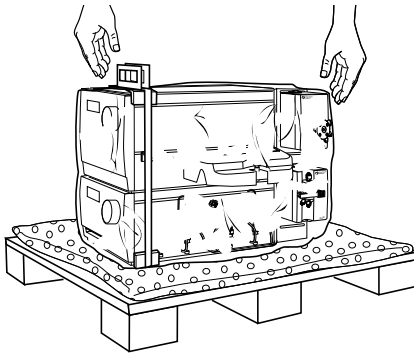
4 Cut the strap at the front and remove the handle and additional packing material.

Note: Take care not to damage the capillaries.

5 Pull off the plastic cover to uncover the system.



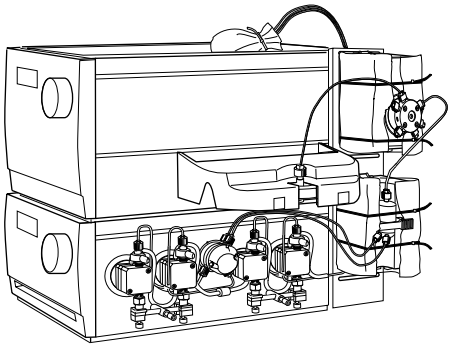
6 Save all the original packing material. If, for any reason, the equipment has to be repacked, for transportation or otherwise, it is important that the system can be safely packed using the original packing material.



7 Turn ÄKTAbasic to access the fluid component side of the system.

8 Detach the following items by cutting straps and removing red tape. Also remove the desiccant bags.

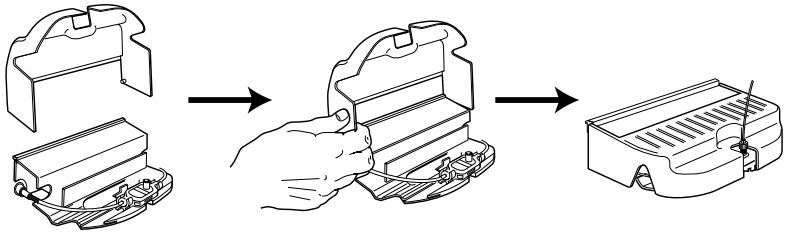
Note: Use pliers. Be careful not to cut any capillaries by accident. Do not lose any of the capillary marking tags.



- Valve INV-907, located at the top right side. Detach the valve from the attachment bracket to remove the protective foam. Reposition the valve to the top slot in the attachment bracket.
- Valve PV-908 (optional). Remove the protective foam and position the valve in the slot on the left side of the UV monitor (towards the front).
- Mixer M-925, located below valve INV-907. Detach the mixer from the attachment bracket to remove the protective foam. Reposition the mixer in the same slot in the attachment bracket.
- UV cell cover, located above the UV flow cell. Remove the red tapes.
- Capillary loops. Remove the red tape holding the capillary loops attached to valve INV-907 (and valve PV-908 if used).
- Place the waste tubings in a waste bottle and place the bottle in front of ÄKTAbasic, or wherever convenient.

- Unpack the inlet tubing contained in a plastic bag taped on top of UV-900.
- Make sure all items are securely fitted and that no capillary connection has worked loose or been tangled.
- Attach the column holder directly above the UV cell. Select a slot to suit the height of the column to be used.

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



1 The cover is a simple push fit onto the cell holder. Two small lugs on the cover locate in holes at the front and rear of the cell holder

2 The cover is then lowered over the cell holder.

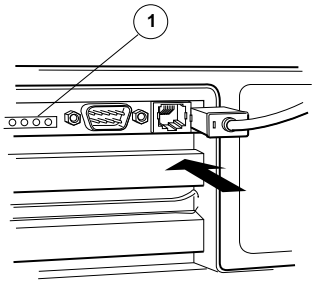
Note: Installation of fraction collectors and outlet valve PV-908 are also described in ÄKTAbasic Optional Configurations User Manual.

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

Note: Do not switch them on yet!

CAUTION! The mains power to ÄKTAbasic must be switched OFF before the UniNet-1 cabling is installed.

CAUTION! The UniNet connection to the computer must be made to the board with four LEDs (1).



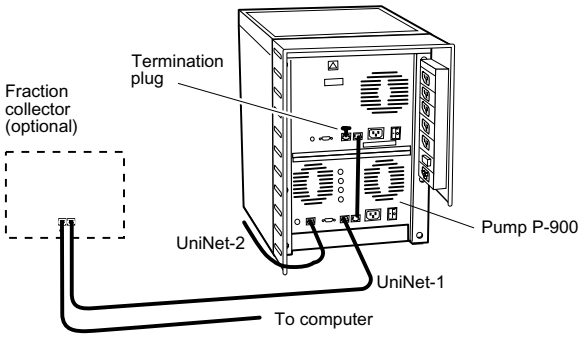
4 Connect a UniNet-1 cable between the computer and Pump P-900.

5 If a fraction collector is used:

- Connect a UniNet-1 cable between Pump P-900 and the fraction collector.

- Connect a UniNet-1 cable between the fraction collector and the computer.

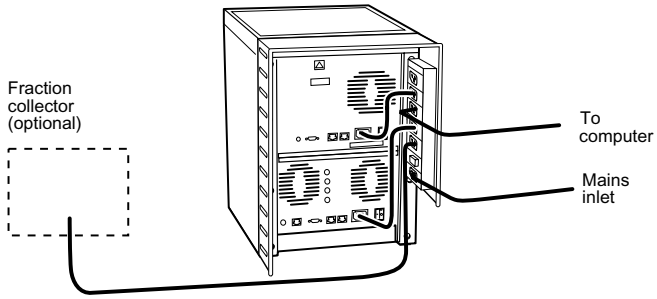
All other UniNet 1 cables are connected at delivery.



WARNING! ÄKTAbasic must be connected to a grounded mains socket.

1 Connect a mains cable supplied between ÄKTAbasic and a properly grounded mains socket according to the figures below.
Do not switch on.

2 If a fraction collector is used, connect a mains cable supplied between the fraction collector and a mains socket at the rear of ÄKTAbasic.



Complete the two first sections of the Installation record

The unpacking and installation phase of ÄKTAbasic is now completed.

Check	*Sign*	*Remarks*
1 Unpacking		
Contents according to packing lists.		
All packing material removed.		
No visible damage.		
2 Installation		
Injection valve waste tubings (port 4 and 5, marked W1 and W2) to waste reservoir.		
Fraction collector (optional) installed.		
Outlet valve tubing (port 2) connected to fraction collector (optional).		
Waste tubing (marked W3) extended to waste reservoir.		
Computer and printer installed.		
UniNet-1 (and UniNet-2, optional) cabling installed.		
Mains power cabling installed.		
Column holder installed.		
3 Installation test		
Solutions prepared.		
Tubings to piston seal rinsing system in 20% ethanol.		
ÄKTAbasic prepared.		
Installation Test method run.		
Installation Test results evaluated.		
Test Record completed.		
Registration Form completed.		
Test Record and copy of Registration form stored in System Logbook.		
Registration form posted to Service Administration.		
Installation Guide stored in User Manual box for future use.		

Table 2-1. Installation Record.

2.5 Installation test

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

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

Correct UV monitoring is tested by monitoring the acetone concentration at 265, 254 and 280 nm and calculating the absorbance ratios 265 nm/254 nm and 265 nm/280 nm.

For installation test instructions with Autosampler A-900 or A-905 connected to ÄKTAbasic 10, refer to *ÄKTAbasic Optional Configurations User Manual*.

2.5.1 Preparation of ÄKTAbasic

Startup the ÄKTAbasic separation unit

1 Switch on the separation unit using the mains switch located at the rear of the system.

Startup the computer and the UNICORN software

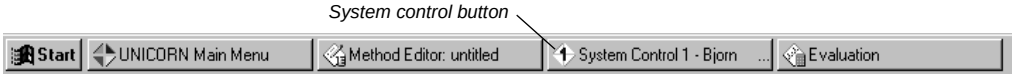
1 Switch on the computer, the display and the printer according to the instructions in the manufacturer manuals.

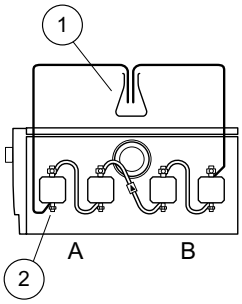
2 Log into Windows® by first pressing **Ctrl-Alt-Del,** and then clicking **OK.**

3 When the Windows desktop appears, start UNICORN by double-clicking on the UNICORN icon.

4 Select user **default** and enter **default** as password. Click **OK**.

5 Click the **System Control** button in the taskbar.





Priming the piston seal rinsing system of Pump P-900

1 Immerse the rinsing tubing in a flask (1) containing 20% ethanol in distilled water.

2 Connect a syringe to the rinsing tubing that is connected to the underside (2) of the left pump head on pump A. Slowly draw rinsing solution into the syringe. When rinsing solution starts to enter the syringe, continue to draw a few milliliters.

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

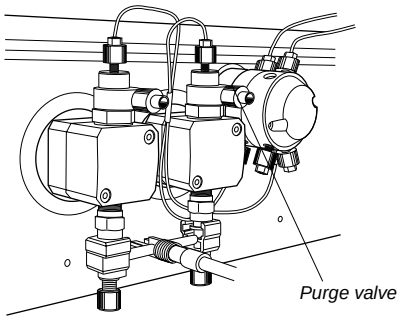
Purging Pump P-900

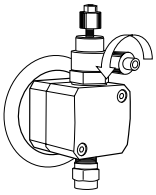
1 Immerse the inlet tubing of all pump modules, with filters, in the distilled water.

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

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

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





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

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

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

Testing pressure stability

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

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

2 **ÄKTAbasic 10**:
Manually, select pump flow 0.2 ml/min and run at 0%B (distilled water). Check on the pump display that the pressure reading is stable (variation less than ±0.02 MPa).

ÄKTAbasic 100:
Manually, select pump flow 10 ml/min and run at 0%B (distilled water). Check on the pump display that the pressure reading is stable (variation less than ±0.2 MPa).

3 **ÄKTAbasic 10**:
Manually, select pump gradient 100%B, length 0 and run with 0.2 ml/min (distilled water). Check on the pump display that the pressure reading is stable (variation less than ±0.02 MPa).

ÄKTAbasic 100:
Manually, select pump gradient 100%B, length 0 and run with 10 ml/min (distilled water). Check on the pump display that the pressure reading is stable (variation less than ±0.2 MPa).

4 Proceed to step 5 if the pressure is stable. If not, consult the *Pump P-900 User Manual* for trouble-shooting instructions.

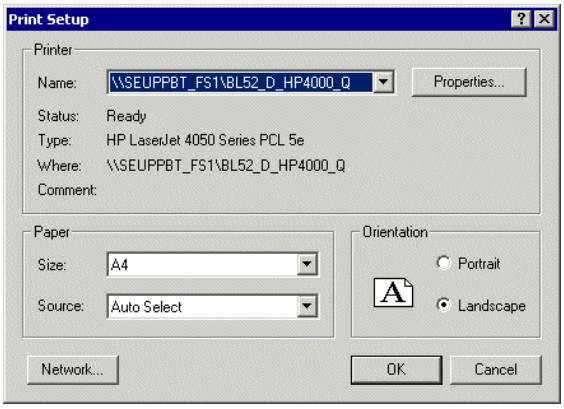
5 Click **END**.

6 Transfer the inlet tubing B into a flask containing 500 ml of 0.4% acetone in distilled water.

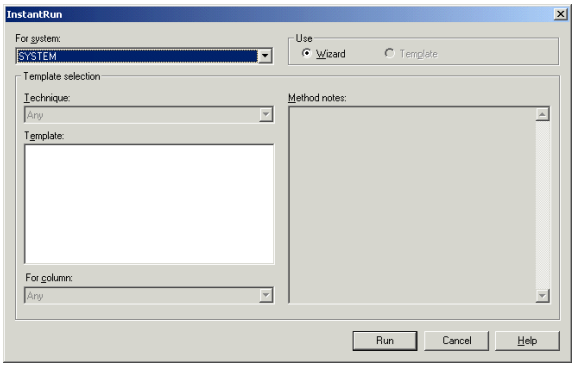
Installation Test Method Guide	
Buffer A:	Distilled water
Buffer B:	0.4% acetone in distilled water
Test flow rate:	5 ml/min in ÄKTAbasic 10 10 ml/min in ÄKTAbasic 100
Test run time:	Approximately 30 minutes

2.5.2 Running the installation test method

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

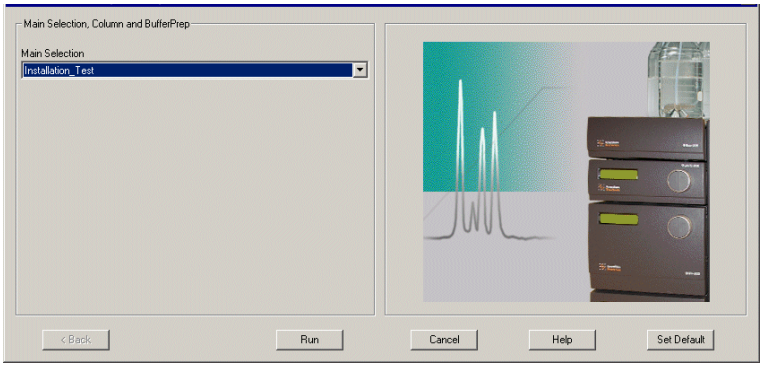


2 Click the **Instant Run** button  . The **Instant Run** window opens.

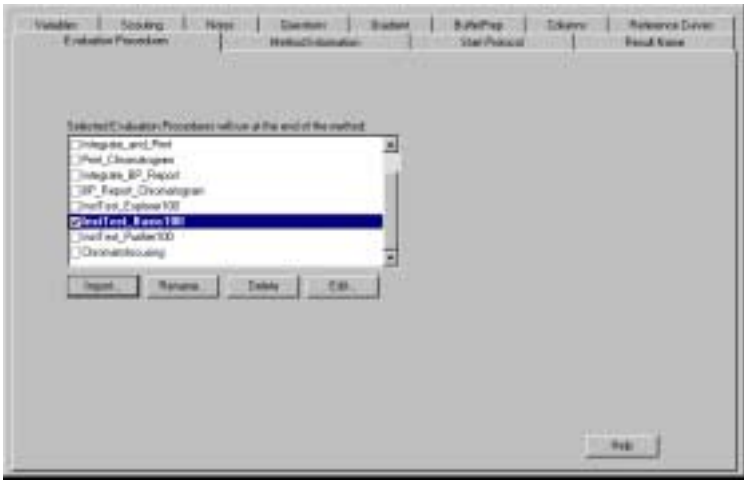


Select the appropriate system and click **Run**.

3 Select **Installation_Test** in the Method Wizard. Click **Run**.



4 In the **Evaluation Procedures** window, select the procedure for your system, for example, **InstTest_Basic100**.



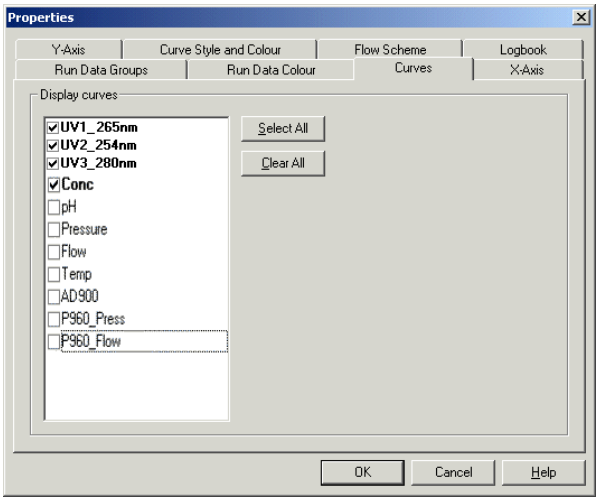
5 Click **Next** in the **Method Information** window.

6 Click **START** in the **Result Name** window to start the installation test.

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

7 To customize the **Curves** pane, right-click in the pane and select **Properties**. Click **Run**.

8 Click the **Curves** tab.

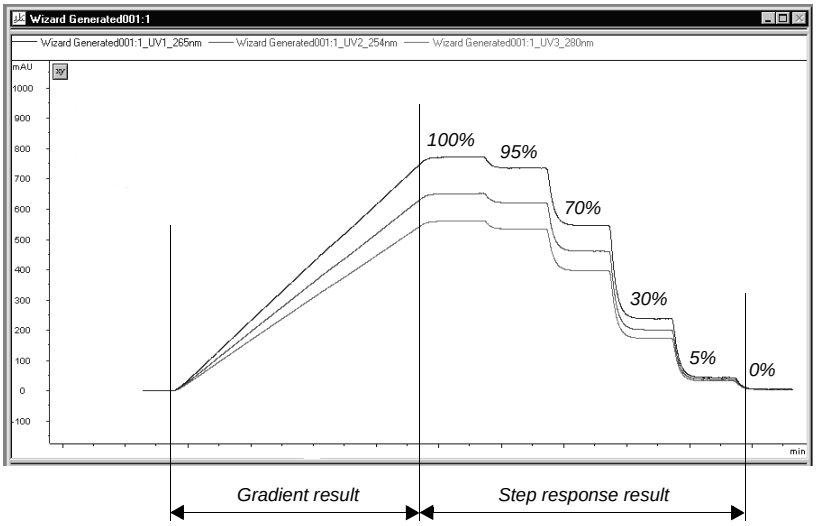


9 Select the following curves to be displayed:

- **UV1_265nm**
- **UV2_254nm**
- **UV3_280nm**
- **Conc**

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

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



2.5.3 Evaluating the installation test results

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

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

If any of the evaluated values falls outside the specified range, go to section *2.5.4 Correcting faulty evaluation results.*

Manual evaluation

If your chromatography system deviate from the standard configuration, e.g. if optional components have been installed, the automatic evaluation will not give a reliable result. A manual evaluation is required.

1 Select the **UNICORN Main menu** module.

2 Click on  in the **Results** window and then double-click on the **Wizard Generated001** icon to open the result file.

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

4 Click the **Curves** tab and select the following curves to be displayed:

- **Wizard Generated001:1_UV1_265nm@01,SMTH**
- **Wizard Generated001:1_UV2_254nm@02,SMTH**
- **Wizard Generated001:1_UV3_280nm@03,SMTH**
- **Wizard Generated001:1_Conc**

5 Click **OK**.

6 Click in the chromatogram window with the right mouse button and select **Marker**.

7 Read the absorbance for the steps corresponding to **Wizard Generated001**:**1_UV1_265nm@01,SMTH.** Move the vertical bar to the constant section of each plateau by dragging it. Enter the absorbance values (in mAU) in column 2 in the Step response table of the Test record (see page 30), leaving out the decimals.

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

- **Installation Test01:1_UV1_265nm@01,SMTH**
- **Installation Test01:1_UV2_254nm@02,SMTH**
- **Installation Test01:1_UV3_280nm@03,SMTH**

and enter the values in column 2 in the UV response table of the Test record (see page 30).

9 Click **Print** under **File** to print the chromatogram.

Evaluating the gradient

Place a ruler along the gradient part of curve **Wizard Generated001:1_UV1_265nm@01,SMTH** in the printed report.

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

Evaluating the step response

Calculate the relative adsorption plateau heights for curve **Wizard Generated001:1_UV1_265nm@01,SMTH** as follows:

- Subtract the base line value (0%B) from each of the values in column 2 in the Step response table of the Test record (see page 30) and enter the results in column 3.
- Divide each value in column 3 by the base line corrected value corresponding to 100%B, multiply by 100 and enter the results in column 4.

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

Evaluating the UV response

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 (see page 30) and enter the results in column 3.
- Calculate the absorbance ratios 265 nm/254 nm and 265 nm/280 nm using the values of column 3 and enter the results in column 4.

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

2.5.4 Correcting faulty evaluation results

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

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

Faulty gradient

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

Faulty step response

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

2.6 Test record

Date: .....

ÄKTAbasic serial no.: .....

2.6.1 Gradient test result

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

2.6.2 Step response test result

Step response table:

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

2.6.3 UV response test result

UV response table:

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

2.7 Registration form

IMPORTANT
WARRANTY REGISTRATION INFORMATION

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

Name: .....

Institute/company: .....

Address: .....

Department/location: .....

Post Code: .....

Phone Number: ..... Fax Number: .....

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

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

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

Support Agreement purchased with the instrument: Y / N

If YES give details: .....

Installer (name): .....

Signature of Installer:.....

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

Note: Fill in serial numbers over-leaf.

2.7.1 Components

ÄKTAbasic system serial numbers: .....

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

3 Operation

3.1 Getting started - basic handling

This section is written for users who are not familiar with the UNICORN software and ÄKTAbasic. Here you will learn the basics of UNICORN and how to operate ÄKTAbasic from UNICORN.

UNICORN is a software package for control and supervision of the ÄKTAdesign chromatography system. It runs on an IBM-compatible PC under Windows NT®, Windows 2000® or Windows XP®, and includes hardware for interfacing the controlling PC to the chromatography liquid handling parts of ÄKTAbasic.

In this chapter you will learn how to:

- create methods
- prepare the system for runs
- perform runs
- make simple evaluations
- make reports

Follow the instructions from page to page in front of the computer. The time will be well spent.

Note: To follow the instructions, it is not necessary to read the comments (written with smaller font) containing additional information.

3.1.1 Pre-requisites

The system and the software must be:

- installed and functioning
- monitors and pumps tested

as described in chapter *2 Installation*.

IMPORTANT! Before using ÄKTAbasic, read all the safety information in section *1.2 Safety*.

3.1.2 Typographical conventions

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

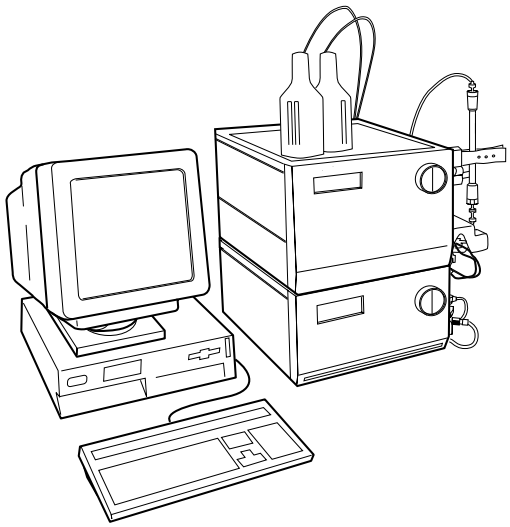
3.2 The system and the software

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

The modules are:

- Pump P-900, a binary high performance gradient pump for flow rates up to:
  - ÄKTAbasic 100 (P-901): 100 ml/min and pressures up to 10 MPa.
  - ÄKTAbasic 10 (P-903): 10 ml/min and pressures up to 25 MPa.
- Monitor UV-900, a multi-wavelength UV-Vis monitor for simultaneous monitoring of up to 3 wavelengths in the range 190-700 nm.

If installing a fraction collector, it should be placed to the right of the system.



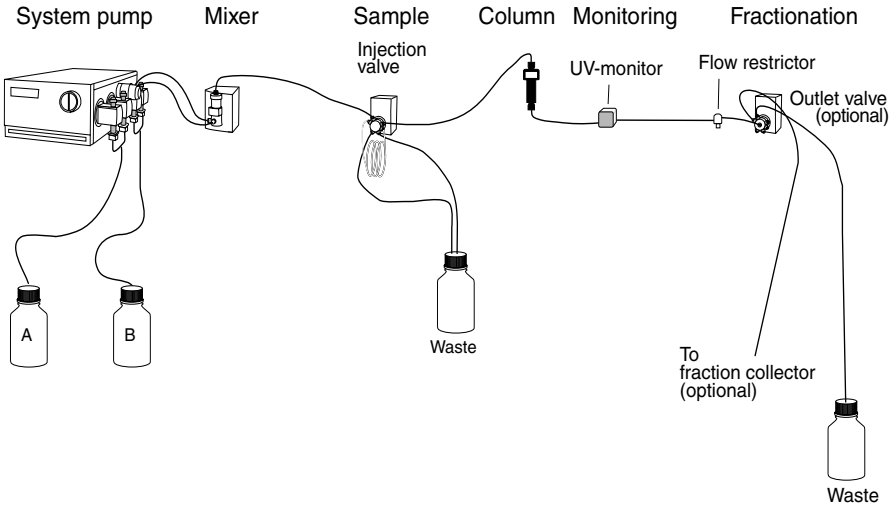
Components, such as the mixer, column and injection valve, are mounted to the right. Columns are snapped in place in column holders.

The separation unit is controlled from UNICORN software.

Pump P-900 and Monitor UV-900 can also be controlled individually from the modules without UNICORN software. In this section, however, you will only learn how to operate the chromatography system from UNICORN.

Switch on the chromatography system with the ON/OFF button located at the rear of ÄKTAbasic.

Comment:
The flow path between the different components in the system is shown and described below. It is not necessary to go through this in detail to make your first runs.



1 The pump has 4 pump heads, two for pump module A and two for pump module B. Pump module A is the one closest to the front.

2 Pump inlets A and B are placed in buffer A and B respectively and the buffer solutions are pumped to a mixer.

3 The flow path continues from the mixer to the injection valve.

4 A sample loop is connected between ports 2 and 6 on the injection valve. The sample loop is filled manually using a syringe. To perform this procedure, connect a fill port to port 3 on the injection valve.

5 After the injection valve, the flow is directed to the column, and then forward to the UV cell located inside the cell holder on Monitor UV-900.

6 The flow path continues to the flow restrictor. The flow restrictor generates a constant back-pressure to eliminate the risk of air bubbles entering the UV flow cell.

7 If fractionation (optional) is included, the outlet flow from the flow restrictor is directed to an outlet valve (optional), which is used to select fraction collection method, and to handle waste and flowthrough.

3.3 UNICORN overview

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

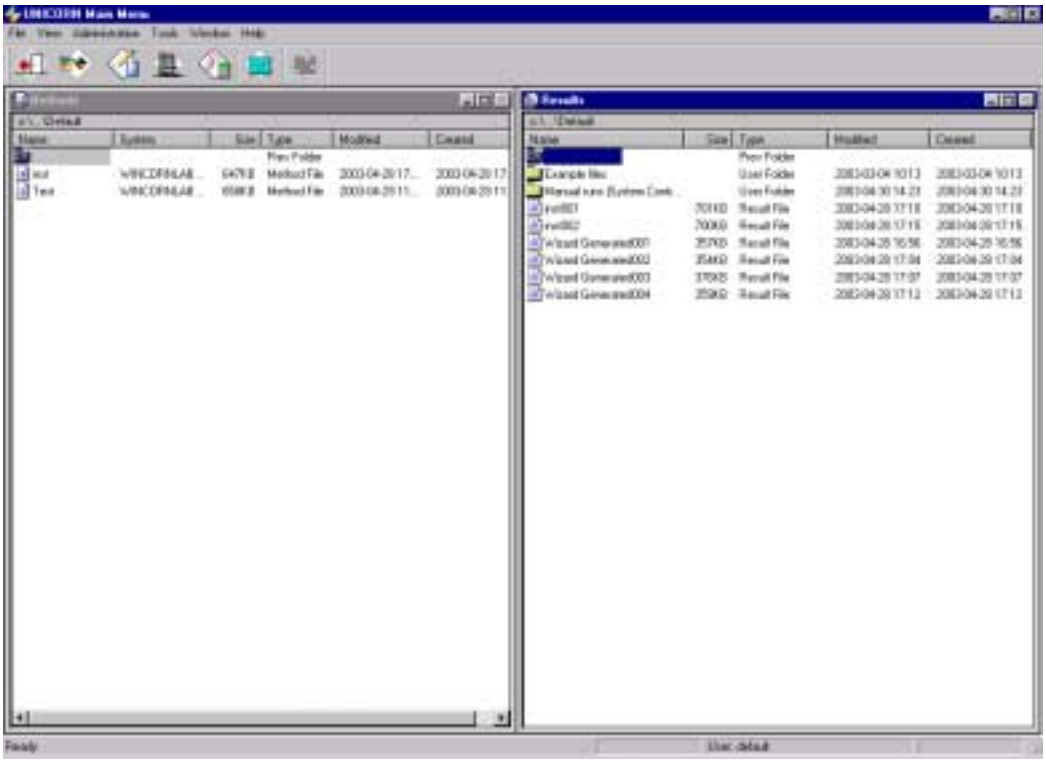
2 Start UNICORN by double-clicking on the UNICORN icon.

3 An information window appears during start-up.

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

Note: You should enter users and individual passwords before starting using ÄKTAbasic on a regular basis.

5 Eventually, the UNICORN **Main menu** window appears on the screen.



6 The **Main menu** window is the central part of the UNICORN displays. It is mainly used for file handling. From this window you navigate through the control system. It is mainly used for file handling.
In the **Methods** pane to the left in **Main menu**, all method files that you create are displayed. A method file contains a series of instructions for controlling a run.
In the **Results** pane to the right, all result files are displayed. A result file is the result from a run, including all documentation (e.g. the method used) and the generated chromatogram.

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

- Method Editor — opens a dialog window for creating new methods.

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

- Evaluation — opens a dialog window for evaluating your results.

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

Additional buttons are provided in the toolbar. These are:

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

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

- Method Queue* — opens a dialog window for defining a new Method Queue.

- Existing Method Queue — opens a dialog window for showing a Method Queue that is running.

* Method Queues are used to link several methods together.

3.3.1 Help

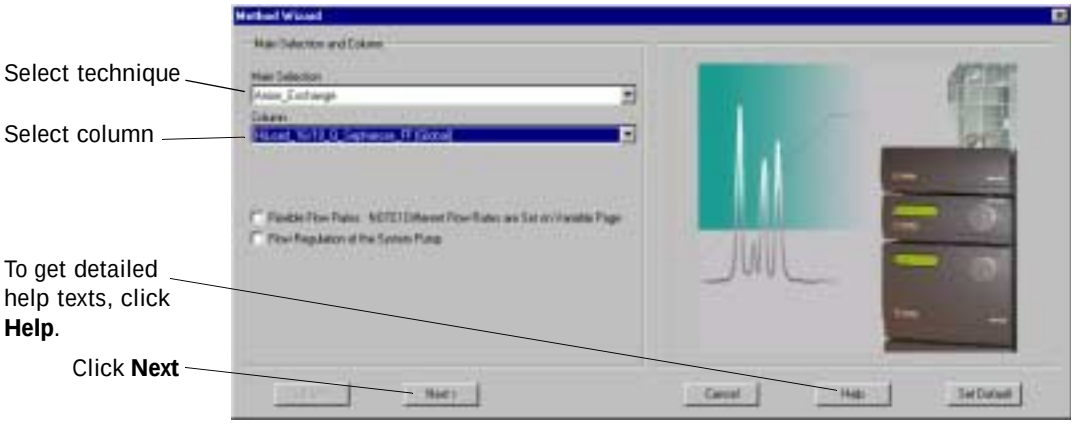
Comprehensive on-line help is available. To get help about an instruction or module, place the cursor on the instruction/module and press the **F1** key. Alternatively, click on the **Help** menu in the upper right corner of each module and select **Help for.....** to get general help about the current instruction or module and find new help topics, or **Index** for a specific topic. In any dialog, click on the **Help** button to get help on how to use the current active dialog.

3.4 Creating a method

The UNICORN software is supplied with a *Method Wizard* used for creating new methods. The wizard is a number of dialog windows with questions and instructions that help you creating the method.

To create a method:

1 Click the **Method Wizard** icon  in the **Method Editor** module. If required, choose which system you want to use and click **OK**. The **Method Wizard** window appears.



Note: You can restore all settings to default values by clicking **Set Default** *(can only be done in this dialog).*

2 Select a chromatographic technique, for example **Anion_Exchange.**

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

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

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

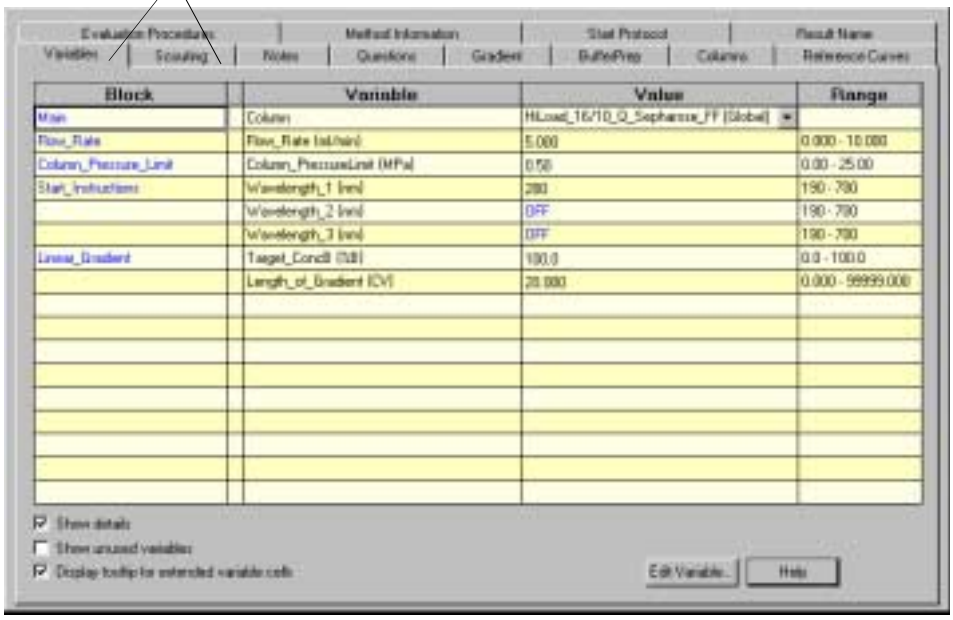
Comment:
If you do not find your column in the list, you can add one. Refer to the UNICORN User Manual.

4 Select if required, **Flexible Flow rates** and/or **Flow Regulation of the System pump**.

5 Click **Next** to go through the subsequent windows. In each window, select the appropriate parameter values.

6 Click **Finish** in the last window. The **Run Setup** window appears.

Click here to select page



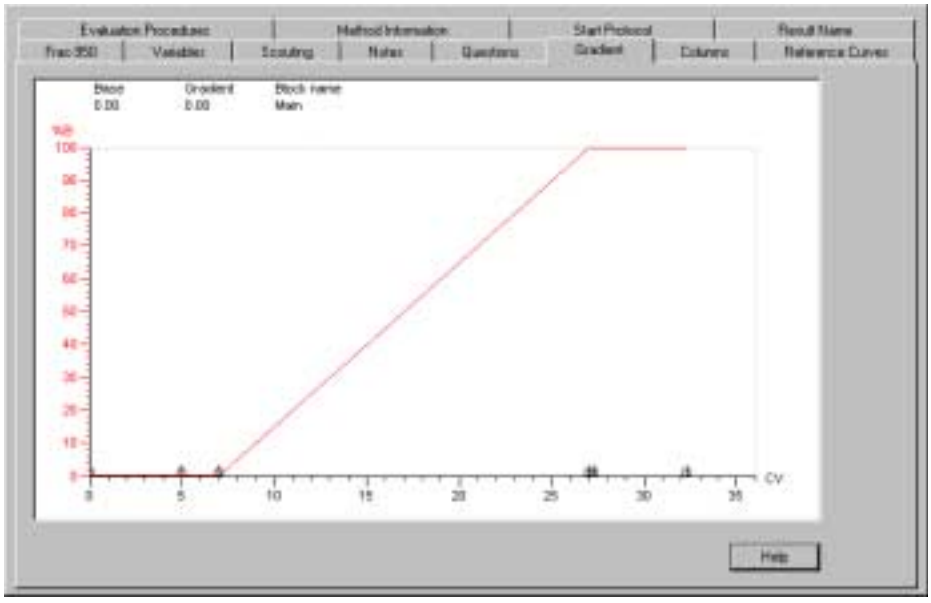
Run setup consists of a number of pages. You will only look at a few now. You select a page by clicking the respective tab at the top of the window.

7 On the **Variables** page, the method is presented by a number of blocks. The blocks represent the typical steps in a chromatographic run:

- Start instructions
- Column equilibration
- Sample injection
- Wash out unbound sample
- Fractionation
- Gradient
- Clean after elution
- Re-equilibration

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

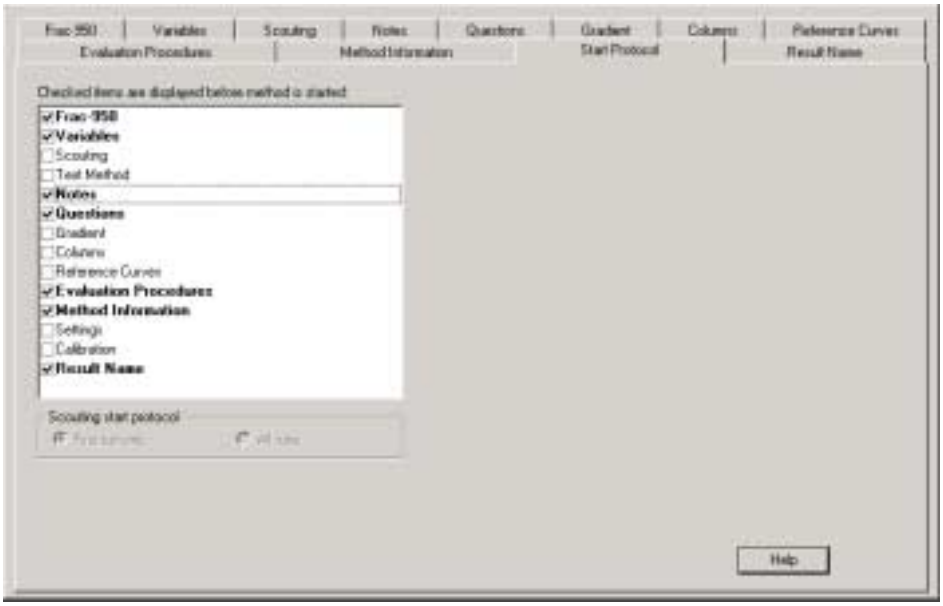
8 Click the **Gradient** page to view the method graphically.



The length of each block is marked at the bottom of the graph.

Click the x-axis to view the method in time, volume or column volumes.

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



Note: When Scouting is selected, check the First run only button to enable automatic scouting runs.

10 To save the method, select **File:Save.** In the **Save** dialog, enter a name. Store the method in the directory of your choice by double-clicking on a directory. Click **OK**. In the **UNICORN Main Menu** module, the method appears in the **Methods** window.

Comment:
The method name, followed by three consecutive numbers starting with 001 will then be used as default name for the result file of your method after runs.

Now you are ready to start a run. Go to section 3.8.

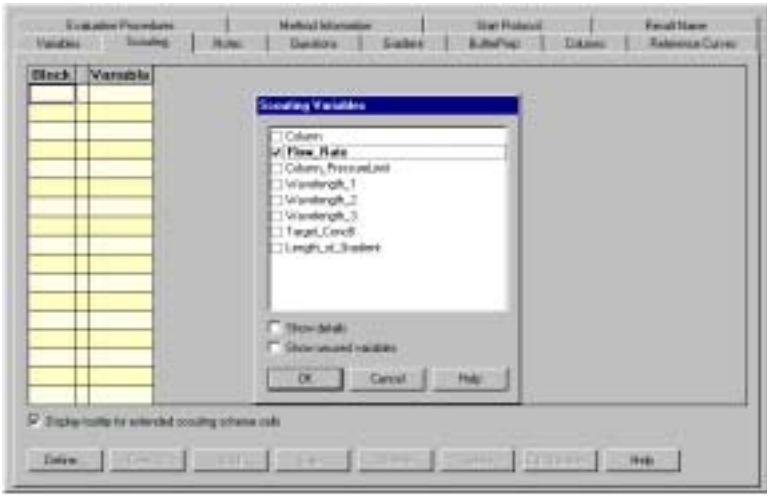
3.5 Scouting

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

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

1 Create a new method as described in section *3.4*.

2 When the **Run Setup** window appears, click the **Scouting** tab.

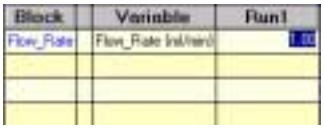


3 A list of all the variables will appear. Select the variable **Flow_Rate** and any other variable you wish to alter.

4 Click **OK**. The selected scouting variables will appear to the left with their default values inserted.

Note: Values for variables selected for scouting are greyed on the Variable page and cannot be changed there.

5 To change a variable value, position the cursor in the Run value field and double-click with the left mouse button. Type the new value.



6 To add a table column for the next run, click **Add**. A second column appears with the values from the previous run copied. Change the values as required.

If you want to insert a new run column after a specific column in the scouting scheme, position the cursor in the column and click **Insert**. A new column with identical values appears directly after the selected column.

7 Repeat step 6 until you have defined all the runs you require. If necessary, use the horizontal scroll bar to see more runs.

8 Click **Run1**, **Run2**, etc. at the top of the scheme with the right mouse button to toggle between **Run** and **Excluded** for the different runs. Those marked **Excluded** will not be run. A scouting scheme is now defined.

9 To save the scouting method, select **File:Save**.

10 Prepare the system, and start the run as described in sections 3.7 and 3.8.

When the method is started, all the runs in the scheme will be performed automatically and the set flow rate for each run will be prepared automatically. Each run in the scouting scheme will generate a separate result file which are all stored in a special scouting directory.

3.6 Fluid handling components

3.6.1 Columns and tubing

A wide range of pre-packed columns ion exchange, size exclusion, hydrophobic interaction, reversed phase and affinity chromatography are suitable for use with ÄKTAbasic 10/100. A comprehensive list of the recommended pre-packed columns is given overleaf together with information on the recommended tubing for each column.

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

ÄKTAbasic 10 tubing

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

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

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

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

ÄKTAbasic 100 tubing

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

3.6.2 Recommended tubing and columns – ÄKTAbasic 10

The tables below shows which tubing kit should be used for each column. It is important that the recommendations in these tables are followed. The tubing to be changed is described in *Reference information,* section *6.5.*

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

X = recommended tubing kit
P = recommended for purity checks only
RF = can be used with indicated tubing if the optimal recommended flow rate in the column list is reduced in order not to exceed the column maximum pressure

Ion Exchange Columns

Code no	*Column name*	*0.25*	*0.50*
17-5177-01	Mini Q™ 4.6/50 PE	X, P	
17-5166-01	Mono Q™ 5/50 GL	X	
17-5167-01	Mono Q 10/100 GL	RF	X
17-0506-01	Mono Q HR 16/10	RF	X
17-5178-01	Mini S™ 4.6/50 PE	X, P	
17-5168-01	Mono S™ 5/50 GL	X	
17-5169-01	Mono S 10/100 GL	RF	X
17-0507-01	Mono S HR 16/10	RF	X
17-5181-01	SOURCE™ 15Q 4.6/100 PE	RF	X
17-5182-01	SOURCE 15S 4.6/100 PE	RF	X
17-1177-01	RESOURCE™ Q, 1 ml		X
17-1179-01	RESOURCE Q, 6 ml		X
17-1178-01	RESOURCE S, 1 ml		X
17-1180-01	RESOURCE S, 6 ml		X
17-1153-01	HiTrap™ Q HP, 1 ml		X
17-1154-01	HiTrap Q HP, 5 ml		RF
17-1151-01	HiTrap SP HP, 1 ml		X
17-1152-01	HiTrap SP HP, 5 ml		RF
17-6002-33	HiTrap IEX Selection Kit, 7 x 1 ml		X
17-5053-01	HiTrap Q FF, 1 ml		X

Code no	Column name	0.25	0.50
17-5156-01	HiTrap Q FF, 5 ml		RF
17-5054-01	HiTrap SP FF, 1 ml		X
17-5157-01	HiTrap SP FF, 5 ml		RF
17-5055-01	HiTrap DEAE FF, 1 ml		X
17-5154-01	HiTrap DEAE FF, 5 ml		RF
17-5056-01	HiTrap CM FF, 1 ml		X
17-5155-01	HiTrap CM FF, 5 ml		RF
17-5162-01	HiTrap ANX FF (high sub), 1 ml		X
17-5163-01	HiTrap ANX FF (high sub), 5 ml		RF
17-5158-01	HiTrap Q XL, 1 ml		X
17-5159-01	HiTrap Q XL, 5 ml		RF
17-5160-01	HiTrap SP XL, 1 ml		X
17-5161-01	HiTrap SP XL, 5 ml		RF
17-5092-01	HiPrep 16/10 Q XL		RF
17-5093-01	HiPrep 16/10 SP XL		RF
17-5090-01	HiPrep 16/10 DEAE FF		RF
17-5091-01	HiPrep 16/10 CM FF		RF
17-5190-01	HiPrep 16/10 Q FF		RF
17-5191-01	HiPrep 16/10 ANX FF (high sub)		RF
17-5192-01	HiPrep 16/10 SP FF		RF
17-1064-01	HiLoad™ 16/10 Q Sepharose™ HP		X
17-1066-01	HiLoad 26/10 Q Sepharose HP		
17-1137-01	HiLoad 16/10 SP Sepharose HP		X
17-1138-01	HiLoad 26/10 SP Sepharose HP		

Reversed Phase Columns

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

Size Exclusion Columns

Code no	*Column name*	*0.25*	*0.50*
17-1458-01	Superdex™ Peptide PC 3.2/30	X, P	
17-5176-01	Superdex Peptide 10/30O GL	X	
17-0771-01	Superdex 75 PC 3.2/30	X, P	
17-5174-01	Superdex 75 10/300 GL	X	
17-1089-01	Superdex 200 PC 3.2/30	X, P	
17-5175-01	Superdex 200 10/300 GL	X	
17-5172-01	Superose™ 6 10/300 GL	X	
17-0673-01	Superose 6 PC 3.2/30	X,P	
17-5173-01	Superose 12 10/300 GL	X	
17-0674-01	Superose 12 PC 3.2/30	X,P	
17-1408-01	HiTrap Desalting, 5 ml		RF
17-5087-01	HiPrep 26/10 Desalting		RF
17-1139-01	HiLoad 16/60 Superdex 30 prep grade		X
17-1140-01	HiLoad 26/60 Superdex 30 prep grade		X
17-1068-01	HiLoad 16/60 Superdex 75 prep grade		X
17-1070-01	HiLoad 26/60 Superdex 75 prep grade		X
17-1069-01	HiLoad 16/60 Superdex 200 prep grade		X
17-1071-01	HiLoad 26/60 Superdex 200 prep grade		X
17-1165-01	HiPrep 16/60 Sephacryl™ S-100 HR		RF
17-1194-01	HiPrep 26/60 Sephacryl S-100 HR		RF
17-1166-01	HiPrep 16/60 Sephacryl S-200 HR		RF
17-1195-01	HiPrep 26/60 Sephacryl S-200 HR		RF
17-1167-01	HiPrep 16/60 Sephacryl S-300 HR		RF
17-1196-01	HiPrep 26/60 Sephacryl S-300 HR		RF

Hydrophobic Interaction Columns

Code no	*Column name*	*0.25*	*0.50*
17-1184-01	RESOURCE ETH 1 ml		X
17-1185-01	RESOURCE ISO 1 ml		X
17-1186-01	RESOURCE PHE 1 ml		X
17-1349-01	HiTrap HIC Selection Kit, 5 x 1 ml		X
17-1085-01	HiLoad 16/10 Phenyl Sepharose HP		X
17-1086-01	HiLoad 26/10 Phenyl Sepharose HP		
17-5095-01	HiPrep 16/10 Phenyl FF (high sub)		RF
17-5094-01	HiPrep 16/10 Phenyl FF (low sub)		RF
17-5096-01	HiPrep 16/10 Butyl FF		RF
17-5097-01	HiPrep 16/10 Octyl FF		RF
17-1355-01	HiTrap Phenyl FF (high sub), 1 ml		X
17-5193-01	HiTrap Phenyl FF (high sub), 5 ml		RF
17-1353-01	HiTrap Phenyl FF (low sub), 1 ml		X
17-5194-01	HiTrap Phenyl FF (low sub) 5 ml		RF
17-1351-01	HiTrap Phenyl HP, 1 ml		X
17-5195-01	HiTrap Phenyl HP, 5 ml		RF
17-1359-01	HiTrap Octyl FF, 1 ml		X
17-5196-01	HiTrap Octyl FF, 5 ml		RF
17-1357-01	HiTrap Butyl FF, 1 ml		X
17-5197-01	HiTrap Butyl FF, 5 ml		RF

Affinity Columns

Code no	*Column name*	*0.25*	*0.50*
17-0408-01	HiTrap Chelating HP, 1 ml		X
17-0409-01	HiTrap Chelating HP, 5 ml		RF
17-0716-01	HiTrap NHS-activated HP, 1 ml		X
17-0717-01	HiTrap NHS-activated HP, 5 ml		RF
17-5130-01	GSTrap™ FF, 1 ml (5 pcs)		X
17-5130-02	GSTrap FF, 1 ml (2 pcs)		X
17-5131-01	GSTrap FF, 5 ml		RF
17-5234-01	GSTPrep™ FF 16/10		RF
17-5079-02	HiTrap rProtein A FF, 1 ml (2 pcs)		X

Code no	Column name	0.25	0.50
17-5079-01	HiTrap rProtein A FF, 1 ml (5 pcs)		X
17-5080-01	HiTrap rProtein A FF, 5 ml		RF
17-0402-03	HiTrap Protein A HP, 1 ml (2 pcs)		X
17-0402-01	HiTrap Protein A HP, 1 ml (5 pcs)		X
17-0403-01	HiTrap Protein A HP, 5 ml		RF
17-0404-03	HiTrap Protein G HP, 1 ml (2 pcs)		X
17-0404-01	HiTrap Protein G HP, 1 ml (5 pcs)		X
17-0405-01	HiTrap Protein G HP, 5 ml		RF
17-0406-01	HiTrap Heparin HP, 1 ml		X
17-0407-01	HiTrap Heparin HP, 5 ml		RF
17-5189-01	HiPrep 16/10 Heparin FF		RF
17-0412-01	HiTrap Blue HP, 1 ml		X
17-0413-01	HiTrap Blue HP, 5 ml		RF
17-5110-01	HiTrap IgM Purification HP, 1 ml		X
17-5111-01	HiTrap IgY Purification HP, 5 ml		RF
17-5112-01	HiTrap Streptavidin HP, 1 ml		X
17-5143-02	HiTrap Benzamidine FF (high sub), 1 ml (2 pcs)		X
17-5143-01	HiTrap Benzamidine FF (high sub), 1 ml (2 pcs)		X
17-5144-01	HiTrap Benzamidine FF (high sub), 5 ml		RF

Chromatofocusing Columns

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

3.6.3 Recommended tubing and columns – ÄKTAbasic 100

The tables below shows which tubing kit should be used for each column. It is important that the recommendations in the table is followed. The tubing to be changed is described in *Reference information* section *6.5*.

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

X = recommended tubing kit
(+) = may improve resolution
(x) = can be used if flow < 5 ml/min
(o) = should be used if flow > 30 ml/min

Ion Exchange Columns

Code no	*Column name*	*0.50*	*0.75*	*1.0*
17-5166-01	Mono Q™ 5/50 GL	(+)	X	
17-5167-01	Mono Q 10/100 GL		X	
17-5168-01	Mono S™ 5/50 GL	(+)	X	
17-5169-01	Mono S 10/100 GL		X	
17-1177-01	RESOURCE™ Q, 1 ml	(+)	X	
17-1179-01	RESOURCE Q, 6 ml		X	(o)
17-1178-01	RESOURCE S, 1 ml	(+)	X	
17-1180-01	RESOURCE S, 6 ml		X	(o)
17-1153-01	HiTrap™ Q HP, 1 ml	(+)	X	
17-1154-01	HiTrap Q HP, 5 ml		X	
17-1151-01	HiTrap SP HP, 1 ml	(+)	X	
17-1152-01	HiTrap SP HP, 5 ml		X	
17-6002-33	HiTrap IEX Selection Kit, 7 x 1 ml	(+)	X	
17-5053-01	HiTrap Q FF, 1 ml	(+)	X	
17-5156-01	HiTrap Q FF, 5 ml		X	
17-5054-01	HiTrap SP FF, 1 ml	(+)	X	
17-5157-01	HiTrap SP FF, 5 ml		X	
17-5055-01	HiTrap DEAE FF, 1 ml	(+)	X	
17-5154-01	HiTrap DEAE FF, 5 ml		X	

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

Size Exclusion Columns

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

Hydrophobic Interaction Columns

Code no	*Column name*	*0.50*	*0.75*	*1.0*
17-1184-01	RESOURCE ETH 1 ml		X	
17-1185-01	RESOURCE ISO 1 ml		X	
17-1186-01	RESOURCE PHE 1 ml		X	
17-1187-01	RESOURCE HIC Test kit, 3x1 ml	(+)	X	
17-1349-01	HiTrap HIC Selection Kit, 5x1 ml	(+)		X
17-1085-01	HiLoad 16/10 Phenyl Sepharose HP		(x)	X
17-1086-01	HiLoad 26/10 Phenyl Sepharose HP		(x)	X
17-5095-01	HiPrep 16/10 Phenyl FF (high sub)		X	
17-5094-01	HiPrep 16/10 Phenyl FF (low sub)		X	
17-5096-01	HiPrep 16/10 Butyl FF		X	
17-5097-01	HiPrep 16/10 Octyl FF		X	
17-1355-01	HiTrap Phenyl FF (high sub), 1 ml		X	
17-5193-01	HiTrap Phenyl FF (high sub), 5 ml		RF	
17-1353-01	HiTrap Phenyl FF (low sub), 1 ml		X	
17-5194-01	HiTrap Phenyl FF (low sub), 5 ml		RF	
17-1351-01	HiTrap Phenyl HP, 1 ml		X	
17-5195-01	HiTrap Phenyl HP, 5 ml		RF	
17-1359-01	HiTrap Octyl FF, 1 ml		X	
17-5196-01	HiTrap Octyl FF, 5 ml		RF	
17-1357-01	HiTrap Butyl FF, 1 ml		X	
17-5197-01	HiTrap Butyl FF, 5 ml		RF	

Reversed Phase Columns

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

Affinity Columns

Code no	*Column name*	*0.50*	*0.75*	*1.0*
17-0408-01	HiTrap Chelating HP, 1 ml	(+)	X	
17-0409-01	HiTrap Chelating HP, 5 ml		X	
17-0716-01	HiTrap NHS-activated HP, 1 ml	(+)	X	
17-0717-01	HiTrap NHS-activated HP, 5 ml		X	
17-5130-01	GSTrap™ FF, 1 ml (5 pcs)	(+)	X	
17-5130-02	GSTrap FF, 1 ml (2 pcs)	(+)	X	
17-5131-01	GSTrap FF, 5 ml		X	
17-5234-01	GSTPrep™ FF 16/10		X	
17-5079-02	HiTrap rProtein A FF, 1 ml (2 pcs)	(+)	X	
17-5079-01	HiTrap rProtein A FF, 1 ml (5 pcs)	(+)	X	
17-5080-01	HiTrap rProtein A FF, 5 ml		X	
17-0402-03	HiTrap Protein A HP, 1 ml (2 pcs)	(+)	X	
17-0402-01	HiTrap Protein A HP, 1 ml (5 pcs)	(+)	X	
17-0403-01	HiTrap Protein A HP, 5 ml		X	
17-0404-03	HiTrap Protein G HP, 1 ml (2 pcs)	(+)	X	
17-0404-01	HiTrap Protein G HP, 1 ml (5 pcs)	(+)	X	
17-0405-01	HiTrap Protein G HP, 5 ml		X	
17-0406-01	HiTrap Heparin HP, 1 ml	(+)	X	
17-0407-01	HiTrap Heparin HP, 5 ml		RF	

Code no	Column name	0.50	0.75	1.0
17-5189-01	HiPrep 16/10 Heparin FF		X	
17-0412-01	HiTrap Blue HP, 1 ml	(+)	X	
17-0413-01	HiTrap Blue HP, 5 ml		RF	
17-5110-01	HiTrap IgM Purification HP, 1 ml	(+)	X	
17-5111-01	HiTrap IgY Purification HP, 5 ml		X	
17-5112-01	HiTrap Streptavidin HP, 1 ml	(+)	X	
17-5143-02	HiTrap Benzamidine FF (high sub), 1 ml (2 pcs)	(+)	X	
17-5143-01	HiTrap Benzamidine FF (high sub), 1 ml (2 pcs)	(+)	X	
17-5144-01	HiTrap Benzamidine FF (high sub), 5 ml		RF	

Chromatofocusing Columns

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

3.6.4 Selecting tubing kit for other columns

For the columns not available in the table above, select the tubing kit as described below.

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

1 Note the maximum specified back-pressure for the column at the variable **Pressure limit** on the **Variables** page in Run Setup.

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

2 Install the column and test to run at the flow rate and with the eluents to be used, with the i.d. 0.75 mm tubing kit.

3 a) If the generated back-pressure at the flow to be used is within the set column pressure limit, use the tubing kit already installed.

b) If the generated back-pressure at the flow rate to be used is well beyond the column pressure limit and the predicted peak volume is less than 1 ml, it is preferable to change to a narrower tubing kit, i.d. 0.5 mm. If the demands on low band-broadening are less critical, use the tubing kit already installed.

c) If the generated back-pressure at the flow rate to be used exceeds the set column pressure limit, change to a wider tubing kit, i.d. 1.0 mm. If the set column pressure limit is still exceeded with the i.d. 1.0 mm tubing kit installed, change to Flow restrictor FR-902, and check the generated back-pressure according to the description below.

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

3.6.5 Extra system pressure measurement

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

1 Set the injection valve (INV-907) in position WASTE.

2 Run the pump at the mandatory or intended flow rate.

3 Make a note of the back-pressure on the pump display or in the **Run Data** pane in UNICORN.

4 Add this value to the pressure limit value for the column (e.g. 0.5 MPa for HiLoad or HiTrap).

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

1 In the **Method Editor**, select **Edit:Column list** to open the **Column List** dialog window. Clicking a column in the list will display its parameters in the field to the right of the box.

2 Click **Edit** to display the **Edit Column** dialog. In the **Parameter** column, enter in the field for **Max pressure** the new unit pressure limit, 0.5 MPa + the measured value. Click **Replace** after the new value has been entered.

3 Click **Save as** and enter a new name of your column. You can choose to save the column globally, i.e. available to all users, by checking the **Save as global** box. However, we recommend to clear the **Save as global** box in this situation. Click **OK**.

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

3.6.6 Sample application overview

With ÄKTAbasic, the sample can be applied in different ways to suit the application and the sample volume.

In the standard system the sample is applied using sample loops, filled manually with a syringe.

The sample can also be applied using sample pump P-960, Autosampler A-900 or A-905, and Superloop™. These are not standard components in ÄKTAbasic. However, they are available as accessories and described in *ÄKTAbasic Optional Configurations User manual.*

The sample injection technique is selected in the **Sample Injection** dialog in the Method Wizard. See also the method notes in UNICORN.

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

Sample application technique	***Volume to inject***
Sample loop manual filling autmated filling	 0–2[1] ml 0.1–2[1] ml
Directly onto the column using Pump P-960[2] or the system pump	> 1 ml
Superloop[3]	1–150 ml
Autosampler[4]	1–500 µl

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

[2] How to apply the sample directly onto the column using Pump P-960 is described in the *ÄKTAbasic Optional Configurations User Manual.*

[3] How to use a Superloop is described in the *ÄKTAbasic Optional Configurations User Manual.*

[4] Depends on the autosampler model and tubing configuration. Refer to the respective user manual.

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

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

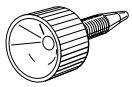
The following section describes manual filling of sample loops. The other sample application techniques are described in the *ÄKTAbasic Optional Configurations User Manual*.

3.6.7 Manual filling of sample loops

Manual sample injection is selected in the **Sample Injection** page in the Method Wizard.

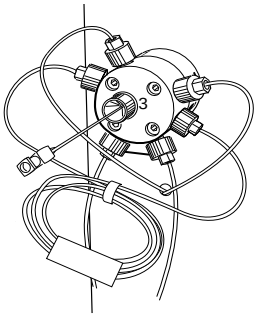
Preparation

Prepare the injection valve as follows:



1 Loosely thread the supplied injection fill port screw into valve port 3.

2 Insert the supplied injection needle (o.d. 0.7 mm) into the injection fill port.



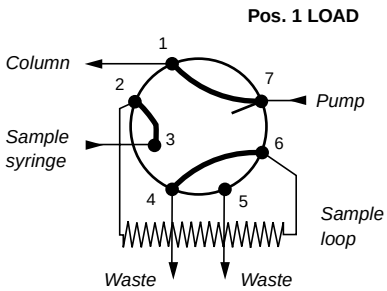
3 Tighten the fill port until the nozzle has formed a seal around the needle's tip, i.e. when it feels as if you are penetrating a septum at the end of the injection fill port. The seal should allow easy insertion and removal of the needle.

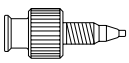
4 Mount the syringe holder on the fill port.

5 Ensure that waste tubing is connected to port 4 of the injection valve.

6 Mount the sample loop between ports 2 and 6 of the injection valve.

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





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

Five sizes of sample loop are available:

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

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

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

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

Partial filling

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

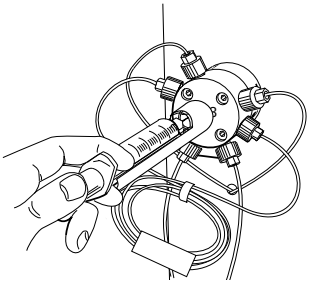
Partial filling is achieved as follows:

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

1 Set the injection valve to position LOAD.

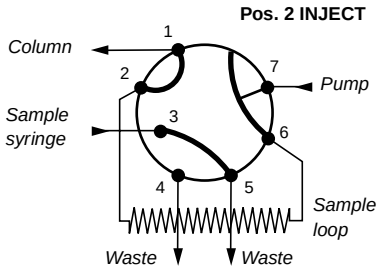
2 Load the syringe with a large volume of buffer (5 times the loop volume).

3 Fill the sample loop carefully with buffer.



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

Note: If the syringe is taken out when the injection valve is in position LOAD, self drainage will occur and air will enter the sample loop.



5 Load the syringe with the required volume of sample.

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

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

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

7 Gently load the syringe contents into the sample loop.

8 Leave the syringe in position. The sample will be injected onto the column when the valve is switched to INJECT in the method.

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

Complete filling

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

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

Complete filling is achieved as follows:

1 Set the injection valve to position LOAD.

2 Load the syringe with sample (2–5 times the loop volume).

3 Gently load the syringe contents into the loop.

4 Leave the syringe in position. The sample will be injected onto the column when the valve is switched to INJECT in the method.

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

Emptying the sample loop

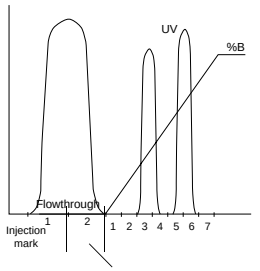
When emptying the sample loop, a buffer volume of at least 5 times the sample loop volume should be used to flush the loop and ensure that all sample is injected onto the column.

3.6.8 Collecting fractions

Fractions can be collected with a fraction collector (optional). The software makes it possible to fractionate in different ways:

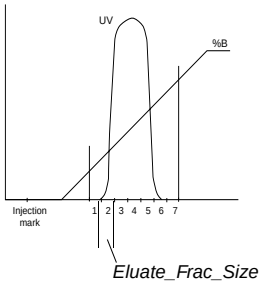
- Flowthrough fractionation.
- Fixed volume fractionation and/or peak fractionation.

Fractionation is selected and specified in the fractionation dialogs in the Method Wizard.
Fraction collection is described in detail in *ÄKTAbasic Optional Configurations User Manual*.



Flowthrough fractionation

Flowthrough fractionation means that fixed volumes are collected before elution fractionation starts. This fractionation method is available in all methods, except gel filtration methods. The fractionation volume is set in the **Flowthrough_Fractionation** dialog in the Method Wizard.

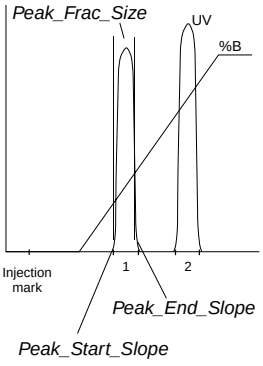


Fixed volume and/or peak fractionation

Fixed volume fractionation allows you to collect fixed volumes during elution. The **Fraction Volume** is set in the **Elution_Fractionation** dialog in the Method Wizard. You will choose elution technique and set interval for the fractionation, for example, interval of %B, in the **Elution** dialog.

Fixed volume fractionation can be combined with peak fractionation, which means collecting peaks during elution. There are two ways to collect peaks:

- Peaks are collected in one fraction each. The peak size is set to a volume which is larger than the actual peak volumes.
- Peaks are collected in several fractions. The peak size is set to a volume which is smaller than the actual peak volume.



The properties for the peak slopes and levels are set in the **Peak_Fractionation** dialog in the Method Wizard. That include variables:

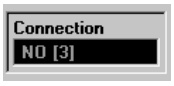
- for control of the start and end points of the peak fractions to be collected,
- for control of the minimum peak width to be collected, and
- that set the peak volume sizes during the fractionation slope interval.

Refer to *UNICORN User manuals* for further description of the peak slopes and levels properties.

3.7 Preparing the system for a run

3.7.1 System connection

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



2 If the text **NO** is written in the **Connection** panel in the **Run Data** window, go to step 3 below. If it says **YES**, go directly to *General system preparation*.

Comment:
Before you can start a run, you must always connect to the system. Connecting means that the System Control window is set up for a particular system. If you are not connected, the text **NO** is written in the **Connect** panel in the **Run Data** window. Once you are connected, the text changes to **YES**.

3 Select **System:Connect...**. The System connect dialog window appears:

4 Select a system from the list. If you are not connected to a network, only one system will be shown. Click **OK**.

5 When connected, the text **YES** is written in the **Connect** panel in the **Run Data** window. You only have to connect once. If you do not select **System:Disconnect,** you will be automatically connected to the system the next time you login to UNICORN.

3.7.2 General system preparation

1 The correct tubing kit for the column you intend to use must be installed. See section 3.6 for an overview over columns with recommended tubing kits. For most columns the 0.5 tubing kit can be used.

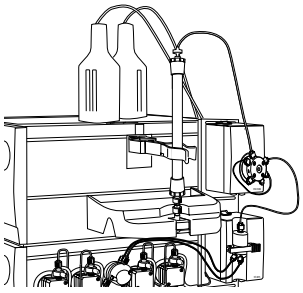
Comment:
If tubing with too large inner diameter is used, the peaks will become broader than necessary. If tubing with too small inner diameter is used, the back pressure from the tubing might become higher than the max. pressure for the column and the run will stop immediately after it is started.

2 Immerse inlet tubing A in buffer A and inlet tubing B in buffer B.

3 If fractionation is included (optional), put the waste tubing from port 1 of the outlet valve (optional) into a waste bottle. Check that the tubing from port 2 of the outlet valve is connected to the fraction collector.

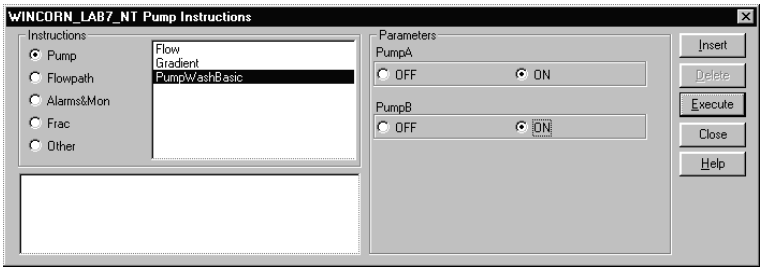
4 If there is air in the inlet tubing or if you suspect air in the pump, purge the pump with a syringe as described in chapter 2 in *Pump P-900 User Manual*.

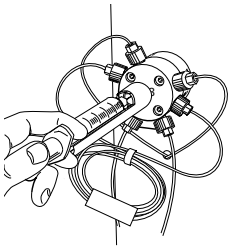
5 Connect the column between port 1 of the injection valve and the top of the UV flow cell. Use a suitable length of PEEK tubing supplied with your system.



6 Insert a sufficient number of tubes into the fraction collector (optional).

7 Click on the **System Control** button. Fill the inlet tubing with the correct solutions by selecting **Manual:Pump**. Then select instruction **PumpWashBasic** and set Pump A and Pump B to **ON**.





8 Click on **Execute** to fill the inlet tubing. The injection valve will automatically switch to waste during the pump wash. Then click on **Close**.

9 Check that the correct loop is mounted between port 2 and 6 on the injection valve.

10 Connect an injection fill port or a union luer female/1/16" male to port 3 on the injection valve and apply the sample manually with a syringe.

3.7.3 Before a run

Selecting a method

UNICORN is supplied with a Method Wizard which is used for creating customised methods.

The steps required to create a method are, in short, as follows:

1 Create the new method as described in the section *3.4 Creating a method*.

2 If necessary, adjust the values for the method variables on the **Variables** page in **Run Setup**.

3 Save the method.

Calibrations

The only type of calibration necessary in ÄKTAbasic is the pressure reading. This should be performed once a year or when required. Refer to the *UNICORN User Manuals* and the *Pump P-900 User Manual* for descriptions of how to perform this calibration. The calibration is performed from UNICORN by selecting **System:Calibrate** in **System Control**.

General preparation

Before starting any method, we recommend you to perform a certain number of checks to make sure that problems are not encountered once the run has been started.

1 Check that the inlet tubings are immersed in the correct bottles for the method selected.

2 Check that there is sufficient eluent available.

3 Check that the waste bottle is not full and will accept the volume diverted to it during the run.

4 Check that the pump has been purged (i.e. no air in the inlet tubing). If not, purge P-900 according to the *P-900 User Manual*.

5 Check that the correct column has been fitted and equilibrated (if not included in the method).

6 Check that the correct mixer chamber and tubing are installed for the method selected.

7 Check that the fraction collector (optional) has sufficient tubes fitted and is connected to the outlet valve (optional) at port 2.

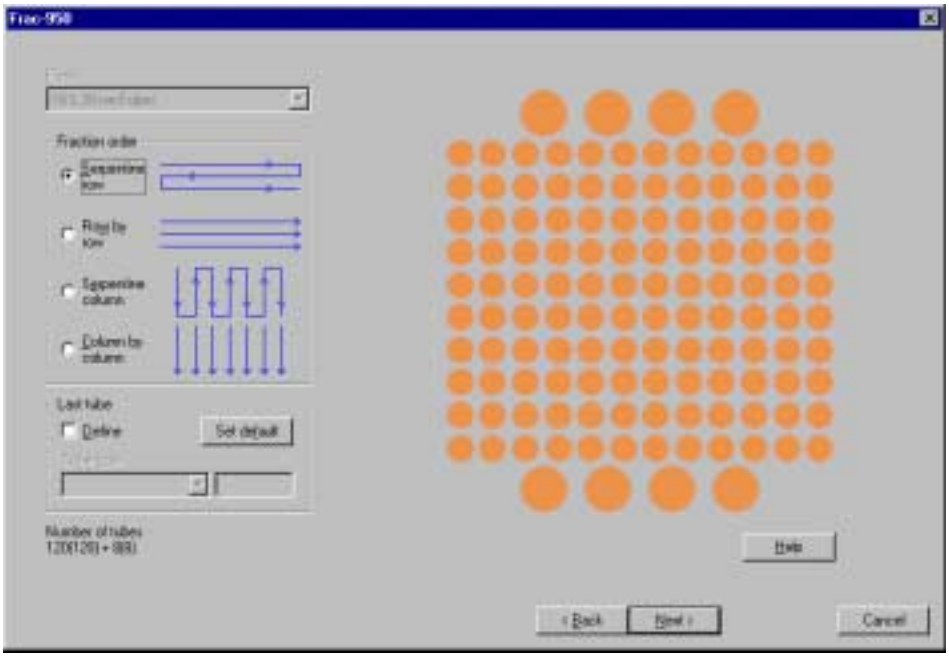
3.8 Starting a run

1 Click the **System Control** icon if it is not open.

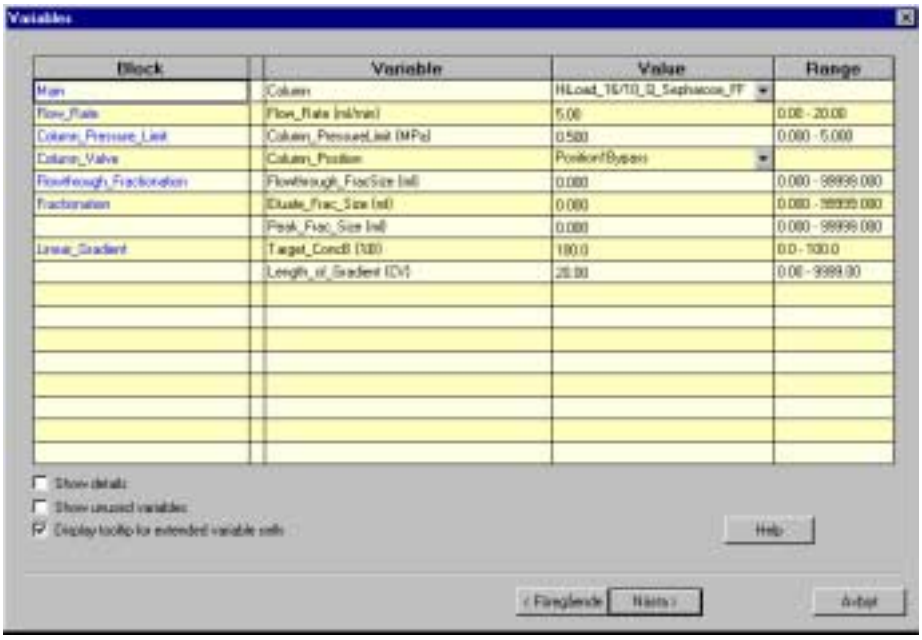
2 Select **File:Run....** Select the method to start. Click **OK** (the method will not start yet).

3 If using a Frac-950, this takes you to **Frac-950.** Otherwise, it takes you directly to **Variables** (next step).

Here you set up the Frac-950 fraction collector. Define the order of fractionation and set up the last tube used in the fractionation. The system will be paused and the fractionation stopped when the last tube is reached.



4 For example, the next page can be **Variables.** This is the same page you were working on in the Method Editor. Here you can verify and fine tune the method before proceeding. This is very convenient when repeating runs with minor adjustments.



5 In the **Variables** page, we recommend to check the **Show Details** box.

Comment:
If using a Frac-950, the Frac-950 page appears first.

Comment:
When starting run no. 2 immediately after run no. 1 with the same method but, for example, a different flow rate, you simply:

1 Click the **Run** button in **System Control**.

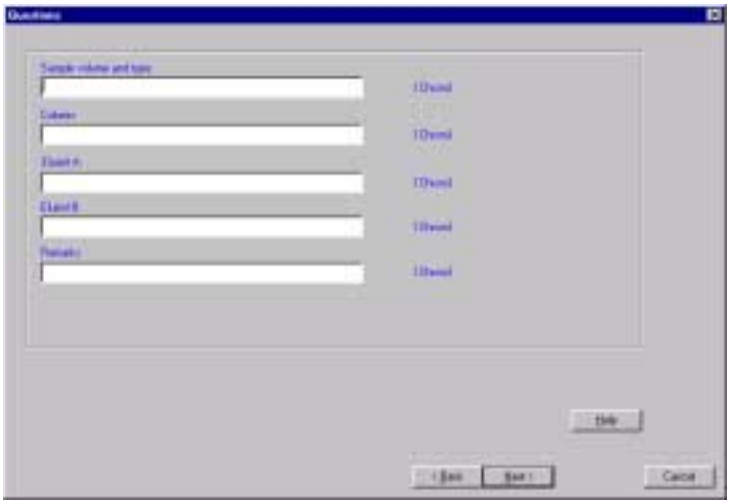
2 Change the flow rate on the **Variables** page.

3 Continue through the start protocol by clicking **Next** and then start the run.

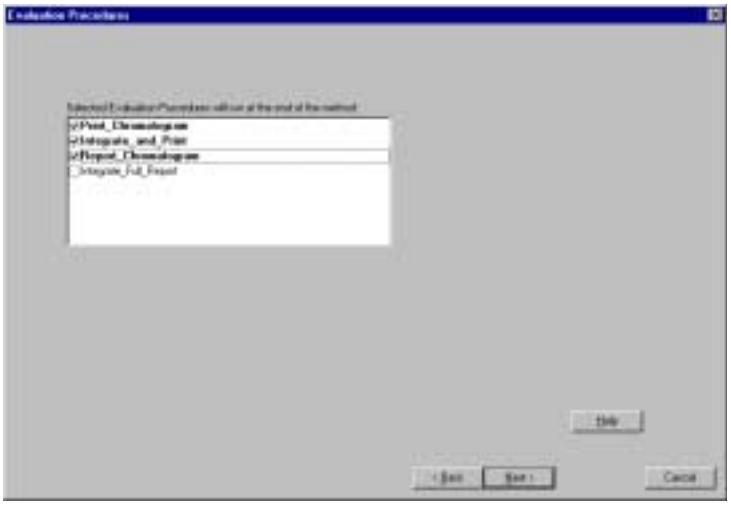
You do not need to change the method in the Method editor.

Go through the **Variables** page to check that the method is OK (this is not necessary if this was done in the Method editor).

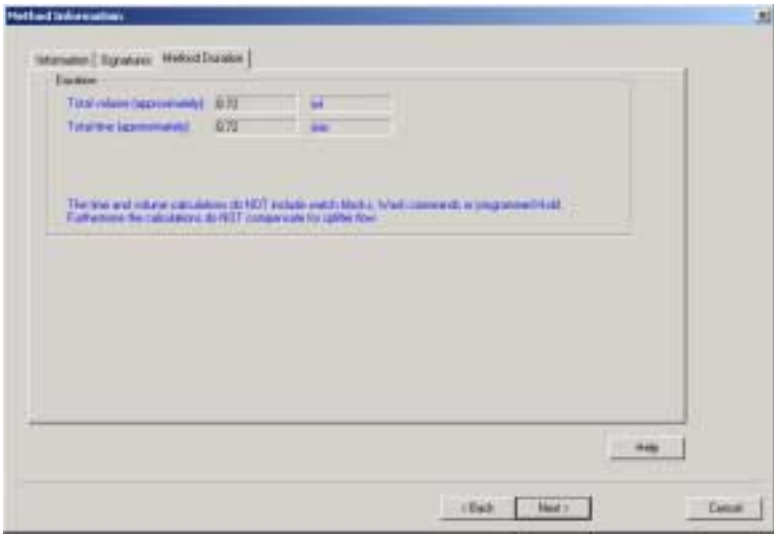
4 Click **Next.** For example, the **Questions** page appears. Type the answers on the questions. The answers will be saved in the result file.



5 Click **Next**. For example, the **Evaluation Procedures** page appears. Evaluation procedures are automated evaluation operations that are performed after the run. For instance, select **Print_Chromatogram** and the chromatogram will automatically be printed after the run.



6 Click **Next.** For example, the **Method Information** page appears. Here you see information about the run. Under the **Method Duration** tab the approximate volume of buffer used (A+B) is shown as well as how long time the method will take.



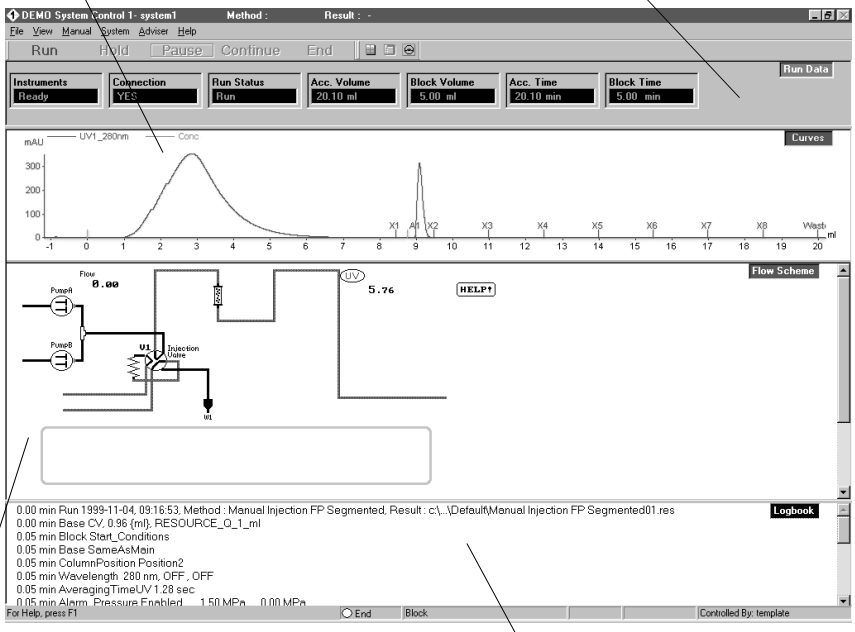
7 Click **Next**. This takes you to **Result Name.**

Here you name the result file and define in which directory the result should be stored. A default name (the method name followed by 001) and a directory are suggested. But you can change the result name and directory (click **Browse...)** if you so wish.

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

The Curves pane shows curves during the run.

The Run Data pane shows current values for running parameters



The Flow scheme is a graphical representation of the chromatography system.

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

3.9 During a run

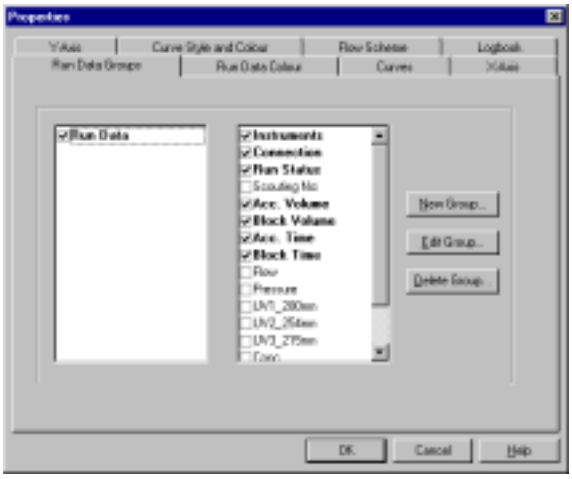
3.9.1 General

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

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

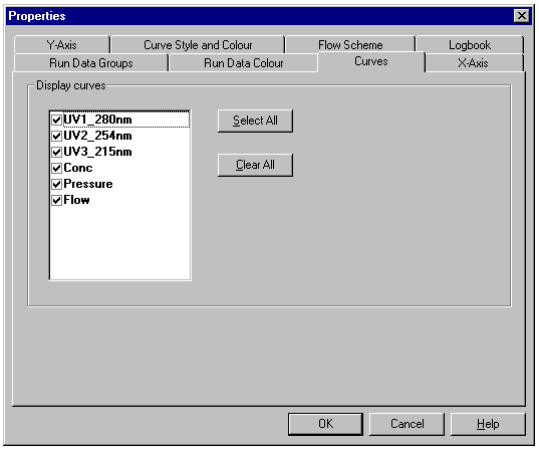
Click the **Customize panes** toolbar button or choose **View:Panes** from the menu to select which panes to display.

Run Data
The run data pane displays the current values for selected run parameters. Right-click in the **Run Data** pane and select **Properties...**, or select **View:Properties** from the **System Control** menu. Select the run data items to be displayed and click **OK.**



Curves

The curves pane displays the monitor signal values graphically. Right-click in the **Curves** pane and select **Properties...**, or select **View:Properties** from the **System Control** menu to select the curves to be displayed. All curves are always stored in the result file.



By clicking the different tabs in the **Curve Properties** pane you can set the properties for the different curves. Normally the curves are scaled with auto scaling, i.e. the scale is adjusted continually to the highest and lowest values for each curve.

To fix the Y-axis scale for a curve, mark the curve, click **Y-axis**, click **Fixed**, and enter the max. and min. values. You can repeat this for other curves. Click **OK**.

To maximise the **Curve Data** pane, position the cursor in the **Curve Data** pane. Click the right mouse button and select **Maximise.** Go back to normal size by clicking **Restore**.

Click the Y-axis scale, or click the curve name at the top of the **Curve Data** pane to shift to a scale for another curve. The color of a curve, its Y-scale, and its name are always the same. Click the X-axis to shift between time and volume.

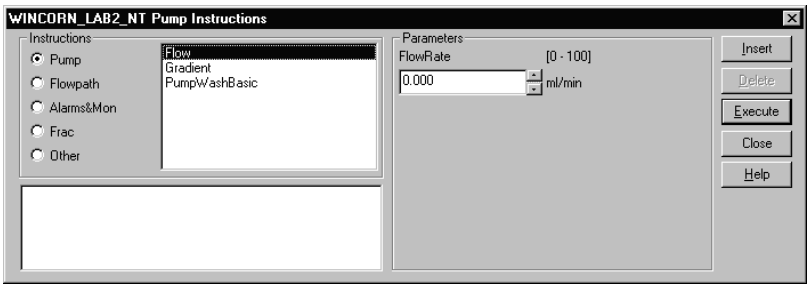
Flow scheme

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

Logbook

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

You can make manual changes during the run. Select **Manual:Pump**. The Instructions box is opened.



If, for example, you want to change the flow rate, select **Pump** and then **Flow**. Enter a new flow rate under **Parameters** and then click on **Execute**. The new flow rate will be used until the end of the run or until a new flow rate instruction is reached in the method. Close the box by clicking **Close**. All manual interactions are recorded in the **Logbook**.

If you want to stop the run before it is finished, click the **End** button at the top.

Front panel display

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

```
Run      13.40 ml/min
2.00MPa        45.5%B
```

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

Run	The pump is running with the set flow rate.
End	The system is not running.
Pause	The pump is stopped but the set flow rate and the gradient values are retained.
Hold	The gradient is held at the value displayed and the pump continues to run. The method is held in its current status.

```
λ1[215]  1.123     AU
λ 1[254]  0.02345  AU
```

The main operating menu of the Monitor UV-900 shows the absorbance values with 5 digits for up to 3 active wavelengths.

```
λ 1[280] 0.1234   AU
```

The display for the third wavelength is reached by turning the dial clockwise.

3.9.2 Changing parameters

From UNICORN

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

The **Manual** menu in **System Control** opens a dialog box similar to the text instruction box in the Method editor. Manual instructions are entered as follows:

1 Highlight the instructions list by clicking on a button on the left of the instruction panel and select the required instruction(s) from the list displayed.

2 Fill in the parameters and click **Execute.**

Some instructions, for example, gradient or fraction instructions, may take time to complete. To print all instructions with explanations, click on **Print** in the **Method Editor:File** menu. This opens a window containing instructions that are printable. Make sure that the **Instruction Set** box is checked and clear any unwanted items. Click **Print** to print the instructions.

From the modules

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

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

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

To select access mode, select **System settings** in **System Control** then **Specials:Keyboard**. Select **Open**, **KeyLocked** or **KeyAndDialLocked**.

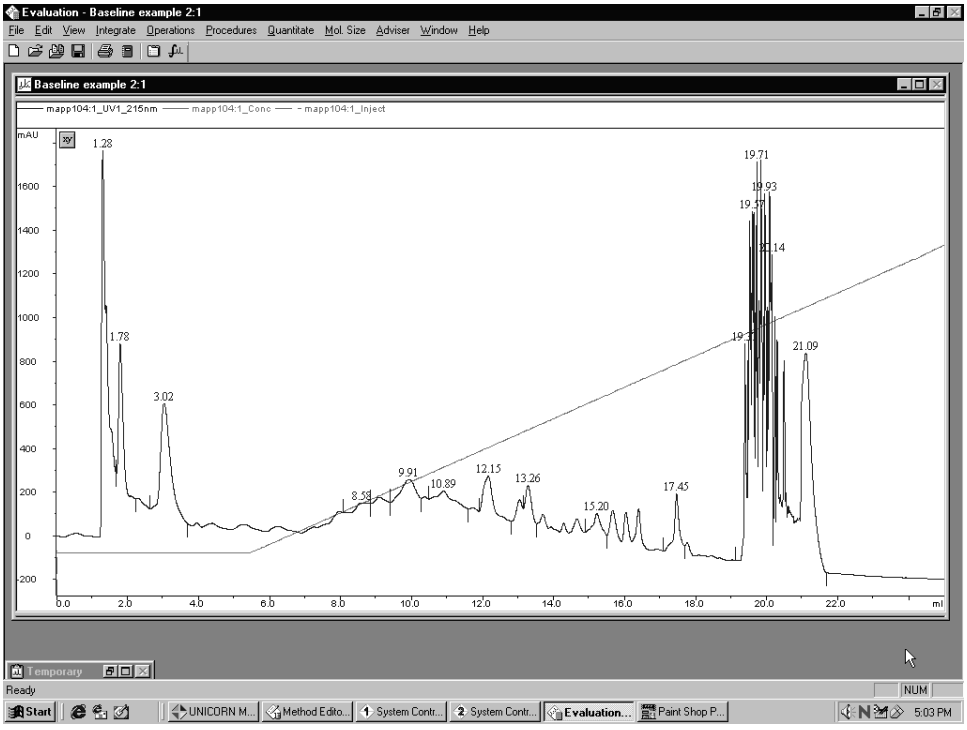
3.10 Viewing and printing the result

If you are satisfied with the automated printout obtained after the run (if selected), you do not need to alter anything described in this section. However, if you want to alter the chromatogram layout, this section will teach you the basics of the evaluation module.

3.10.1 Viewing

1 After a run you can view the result. Click the **UNICORN** menu icon. Double-click a result file icon in the list to the right.

2 The **Chromatogram** window is opened automatically in the Evaluation workspace when you open a result file. The **Chromatogram** window contains all the curves. Note that the term chromatogram is used here when talking about the whole window containing all the different curves.



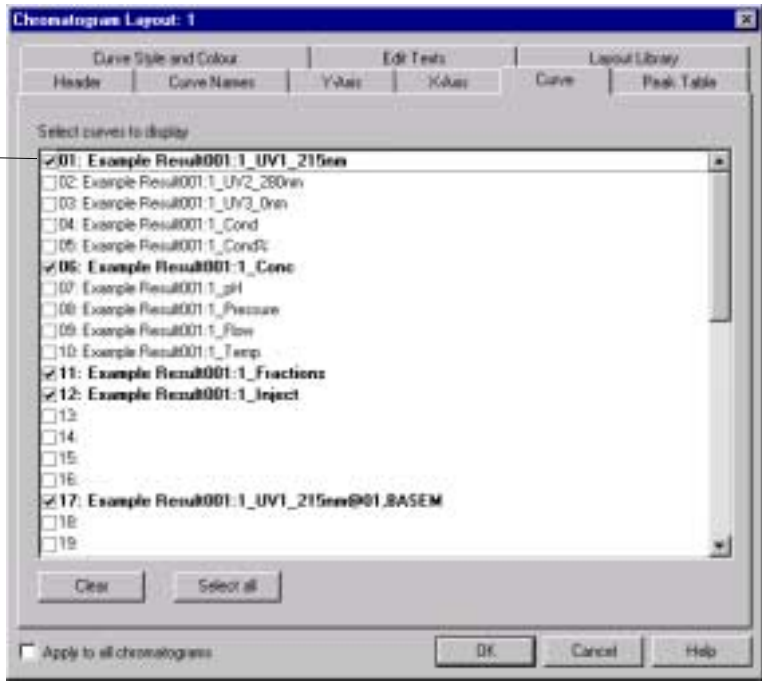
The result file from a run holds a complete record of the run, including method, system settings, curve data and run log.

Comment:
Original raw data curves can never be modified, renamed, or deleted from a result file.

3 Maximise the **Chromatogram** window by clicking on the larger square in the upper right corner.

4 All changes regarding the presentation of the curves are done in the **Chromatogram Layout** window. Position the cursor in the **Chromatogram** window. Click the right mouse button and select **Properties....**, or select **Edit: Chromatogram layout...** to activate this window.

Highlight curves to view



5 Select the curves to view under Curves. Curves are named as **Result001:1_"curve"** where a curve can be, for example, UV_wavelength, pressure...etc. Clear all curves except, for example, the UV and Conc. curves. Click **OK** at the bottom of the **Chromatogram Layout** window.

6 You can easily zoom in on the curves. Place the cursor in the chromatogram, click on the mouse button and holding it pressed down, move the mouse. A rectangle appears on the screen. When you release the mouse button, the part within the rectangle will be enlarged. You can zoom further on the enlarged part. Click the right mouse button and select **Undo** or **Reset zoom** to return to the complete chromatogram.

7 Click on the **Y-axis** scale to change to a scale for another curve. The style and color of a curve, its Y-scale and its X-scale can all be changed.

8 Open the **Chromatogram Layout** window again. Click on the **Y-axis** and **X-axis** tabs to set the scale for the different curves. Normally, the curves are scaled with auto scaling, i.e. the highest and lowest values for each curve set the scale.

9 To fix the Y-axis scale, mark a curve, click **Fixed**, and enter the max. and min. values for that curve. You can repeat this for other curves.

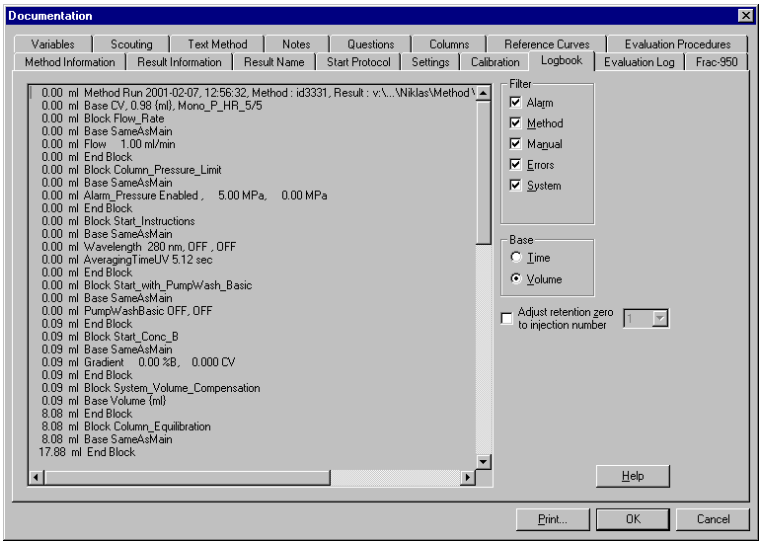
10 To fix the X-axis scale, click **Fixed** in the X-axis field, and enter the min. and max. values for the X-axis. Click **OK.**

11 Click **OK** at the bottom of the **Chromatogram Layout** window to execute all the changes.

Comment:
When you have made the necessary changes in the Chromatogram layout box, they can be saved as a layout. Click on the **Save as** button at the top of the Chromatogram layout box to save the layout. Give the layout a name and click on **OK**. Layouts can be selected in **Apply layout** at the top of the box and all your saved selections will apply. Saved layouts can be applied to any result file.

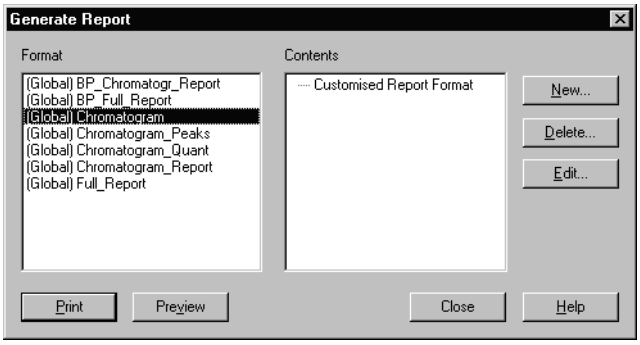
12 Minimise the chromatogram window by clicking on the smaller squares in the upper right corner.

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



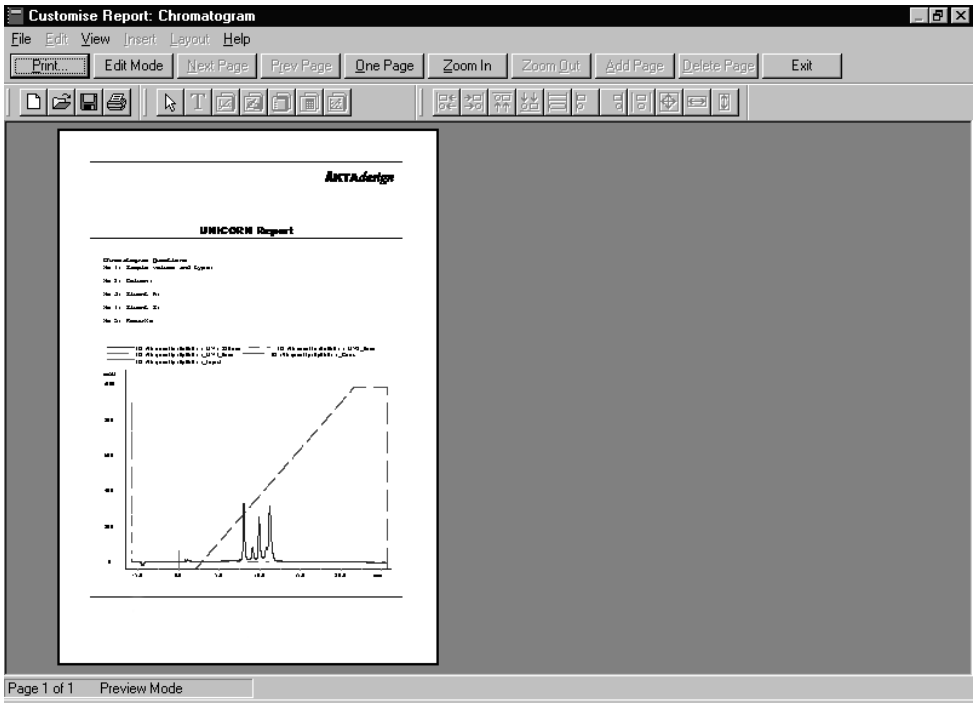
3.10.2 Printing and making a report

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



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

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



Add information to the report

1 Click **Edit Mode** to enable changes in the report.

2 To add an empty page to the report, click **Add Page**.

3 Select from the **Insert** menu**,** the item to include. Items available are:

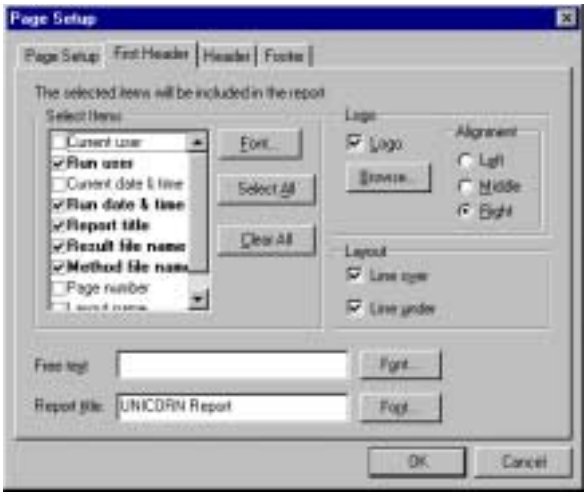
- Free text
- Picture
- Text method
- Chromatogram
- Documentation
- Evaluation log
- Quantitate and molsize (optional)
- Frac 950 (optional).

4 Move the mouse pointer into the page area of the window. You will notice that the mouse pointer has an additional symbol according to the item type you selected to insert.

5 Click-and-drag to create a box of the desired size. Release the mouse button. A dialog is displayed specific to the type of item inserted. Make the appropriate selections in the dialog and then click **OK** to view the inserted item.

Change page layout

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



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

3.11 Completion of a run and storage

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

3.11.1 Storage

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

Weekend and long term storage: Flush the system with water and then fill it with 20% ethanol.

3.12 Coldroom operation

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

3.12.1 Preparation

1 Place the separation unit in the cold room.

2 Place the computer outside the cold room. A 15 m UniNet cable is available as an accessory and should be used to connect the computer to the separation unit.

3 Allow the separation unit to stabilise at the new temperature for at least 12 hours.

4 Tighten all connections and pump water through the system to check for leaks.

5 Tighten any leaking connector.

3.12.2 Running

Before starting a run, check the following:

1 Ensure that the temperature of the buffers has reached the ambient temperature.

3.12.3 Removal from cold room

1 Loosen all connections to prevent them sticking when the system returns to room temperature.

2 Allow the separation unit to stabilise at room temperature for at least 12 hours.

3 Tighten all connections and pump water through the system to check for leaks.

4 Tighten any leaking connector.

3.13 Feedback tuning

Feedback tuning of the sample pump and the system pump is used for:

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

Feedback tuning is useful in applications where high back-pressure can be expected, or when the back-pressure fluctuates, for example, when using samples with high vicosity.

The default feedback tuning is set in the Method Wizard, and activated only during sample application and wash-out of unbound sample.

Feedback tuning is set up and used in slightly different ways when applied to the system pump and the sample pump, which is described in the following sections.

3.13.1 Feedback tuning of the system pump

Tuning principle

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

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

Feedback tuning of the system pump in a method

1 To include feedback tuning in the **Method Wizard**, select **Flow Regulation of the System pump.**

2 Type the pressure control set point (choose a value slightly below the column pressure limit) and the minimum allowed flow.

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

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

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

Feedback tuning can also be applied to an existing method in the **Method Editor** module.

Feedback tuning instructions

Feedback tuning can also be used when running the system pump manually. The instructions are found in the **System Control** module by selecting **Manual:Pump** and are explained in Table 3-1 .

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

Table 3-1. Feedback tuning instructions

Optimizing the PID parameters

The two regulators for the system pump have separate PID parameters. The default PID parameters in UNICORN provide a robust feedback tuning that is suitable for most purposes. However, the parameters can be further optimized to suit a specific application.

The table below describes the three PID parameters.

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

Table 3-2. PID parameters

Rules of thumb for optimizing the PID parameters:

- Use the default parameter values as a start.
  To set the default values, select **System:Settings** in the **System Control** module. The parameters are found in **Specials**.
- Keep the D parameter set to zero, i.e. use a simple PI-regulator.
- Start the pump before activating the regulator.
- Increasing P makes the regulator faster.
- Increasing I reduces oscillations.

See also the *UNICORN Administration and Technical Manual* for more information about feedback tuning.

3.13.2 Feedback tuning of the optional sample pump

Tuning principle

The feedback tuning of the sample pump is simpler than the feeback tuning of the system pump. When the pressure reaches the maximum allowed pressure, the flow is decreased. After a short while, the flow slowly increases towards the set flow rate, and so on.

The tuning regulator is rather simple and does not use PID-parameters. The parameters that control the tuning characteristics can not be changed.

Feedback tuning of the sample pump in a method

1 To include feedback tuning in the **Method Wizard**, select **Sample Pump Direct Loading** in the **Sample Injection** dialog.

2 Select **Pressure Control for Sample pump**.

The default value for the maximum allowed pressure is 2.0 MPa and for the minimum allowed flow 0.1 ml/min.

3 Click **Finish** in the last dialog.

Note: If the flow rate falls below the MinFlow value, an Alarm is raised and the system set to Pause. Therefore, we recommend using a Watch instruction (WatchPar_SampleFlow_960) for the flow that is activated above this value. A suitable action is to continue to the next block.

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

Feedback tuning can also be applied in an existing method in the **Method Editor** module.

To change the maximum allowed pressure:

1 Select **View:Text Instructions**.

2 Expand **Block Alarm_Sample_PressureLimit.**

3 Select the **Alarm_SamplePressure_960** instruction.

4 Type the desired **HighAlarm** value (maximum allowed pressure) in the **Parameters** field.

To change the minimum allowed flow:

1 Select **View:Text Instructions**.

2 Expand **Block Direct_Sample_Loading.**

3 Expand **Block PressureReg_Sample_Pump.**

4 Select the **SamplePumpControlMode_960** instruction.

5 Type the desired **MinFlow** value (minimum allowed flow) in the **Parameters** field.

Feedback tuning with manual instructions

To use feedback tuning when running the sample pump manually:

1 In **System Control** select **Manual:Alarm&Mon.**

2 Select **Alarm_SamplePressure_960**.

3 Select **Enabled** and set the **HighAlarm** value (maximum allowed pressure. Click **Insert**.

4 Select **Pump:SamplePumpControlMode_960**.

5 Select **PressFlowControl** and set the **MinFlow** value.

6 Click **Execute** to start feedback tuning.

Note: Start the sample pump at a low flow rate after running the sample pump with feedback tubing.

4 Maintenance

4.1 Periodic maintenance

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

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

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

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

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

WARNING! When using hazardous chemicals, make sure that the entire system has been flushed thoroughly with distilled water before service and maintenance.

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

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

Interval	*Action*
Every day	
System	• Inspect the complete system for eluent leakage. • The system can be left filled with buffer overnight. If you are not using the separation unit for a few days, wash the flow path with distilled water. Remove the column and the pH electrode (optional). Replace the column by a bypass capillary and fit the pH dummy electrode (if applicable). Then wash the system with 20% ethanol and store it in 20% ethanol. Make sure that all tubing and all flow paths used are rinsed.
Pump P-900	• Check for leakage. If there are signs of liquid leaking between the pump head and the housing side panel or increased or decreased volume of rinsing solution, replace the piston seals. Refer to chapter 4 in *Pump P-900 User Manual*. • When changing eluent, it is important to remove trapped air. Purge the pump according to chapter 2 in the *Pump P-900 User Manual*. If there is still air in the inlet tubing, stop and purge the pump according to chapter 5 in the *Pump P-900 User Manual*. ***Note**: If air is allowed to enter the columns, their performance can be adversely affected or destroyed.*

Interval	*Action*
Every week	
Inlet filters	• Check the inlet filters visually and replace them if necessary.
Pump rinsing solution	• Change rinsing solution. Always use 20% ethanol as rinsing solution. • If the volume of rinsing solution in the storage bottle has increased, it can be an indication of internal pump leakage. Replace the piston seals according to chapter 4 in *Pump P-900 User Manual*. • If the volume of rinsing solution in the storage bottle has decreased significantly, check if the rinsing system connectors are mounted properly. • If the rinsing system connectors are not leaking, the rinsing membranes or piston seals may be leaking. Replace the membranes and piston seals according to chapter 4 in *Pump P-900 User Manual*.

Interval	*Action*
Every month	
Flow restrictor	• Check that the flow restrictor generates the following back-pressures: FR-904..... 0.4 ± 0.05 MPa FR-902..... 0.2 ± 0.05 MPa Checking the back-pressure: 1 Disconnect the flow restrictor. 2 Connect a piece of tubing (approx. 1 m, i.d. 1.0 mm) to a free port in injection valve V2. Set the valve manually to this port. Put the open end in a waste container. 3 Run the pump at 10 ml/min with water. *Note the back-pressure (Bp1) on the pump display, or in the RUN DATA window.* 4 Connect flow restrictor to the open end of the tubing (observe the IN marking). Put the flow restrictor in the waste container. 5 Run the pump at 10 ml/min with water. *Note the back-pressure (Bp2) on the pump display, or in the RUN DATA window.* 6 Calculate the back pressure (Bp2-Bp1) of the flow restrictor. If is not within the limit, replace or contact Amersham Biosciences.
Every 3 months	
Monitor UV-900	• Check the instrument according to chapter 4 in *Monitor UV-900 User Manual*.
Every 6 months	
Monitor UV-900	• Clean the flow cell and optical fiber connectors according to chapter 4 in *Monitor UV-900 User Manual*.
Mixer M-925	• Check that the mixer chamber is clean and without damage. Check the tubing connectors. Replace if required.

Interval	*Action*
Yearly	
Valve INV-907 and PV- 908 (optional)	• Check for external and/or internal leakage. Replace the distribution plate yearly or when required. Refer to chapter 4 in the relevant valve instruction.
Every 2 years	
Mixer M-925	• Replace the complete mixer chamber.
When required	
Pump P-900	• Replace piston seals. Refer to chapter 4 in the *Pump P-900 User Manual*. • Replace piston. Refer to chapter 4 in the *Pump P-900 User Manual*. • Clean or replace the inlet our outlet check valves. Refer to the chapter 4 in *Pump P-900 User Manual*.

4.2 Cleaning the system

The procedures described below are for system cleaning.

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

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

4.2.1 At the end of the day

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

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

1 Submerge the inlet tubings in distilled water.

2 Run the **PumpWash Basic** instruction.

4.2.2 Leaving the system for a few days

Perform a **PumpWash Basic** with distilled water. Repeat with a bacteriostatic solution, i.e. 20% ethanol.

4.2.3 Additional wash of outlet valve (option)

Wash the valve as follows:

1 In **System Control**, start the system flow rate.

2 Rinse the valve by switching between the ports manually from **System Control.**

4.2.4 Monthly cleaning

Clean the system every month or when problems such as ghost peaks occur:

1 Disconnect the column and replace it with bypass tubing.

2 If using a pH electrode, replace it with a dummy electrode.

3 Place all the inlet tubings in 1 M NaOH.

4 Manually, perform **PumpWash Basic** for both inlet tubings.

5 Flush the whole system with 1 M NaOH for 20 minutes (1 ml/min).

6 Immediately, repeat steps 3 and 4 with distilled water to rinse the system of NaOH.

4.2.5 Cleaning-in-place

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

A method for cleaning-in-place, **CIP**, is available in the UNICORN Method Wizard. It gives many possibilities to design a powerful cleaning protocol for individual problems, with up to 9 cleaning segments.

4.3 Moving the system

Two persons are recommended to lift the system.

CAUTION! Never lift the system by the valves.

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

5 Trouble-shooting

5.1 Faults and actions

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

Type	*Page*
Pressure curve	102
UV curve	103
Monitor UV-900	104
Pump P-900	104
Mixer M-925	105
Valve PV-908, INV-907	106

If the suggested actions do not correct the fault, contact the local Amersham Biosciences service representative.

5.2 Pressure curve

Fault	*Action*
Erratic flow, noisy baseline signal, irregular pressure trace **Possible causes are:**	
Air bubbles passing through or trapped in the pump	1 Check that there is sufficient eluent present in the reservoirs. 2 Check all connections for leaks. 3 Follow the instructions in chapter 5 of *Pump P-900 User Manual*.
Inlet or outlet check valves not functioning correctly	1 Clean the valves according to chapter 4 of *Pump P-900 User Manual*.
Piston seal leaking	1 Replace the piston seal according to the instructions in chapter 4 of *Pump P-900 User Manual*.
Blockage or partial blockage of flow path	1 Flush through to clear the blockage. 2 If necessary, replace tubing. 3 Check the inlet tubing filter. It can become clogged if unfiltered buffers or samples are applied. 4 See instructions for flushing through at the end of the run in chapter 3 of *Pump P-900 User Manual*.

5.3 UV curve

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

5.4 Monitor UV-900

Fault	*Action*
No text on the front display	1 Check that the mains cable is connected and the power switch is in ON-position I.
Unstable baseline	1 Try using a larger mixer chamber instead of the standard mixer chamber.

5.5 Pump P-900

Fault	*Action*
No text on the front display	1 Check that the mains cable is connected and the power switch is in ON-position I.
Liquid leaking between the pump head and the side panel	Piston seal or rinsing membrane incorrectly fitted or worn. 1 Replace or re-install the seal or membrane. 2 Run-in carefully, see chapter 4 of *Pump P-900 User Manual*.
Low eluent flow and noise as the piston moves	1 Disassemble pump cylinder and examine the piston spring according to chapter 4 of *Pump P-900 User Manual*. Replace if necessary. 2 If the spring is corroded, check piston seal and rinse membrane. Make sure that piston rinsing system is always used when working with aqueous buffers with high salt concentration. 3 Check the piston for damage. If damaged, replace the piston according to chapter 4 of *Pump P-900 User Manual*. 4 Remember to replace the piston seal with new parts.
Leaking connection and/or crystallised material around a connector	1 Unscrew the connector and check if it is worn or incorrectly fitted. If so, replace the connector. 2 Tighten the connector with your fingers.

Fault	*Action*
Erratic pump pressure	To check the pump function, record the pressure or check it in UNICORN. By observing the piston status indicator in the Check menu together with the pressure trace, the pump cylinder that is functioning abnormally can be identified, see Reference information in the *Pump P-900 User Manual*. There can be several causes of an abnormal pressure recording, for example: • air trapped in the pump cylinders • partially blocked solvent filters • leaking connections • piston seal leakage • pump valve malfunction • piston damaged. Some examples of normal and abnormal pressure traces together with comments are shown in chapter 5 of *Pump P-900 User Manual*.

5.6 Mixer M-925

Fault	*Action*
Leakage	1 Check the tubing connections. Retighten or replace if necessary. 2 Check the mixer chamber. Replace if liquid has penetrated the mixer chamber walls and sealings.
Function test	1 Test the mixer function by placing a stirrer bar on top of the mixer housing. The stirrer bar should rotate when the system is in **RUN** mode. 2 The mixer function can also be checked by running the installation test.

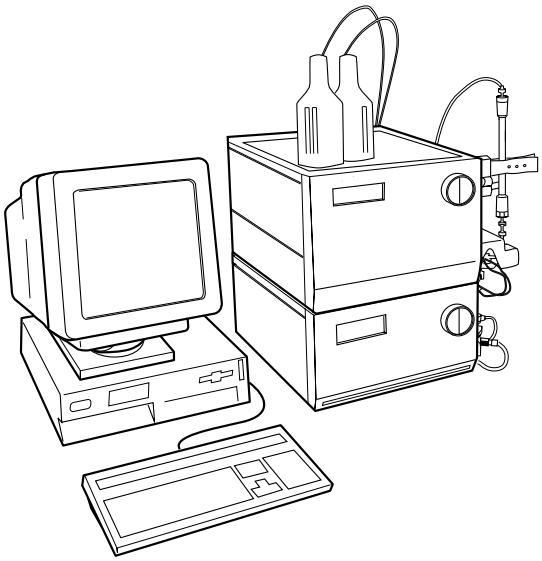
5.7 Valves PV-908 and INV-907

Fault	*Action*
The valve is not switching	1 Check the connection to the pump. The valve should be connected to the UniNet-2 socket. 2 Check the ID-switch on the valve. The ID number should correspond to the number set in UNICORN. 3 Check the UniNet cable and replace if required.
The valve is switching to wrong position	The valve parts may have been incorrectly reassembled after replacement. 1 Check that the distribution plate marking **i/o** or **3** is horizontal.
External leakage	1 Check the tubing connections. Tighten or replace if required.
Internal leakage	Internal leakage can be detected at the small hole on the underside of the valve body. 1 Internal parts may be worn. Change channel plate and distribution plate according to chapter 4 of the relevant valve instruction.
High back-pressure	1 Perform cleaning-in-place according to chapter 4 of the relevant valve instruction. 2 Change channel plate and distribution plate according to chapter 4 of the relevant valve instruction.

6 Reference information

6.1 System description

6.1.1 The system



ÄKTAbasic consists of a compact separation unit and a personal computer running UNICORN software version 4.12 or higher. Fraction collectors are available as accessories.

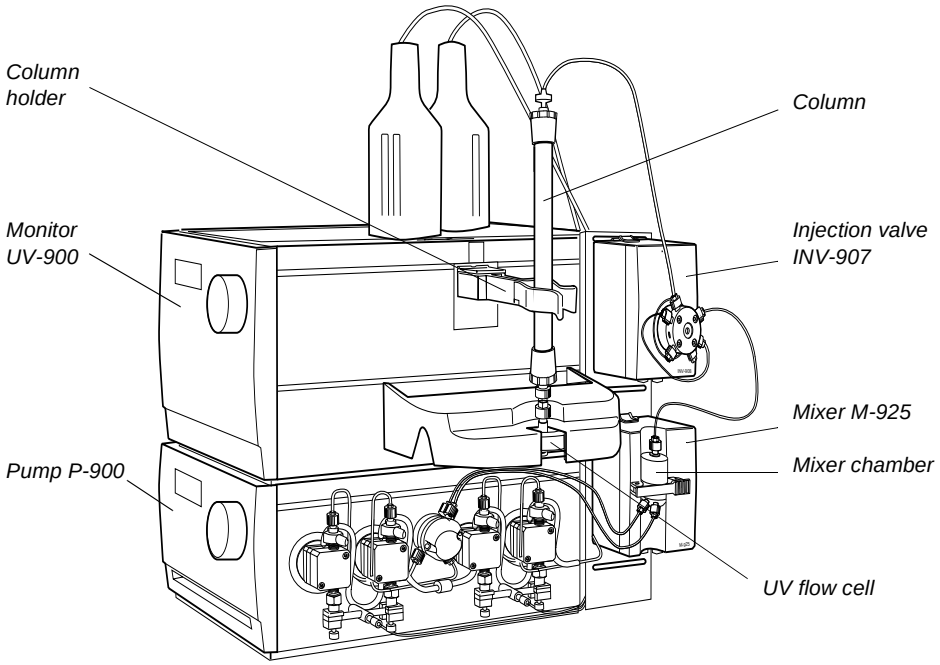
A brief description of the individual modules and components is given in this chapter. Detailed information on the components can be found in their respective User Manuals and Instructions. UNICORN is described in the separate *UNICORN User Manuals*.

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

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

6.1.2 Component locations

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

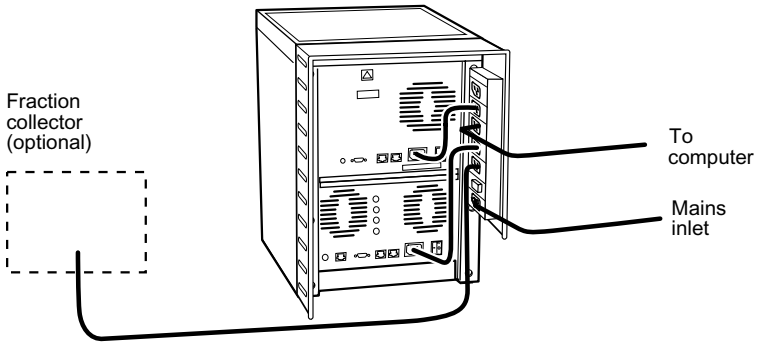


6.1.3 Electrical connections

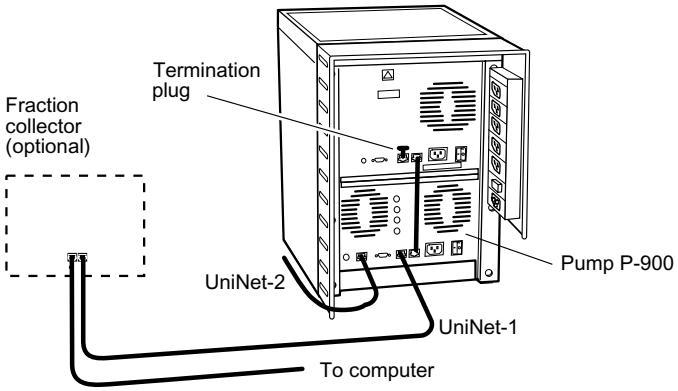
WARNING! Never attempt to remove the mains fuse while mains voltage is applied to the system. For continued protection against risk of fire, replace only with a fuse of the specified type and rating. Refer to the Technical Specifications for fuse data.

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

Mains cables

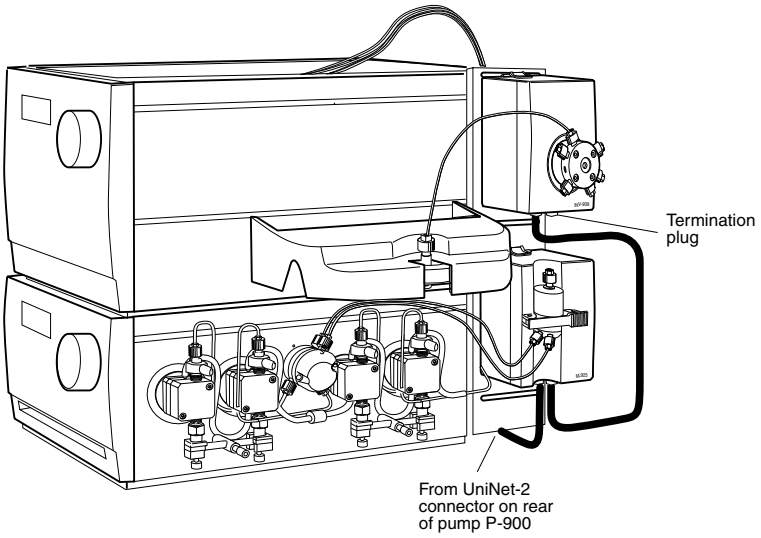


UniNet-1



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

UniNet-2

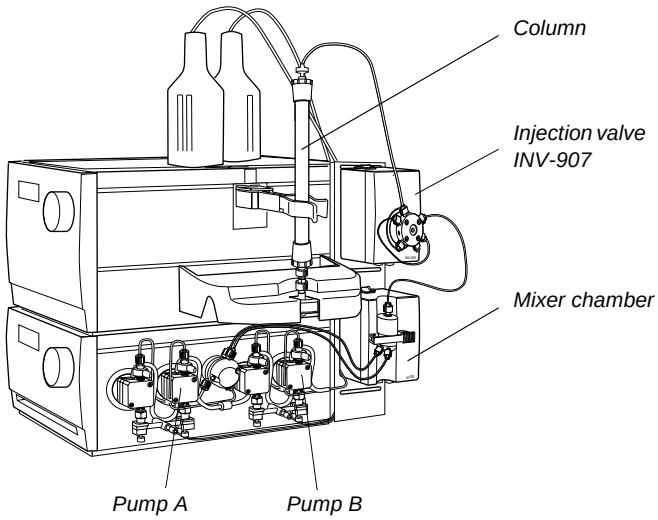


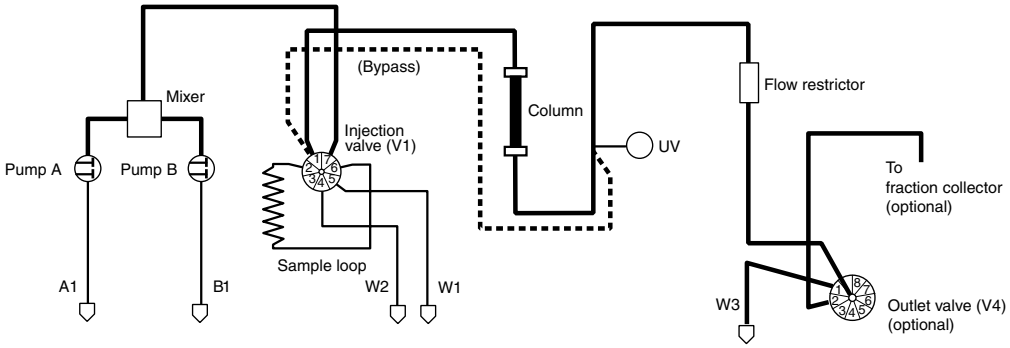
The UniNet-2 data communication cable which supplies the valves and mixer with control signals is routed from the rear of Pump P-900, and links components on the right side of the system. The cable is terminated at the injection valve, V1, with a termination plug.

If using Pump P-960, it should be installed as the last component in the UniNet-2 chain. Refer to *Pump P-960 User Manual* and *ÄKTAbasic Optional Configurations User Manual* for instructions how to connect the pump to the instrument.

6.1.4 Fluid handling path

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





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

ÄKTAbasic 10

The column is installed either using the tubing supplied with the column, or with PEEK tubing cut by the user to suitable lengths (i.d. 0.5 mm orange PEEK tubing is supplied with ÄKTAbasic 10).

Tubing i.d.	*Tubing o.d.*	*Material*	*Color*	*Max. pressure*	*Volume of 10 cm*	*Connected*
0.25 mm	1/16"	PEEK	Blue	25 MPa	4.9 µl	From injection valve to UV flow cell
0.25 mm	1/16"	PEEK	Blue	25 MPa	4.9 µl	From UV flow cell to fraction collector (optional) or via outlet valve (V4) (optional)
0.50 mm	1/16"	PEEK	Orange	25 MPa	19.6 µl	From Pump P-900 to injection valve
0.75 mm (W1–W3)	1/16"	Tefzel	Clear	7 MPa	44.2 µl	Waste tubing
1.6 mm (A, B)	1/8"	Teflon	Clear	3.4 MPa	201.1 µl	Inlet tubing

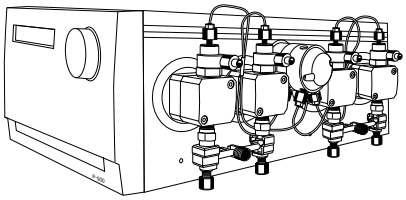
ÄKTAbasic 100

The column is installed either using the tubing supplied with the column, or with PEEK tubing cut by the user to suitable lengths (i.d. 0.75 mm green PEEK tubing is supplied with ÄKTAbasic 100).

Tubing i.d.	*Tubing o.d.*	*Material*	*Color*	*Max. pressure*	*Volume of 10 cm*	*Connected*
0.75 mm	1/16"	PEEK	Green	10 MPa	44.2 µl	From Pump P-900 to fraction collector (optional)
0.75 mm (W1-W3)	1/16"	Tefzel	Clear	7 MPa	44.2 µl	Waste tubing
2.9 mm (A, B)	1/16"	Teflon	Clear	3.4 MPa	660 µl	Inlet tubing

6.2 Component description

6.2.1 Pump P-900

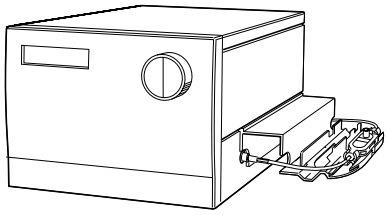


Pump P-900 is the collective name for a pump family. P-900 is a high performance laboratory pump for use where accurately controlled liquid flow is required. It is a low pulsation pump equipped with 2 pump modules; A and B. This allows for binary gradients with high pressure mixing. A pressure sensor is connected to pump module A (left hand pair of pump heads).

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

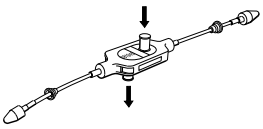
The model installed in *ÄKTAbasic 10 has 10 ml pump heads* and is referred to as Pump P-903 in the accessories list. P-903 has an operating flow rate range of 0.001–10 ml/min in both isocratic and gradient mode, and a *pressure range of 0–25 MPa* (250 bar, 3625 psi).

6.2.2 Monitor UV-900



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

Monitor UV-900 consists of a main unit, optical fibres and a choice of two flow cells (optical pathlength 2 mm, internal volume 2 µl, or pathlength 10 mm, internal volume 8 µl).



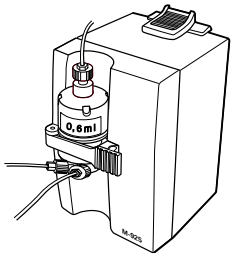
6.2.3 UV flow cells

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

ÄKTAbasic 10 is delivered with the 10 mm cell. A 2 mm cell is available as an accessory. If a lower detection sensitivity is desired due to output signal limitation, use the 2 mm cell.

ÄKTAbasic 100 is delivered with the 2 mm cell. A 10 mm cell is available as an accessory. For higher detection sensitivity, use the 10 mm cell

6.2.4 Mixer M-925

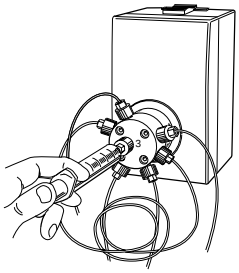


Mixer M-925 is a dynamic, single chamber mixer powered and controlled from Pump P-900. All eluents commonly used in ion exchange, hydrophobic interaction, affinity and reversed phase chromatography can be mixed with a high degree of accuracy and reproducibility. The mixer is positioned directly after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

Mixer M-925 has three interchangeable mixing chambers, 0.6, 2 and 5 ml, for optimal mixing over the entire flow rate range of ÄKTAbasic.

6.2.5 Valve INV-907

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



Three different operating positions make it possible to:

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

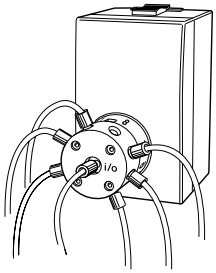
Sample volumes up to 150 ml can be applied via loops connected to the injection valve:

- Using a range of fixed volume loops for applying samples from 10 µl to 1000 µl with accuracy and precision.
- Using Superloop 10 ml, Superloop 50 ml, or Superloop 150 ml for applying samples in the range 1–10 ml, 1–50 ml, and 1–150ml respectively. All three are loaded by a syringe.

6.2.6 Valve PV-908 (optional)

PV-908 valve is utilised as an outlet valve, connecting the outlet to waste (port 1) and the fraction collector (for example, port 2).

The valve is a motorised 8-way valve and has a pressure limit of 25MPa.



6.2.7 Flow restrictor FR-902/904

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

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

FR-904 is set at the factory to 0.4 MPa and is mounted in the system at delivery.

6.2.8 Fraction collector (optional)

A fraction collector can be used for both small scale and preparative scale purifications with ÄKTAbasic. A number of racks for different tubes sizes are supplied with the fraction collector.

In ÄKTAbasic, the fraction collector allows fixed volume fractionation, or automatic peak fractionation. The latter function is based on UV peak detection using slopes or level sensing.

Fraction marks and fraction numbers make it easy to identify fractions and peaks.

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.

6.3 Technical specifications

For the complete specifications for each component, refer to the individual User Manuals and Instructions.

The relevant system specifications are listed below.

6.3.1 Operating data

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

Gradient composition Accuracy Reproducibility	 ± 1% at 0.05–10 ml/min rsd < 0.25% at flow rate <0.05-10 ml/min
Internal volume Stroke volume Total volume	 35.6 µl 556 µl
Monitor UV-900	
Wavelength range	190-700 nm in steps of 1 nm 3 wavelengths simultaneously
Bandwidth	4 nm
Wavelength accuracy	± 2 nm
Wavelength reproducibility	± 0.01 nm
Wavelength switch time	< 500 ms (one cycle from 214 nm to 254 nm and back to 214)
Linearity	< 2% deviation up to 2 AU at 260 nm with Uracil at pH 2
Noise[1] (at 230 nm)	< $6x10^{-5}$ AU, with 10 mm cell, H_2O at 1 ml/min
Drift[1] (at 254 nm)	< $2x10^{-4}$ AU/h
Flow cell Max flow rate Max pressure	 100 ml/min 2 MPa (20 bar, 290 psi)

[1] Typical values at room temperature after warm up.

6.3.2 Physical data

Degree of protection	IP 20
Power requirement	100–120/220–240 V ~, 50–60 Hz
Power consumption	370 VA
Fuse specification	T 6.3 AL
Dimensions, W x D x H	300 x 460 x 500 mm
Weight	40 kg
Environment	+4 to +40 °C, 10–95% relative humidity (non-condensing), 84–106 kPa (840–1060 mbar atmospheric pressure).

6.3.3 Hardware requirements

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

6.3.4 Software requirements

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

6.3.5 Network requirements

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

6.3.6 ÄKTAbasic component materials

The wetted materials of ÄKTAbasic are listed below:

	Pump P-900	Monitor UV-900	Flow restrictor	Mixer M-925	INV-907	PV-908 (optional)	Unions/ Connectors	Inlet filters
Ruby/ sapphire	X							
Stainl. st. (Elgiloy)	X							
Alum. oxide	X							
Gold			X					
Glass								
Quartz		X						
Titan. alloy	X	X						X
PE	X							
PVDF	X							
PP								X
ECTFE	X							
CTFE								
ETFE			X				X	
FEP								
PTFE	X	X	X	X				
PEEK	X	X	X	X	X	X	X	
Silicone								
FFKM				X				

FFKM = perfluororubber
PEEK = polyetheretherketone
PTFE = polytetrafluoroethylene
FEP = perfluoroethylenepropylene copolymer
ETFE = ethylenetetrafluoroethylene
CTFE=chlorotrifluoroethylene
ECTFE = ethylenechlorotrifluoroethylene
PP = polypropylene
PVDF = polyvinylidenefluoride
PE = polyethylene

6.4 Chemical resistance guide and chemical compatibility

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

The ratings are based on the following assumptions:

1 The synergistic effects of the chemical mixtures have not been taken into account.

2 Room temperature and limited over-pressure is assumed.

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

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

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

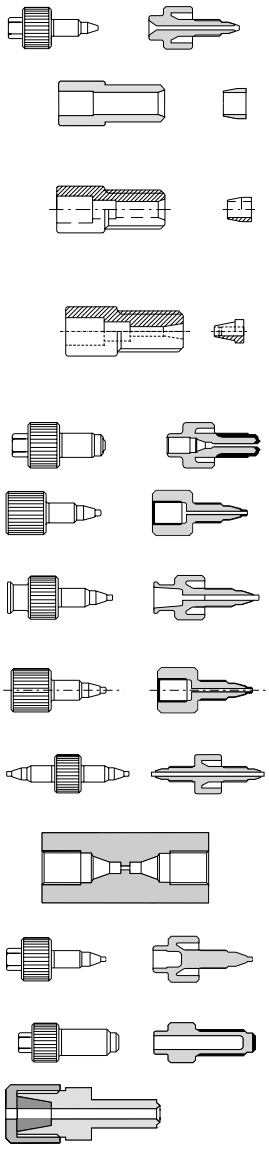
Chemical	*Exposure*		*Comments*
	< 1 day	*up to 2 months*	
Tetrachloroethylene	Avoid	Avoid	Silicone, PP and PE are not resistant
Tetrahydrofuran	Avoid	Avoid	Silicone, ETFE, CTFE, PP and PE are not resistant
Toluene	OK	Avoid	Pressure limit for PEEK decreases
Trichloroacetic acid, 1%	OK	OK	
Trifluoroacetic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

6.5 Accessories, spare parts and consumables

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

Item	*Quant./ pack*	*Code no.*
Monitor UV-900		
Monitor UV-900 complete but without flow cells	1	18-1108-35
Flow cell UV-900, 2 mm	1	18-1111-10
Flow cell UV-900, 10 mm	1	18-1111-11
Fiber detachment tool	1	18-1111-16
Mixer M-925		
Mixer M-925 including one UniNet cable	1	18-1118-89
Mixing chambers 0.6 ml 2 ml 5ml 12 ml	 1 1 1 1	 18-1118-90 18-1118-91 18-1118-92 18-1118-93
Valve PV-908		
Valve PV-908 including one UniNet cable	1	18-1108-41
Valve kit, including channel plate and distribution plate PV-908	1	18-1109-06
Number plates 0-9	1	18-1109-09
Mounting bracket	1	18-1109-11
Valve INV-907		
Valve INV-907 including one UniNet cable (fill port, needle and syringe holder are not included)	1	18-1108-40
Injection kit INV-907 including: fill port 0.7 mm, needle and syringe holder	1	18-1110-89
Valve kit INV-907 including channel plate and distribution plate	1	18-1109-05
Sample loops: 10 µl 100 µl 500 µl 1 ml 2 ml	 1 1 1 1 1	 18-1120-39 18-1113-98 18-1113-99 18-1114-01 18-1114-02
Mounting bracket	1	18-1109-11

Item	*Quant./ pack*	*Code no.*
Cables		
UniNet, 0.18 m	1	18-1109-72
UniNet, 0.3 m	1	18-1109-73
UniNet, 0.7 m	1	18-1109-74
UniNet, 1.5 m	1	18-1117-75
UniNet, 3.0 m	1	18-1109-75
UniNet, 15.0 m	1	18-1117-74
Mains cable, US standard	1	19-2447-01
Mains cable, EU standard	1	19-2448-01
Signal Cable, 6 pin mini DIN-open	1	18-1110-64
Tubing		
Inlet Filter assembly, 1 net, 1 filter	1	18-1113-15
Inlet filter set, 10 nets and 10 filters	10	18-1114-42
PEEK tubing, i.d. 0.25 mm, o.d. 1/16"	2 m	18-1121-36
PEEK tubing, i.d. 0.50 mm, o.d. 1/16"	2 m	18-1113-68
PEEK tubing, i.d. 0.75 mm, o.d. 1/16"	2 m	18-1112-53
PEEK tubing, i.d. 1.0 mm, o.d. 1/16"	2 m	18-1115-83
Tefzel tubing, i.d. 0.25 mm, o.d. 1/16"	2 m	18-1121-36
Tefzel tubing, i.d. 0.50 mm, o.d. 1/16"	2 m	18-1120-96
Tefzel tubing, i.d. 0.75 mm, o.d. 1/16"	2 m	18-1119-74
Teflon tubing, i.d. 0.75 mm, o.d. 1/16"	2 m	18-1112-54
Teflon tubing, i.d. 1.6 mm, o.d. 1/8"	3 m	18-1121-16
Teflon tubing, i.d. 2.9 mm, o.d. 3/16"	3 m	18-1112-47



Item	*Quant./ pack*	*Code no.*
Connectors and unions		
Fingertight connector, 1/16" for 1/16" o.d. tubing, PEEK	10	18-1121-17
Tubing connector, for 3/16" o.d. tubing, PEEK	10	18-1112-49
Ferrules, for 3/16" o.d. tubing, PEEK	10	18-1112-48
Tubing connector for 1/8" o.d. tubing, PEEK	10	18-1121-17
Ferrules for 1/8" o.d.tubing, PEEK	10	18-1121-18
Tubing connector for 1/16" o.d. tubing, PEEK	10	18-1127-07
Ferrules for 1/16" o.d.tubing, PEEK	10	18-1127-06
Union, 1/16" female/M6 male, PEEK	6	18-1112-57
Union, luer female/1/16" male, PEEK	2	18-1112-51
Union, M6 female/1/16" male, PEEK	8	18-1112-58
Union 5/16"–32 female/HPLC male, PEEK	8	18-1142-08
Union, 1/16" male/1/16" male, for 1/16" o.d. tubing, PEEK	10	18-1120-92
Union, 1/16" female/1/16" female, for 1/16" o.d. tubing, titanium	1	18-3855-01
Stop plug, 1/16", PEEK	5	18-1112-52
Stop plug, 5/16", PEEK	5	18-1112-50
Tubing connector/M6 male	1	18-1017-98

Item	*Quant./ pack*	*Code no.*
Accessories		
Flow restrictor, FR-902, 0.2 MPa	1	18-1121-35
Flow restrictor, FR-904, 0.4 MPa	1	18-1119-63
Column holder, for one column	1	18-1113-17
Column holder, for up to six small columns	1	18-1113-18
Lab rod holder	1	18-1113-19
Tubing cutter	1	18-1112-46
User Manuals		
ÄKTAbasic Manual Box complete, containing all User Manuals and Instructions for all components in ÄKTAbasic10/100	1	18-1140-99
UNICORN Getting started	1	56-3207-99
UNICORN version 4.10 manuals	3	18-1164-09
ÄKTAbasic User Manual	1	18-1140-76
Pump P-900 User Manual	1	18-1120-04
Pump P-960 User Manual	1	18-1172-73
Monitor UV-900 User Manual	1	18-1120-05
Short Instruction Pump P-900	1	18-1120-08
Short Instruction Monitor UV-900	1	18-1120-09

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TC information, Uppsala. Printed in Sweden by TK i Uppsala AB