ÄKTA*design*

ÄKTA*FPLC*

System Manual

18-1140-45

amersham pharmacia biotech

Important user information

Meaning: Consult the instruction manual to avoid personal injury or damage to the product or other equipment.

WARNING!
The Warning sign is used to call attention to the necessity to follow an instruction in detail 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 shall be followed to avoid damage to the product or other equipment. 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 or optimal use of the product.

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

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About this manual

This manual describes the operation of the ÄKTA™*FPLC*™ system.

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

The installation of the system is described in the separate Installation Guide. The installation of the fraction collctor is described in a separate manual designated *ÄKTAdesign Optional Configurations User Manual*.

Basic information on how to operate the system is not described in this manual. The user must first read the "Making your first run" booklet to take full advantage of the contents of this manual.

Depending on the application, different optional configurations might be required. Information about these options can be found in *ÄKTAdesign Optional Configurations User Manual* which describes the extended functions of ÄKTA*FPLC*.

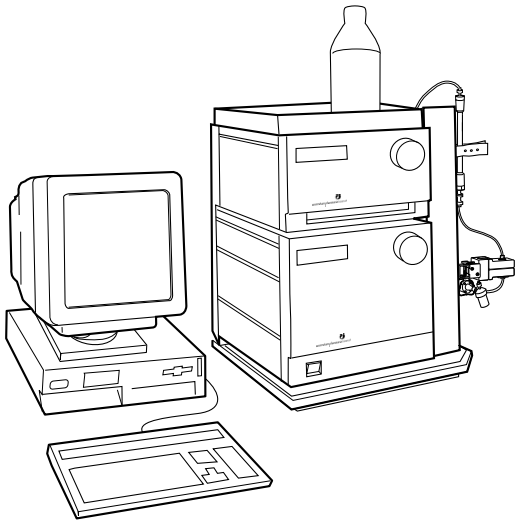
1 Introduction

1.1 General

ÄKTA*FPLC* is a fully automated liquid chromatography system designed for research scale purification of proteins. The system simplifies the transition from laboratory to full scale production. Scale-up to production is predictable and trouble-free.

ÄKTA*FPLC* features:

- Flow rates up to 20 ml/min and pressures up to 5 MPa.
- One working platform for all liquid chromatography techniques suitable for protein purification, from micro-gram to gram scale.
- Method templates as a basis for creating customised methods.

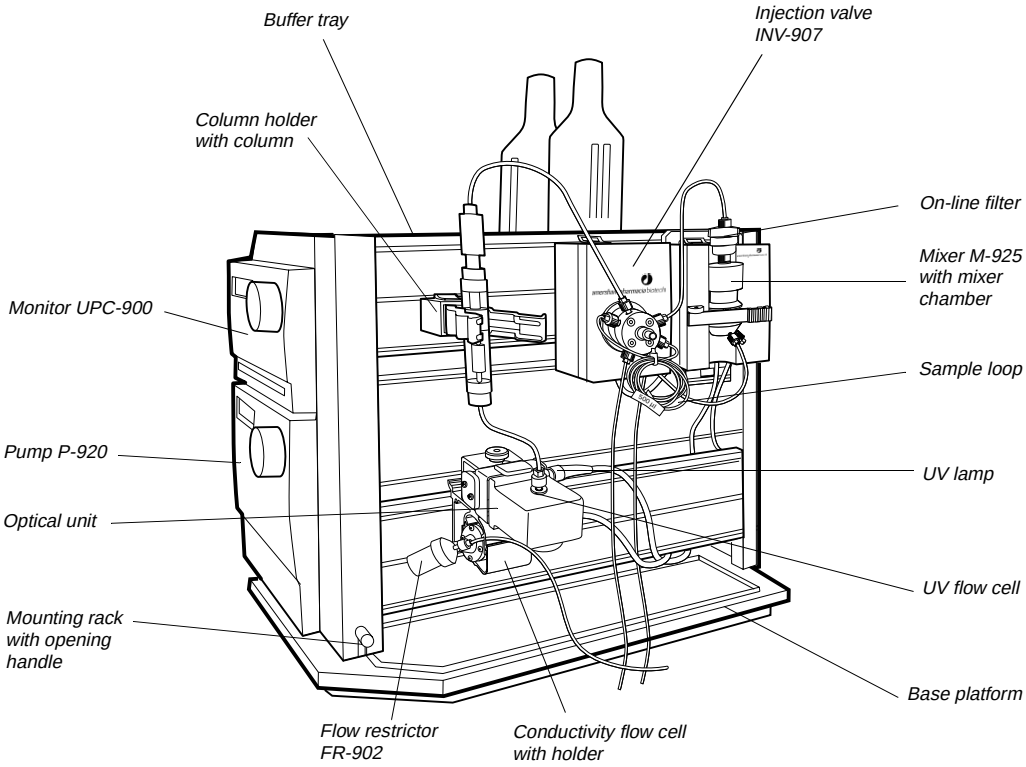


In its standard configuration, ÄKTA*FPLC* consists of a compact separation unit including modules and components, and a personal computer running UNICORN™ software version 3.2 or higher to control the separation unit. A fraction collector, Frac-900 or Frac-950, is also included. A hinged rack allows easy attachment of optional equipment when expanding the standard configuration.

ÄKTA*FPLC* is described in detail in section *A* of *Reference information* in this manual and brief descriptions of the individual modules and components are given in section *B* of *Reference information*. Detailed information on the modules and components can be found in their respective User Manuals and Instructions. UNICORN software is described in the separate UNICORN User Manual.

ÄKTAdesign Optional Configurations User Manual describes the installation and operation of the fraction collector. It also includes information on optional equipment.

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



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! The optical unit of Monitor UPC-900 uses high intensity ultra-violet light. Do not disassemble the optical unit 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 may penetrate the system causing a short-circuit.

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

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

WARNING! NaOH is injurious to health. Avoid spillage.

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 Pharmacia Biotech may be used for maintaining or servicing the system.

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

WARNING! When using hazardous chemicals, ensure 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! Make sure that the locking screws holding the upper part of the system rack are tightened sufficiently when it is raised to upper position.

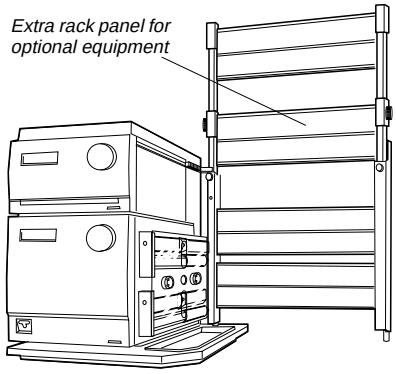
WARNING! There must always be a sample loop or Superloop connected to ports 2 and 6 of the injection valve. This is to prevent liquid spraying out of the ports when switching the valve. This is especially dangerous if hazardous chemicals are used.
If the system is configured for sample application directly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

1.3 Optional configurations

The ÄKTA*FPLC* standard system configuration can easily be changed to optional configurations. This built-in flexibility in the standard ÄKTA*FPLC* system allows the user to enhance already used purification methods and also to develop new, more complex methods.

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

To support the process of implementing optional configurations, general guidelines regarding installation and operation are given in the separate manual *ÄKTA Design Optional Configurations User Manual* included in the ÄKTA*FPLC* Manual Box.



Optional configurations are monitored and controlled via methods run by the UNICORN control system in the same way as the ÄKTA*FPLC* standard configuration.

Optional configurations supported by ÄKTA*FPLC* are:

- Connection of up to 9 motorised multi-port valves. These valves can be used to accomplish the following functions:
  - Column selection.
  - Buffer selection.
  - Flowthrough fractionation and collection of large fractions.
  - Optional functions.
- On-line pH measurement.
- Connection of a dedicated sample pump.
- Connection of a Superloop™.
- Connection of external equipment using digital input/output signals through the system pump P-920 REMOTE connector.
- Connection of up to three air sensors.

2 Operation

This chapter describes how to optimise and operate ÄKTA*FPLC* for different applications. The options available are discussed in the following sections:

- Columns and tubing (section 2.1).
- Sample application techniques (sections 2.2 - 2.3).
- Gradient forming techniques (section 2.4).
- Changing UV flow cell and wavelength (section 2.5).
- Collecting fractions (section 2.6).
- Scouting (section 2.11).

The chapter also discusses how methods are selected and system handling while preparing a run (section 2.7), during runs (section 2.8), after runs (section 2.9), and cold room operation (section 2.10).

2.1 Columns and tubing

A wide range of pre-packed columns for techniques such as ion exchange, gel filtration, hydrophobic interaction and affinity chromatography are suitable for use with ÄKTA*FPLC*. A comprehensive list of the recommended pre-packed columns is given overleaf.

On delivery, the system is equipped with 0.50 mm i.d. tubing (marked G, PEEK tubing, orange) from the pump to the outlet.

When running columns with a low maximum pressure and high flow rates, PEEK tubing with a larger inner diameter may be used instead to prevent increased back-pressure after columns, which could cause the columns to rupture.

Note: *It is very important to use the correct tubing diameter and to take into consideration the maximum allowed pressure for the column and the size of the column.*

When ÄKTA*FPLC* is extended with optional functions, it may also be necessary to use PEEK tubing with a larger inner diameter to prevent increased back-pressure. Refer to the *ÄKTA Design Optional Configurations User Manual* for further details.

Recommended columns

The tables below list recommended columns.

Ion Exchange Columns

Code no.	*Column name*
17-1153-01	HiTrap™ Q, 1 ml
17-1154-01	HiTrap Q, 5 ml
17-1151-01	HiTrap SP, 1 ml
17-1152-01	HiTrap SP, 5 ml
17-5092-01	HiPrep™ 16/10 Q XL
17-5093-01	HiPrep 16/10 SP XL
17-5091-01	HiPrep 16/10 CM
17-5090-01	HiPrep 16/10 DEAE
17-1064-01	HiLoad™ 16/10 Q
17-1066-01	HiLoad 26/10 Q
17-1137-01	HiLoad 16/10 SP
17-1138-01	HiLoad 26/10 SP
17-1177-01	RESOURCE™ Q, 1 ml
17-1179-01	RESOURCE Q, 6 ml
17-1178-01	RESOURCE S, 1 ml
17-1180-11	RESOURCE S, 6 ml
17-5065-01	SOURCE™ 15Q,PE 4.6/100
17-5067-01	SOURCE 15S,PE 4.6/100
17-0546-01	Mono Q™ HR, 5/5
17-0556-01	Mono Q HR, 10/10
17-0506-01	Mono Q HR, 16/10
17-0547-01	Mono S™ HR, 5/5
17-0557-01	Mono S HR, 10/10
17-0507-01	Mono S HR, 16/10

Size Exclusion (Gel filtration) Columns

Code no.	*Column name*
17-1165-01	HiPrep 16/60 Sephacryl S100 HR
17-1194-01	HiPrep 26/60 Sephacryl S100 HR
17-1166-01	HiPrep 16/60 Sephacryl S200 HR
17-1195-01	HiPrep 26/60 Sephacryl S200 HR
17-1167-01	HiPrep 16/60 Sephacryl S300 HR
17-1196-01	HiPrep 26/60 Sephacryl S300 HR

Code no.	Column name
17-1139-01	HiLoad 16/60 Superdex 30 prep grade
17-1140-01	HiLoad 26/60 Superdex 30 prep grade
17-1068-01	HiLoad 16/60 Superdex 75 prep grade
17-1070-01	HiLoad 26/60 Superdex 75 prep grade
17-1069-01	HiLoad 16/60 Superdex 200 prep grade
17-1071-01	HiLoad 26/60 Superdex 200 prep grade
17-1047-01	Superdex™ 75 HR 10/30
17-1088-01	Superdex 200 HR 10/30
17-1453-01	Superdex Peptide HR 10/30
17-5003-01	Superdex Peptide PE 7.5/300
17-0538-01	Superose™ 12 HR 10/30
17-0537-01	Superose 6 HR 10/30

Hydrophobic Interaction Columns

Code no.	Column name
17-5095-01	HiPrep 16/60 Phenyl (high sub)
17-5094-01	HiPrep 16/60 Phenyl (low sub)
17-5097-01	HiPrep 16/60 Octyl
17-5096-01	HiPrep 16/60 Butyl
17-1085-01	HiLoad 16/60 Phenyl
17-1086-01	HiLoad 26/60 Phenyl
17-1084-01	RESOURCE ETH 1 ml
17-1085-01	RESOURCE ISO 1 ml
17-1086-01	RESOURCE PHE 1 ml
17-5071-01	SOURCE 15 PHE, PE 4.6/100
17-0519-01	Phenyl Superose HR 5/5
17-0530-01	Phenyl Superose HR 10/10
17-0586-01	Alkyl Superose HR 5/5
17-0587-01	Alkyl Superose HR 10/10

Chelating Columns

Code no.	Column name
17-0408-01	HiTrap Chelating 1 ml
17-0409-01	HiTrap Chelating 5 ml

Affinity Columns

Code no.	*Column name*
17-0402-01	HiTrap Protein A 1 ml
17-0403-01	HiTrap Protein A 5 ml
17-0404-01	HiTrap Protein G 1 ml
17-0405-01	HiTrap Protein G 5 ml
17-0406-01	HiTrap Heparin, 1 ml
17-0407-01	HiTrap Heparin, 5 ml
17-5079-01	HiTrap rProtein A, 1 ml
17-5080-01	HiTrap rProtein A, 5 ml
17-0412-01	HiTrap Blue, 1 ml
17-0413-01	HiTrap Blue, 5 ml
17-0716-01	HiTrap NHS-activated, 1 ml
17-0717-01	HiTrap NHS-activated, 5 ml
17-5105-01	HiTrap Con A, 1 ml
17-5106-01	HiTrap Lentil Lectin, 1 ml
17-5108-01	HiTrap Peanut Lectin, 1 ml
17-5107-01	HiTrap Wheat Germ Lectin, 1 ml
17-5110-01	HiTrap IgM Purification, 1 ml
17-5111-01	HiTrap IgY Purification, 5 ml
17-5112-01	HiTrap Streptavidin, 1 ml
17-5130-01	GSTrap™, 1 ml (5 pcs)
17-5130-02	GSTrap, 1 ml (2 pcs)
17-5131-01	GSTrap, 5 ml

Chromatofocusing Columns

Code no.	*Column name*
17-0548-01	Mono P HR 5/20
17-0611-01	Mono P HR 5/5

Buffer Exchange/Desalting Columns

Code no.	*Column name*
17-1408-01	HiTrap Desalting
17-0591-01	Fast Desalting HR 10/10
17-5087-01	HiPrep 26/10 Desalting

Extra system pressure measurement

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

1 Set the injection valve (INV-907) to position Waste.

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

3 Make a note of the back-pressure value read on the pump display or in the **Run Data** window 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 entered into the UNICORN column list and defined as a personal column:

1 Select **File:Column list** to open the Columns dialogue box. Selecting a column in the list will display its parameters in the field to the right of the box.

2 Click on **EDIT** to display the column. In the **Column Parameters** dialogue box, enter in the field for Max pressure the new unit pressure limit, 0.5 MPa + the measured value. Click on **Replace** after the new value has been entered.

3 Click on **Save as** and enter a new name for 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 you deselect (uncheck) the **Save as** global box in this situation.

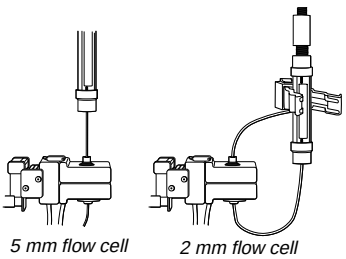
4 Click on **Save as** again to add the updated column parameters to the column list.

For further information, refer to section *5.9.2 Editing column parameters* in the UNICORN User Manual.

Connecting the column

The column is connected between the injection valve, port 1, and the inlet port of the UV cell.

Note: *The inlet port of the 5 mm UV cell is above the optical unit. The inlet port of the 2 mm UV cell is below the optical unit.*



2.2 Sample application overview

In ÄKTA*FPLC* standard configuration, the sample is applied by using a sample loop. For application of large sample volumes, Superloops and a sample pump are available as optional components. These are described in *ÄKTAdesign Optional Configurations User Manual*.

The following table shows which technique is recommended for different sample volumes. For a description of the available method templates, and their contents, please refer to the method notes in UNICORN.

Sample application technique	***Volume to inject***	***Template name begins with***
Sample loop, manual filling	0–2 ml	Manual injection
Superloop[1]	1 ml - 150 ml	Manual injection
Sample pump[2]	>1 ml	Manual injection (modified)

[1] How to use a Superloop is described in ÄKTAdesign Optional Configurations User Manual.

[2] How to use the sample pump and the template modifications required are described in the ÄKTAdesign Optional Configurations User Manual. Refer also to the User Manual for the sample pump.

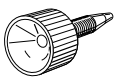
2.3 Manual filling of sample loops

Use the Manual Injection FP Type of gradient method template to apply the sample manually.

Preparation

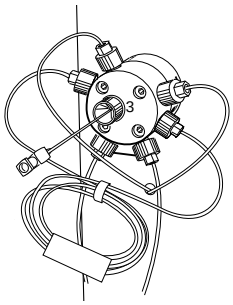
Prepare the injection valve as follows:

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



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

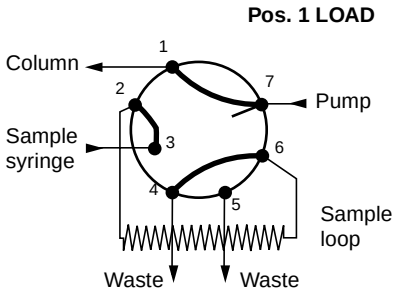
3 Tighten the fill port until the nozzle has formed a seal around the needle's tip, i.e. when it feels as if you are penetrating a septum at the end of the injection fill port. The seal should allow easy insertion and removal of the needle.



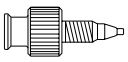
4 Mount the syringe holder on the fill port.

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 taken out 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 ÄKTA*FPLC* 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

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

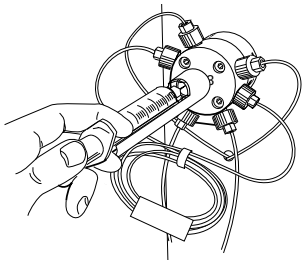
Partial filling

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.*

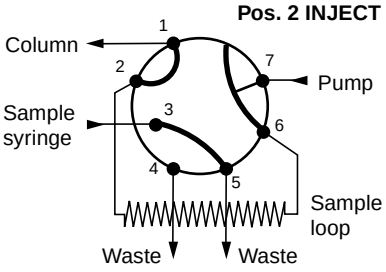
1 Set the injection valve to position LOAD.



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.

When template methods are used, set the volume in the variable **Empty_loop_with**.

2.4 Mixing gradients

Gradients

Gradients are mixed using two separate buffers connected to the A and B pump modules of P-920. The output flow from the pump is routed to Mixer M-925.

Mixer

The mixer is supplied with a 0.6 ml mixer chamber. It can be used at all flow rates up to 20 ml/min.

Note: *When using flow rates above 10 ml/min or gradient volumes larger than 100 ml the result may improve with a 2 ml optional mixer chamber instead of the 0.6 ml standard mixer chamber.*

Other mixer chambers with 2 ml and 5 ml mixer volumes are available as accessories. See section *E* in *Reference Information*.

When using eluents that are more difficult to mix, such as isopropanol and water, a larger mixer volume will give better mixing.

Note: *If the pH (optional) and conductvity curves indicate uneven mixing of your buffers (unstable curves), change to a 2 ml or 5 ml mixer chamber.*

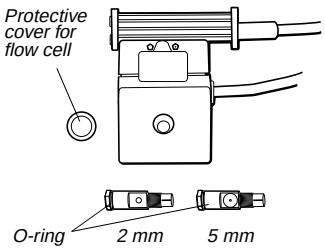
2.5 Changing UV flow cell and wavelength

Changing UV flow cell

There is one analytical flow cell (5 mm, delivered with the system) and one preparative flow cell (2 mm, available as an accessory) available. The flow cell can be changed when required, for example from 2 mm to 5 mm to increase the sensitivity, or from 5 mm to 2 mm to decrease the sensitivity.

Change the flow cell as follows:

1 Disconnect the inlet and outlet capillaries from the flow cell.



Note: *Avoid spillage for prolonged monitor life-time.*

2 Untighten the flow cell, by turning the locking nut, and remove it.

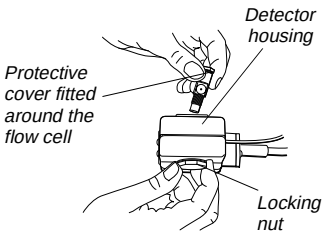
3 Remove the protective cover from the old flow cell and transfer it to the new flow cell.

4 Place the new flow cell into the detector housing from above.

5 Secure the flow cell by turning the locking nut until it reaches its stop position.

Note: *If the locking nut is not tightened sufficiently, the monitor will function poorly (e.g. drifting base-line).*

6 Place the protective cover around the flow cell to protect the electronics inside the optical unit from liquid spillage.

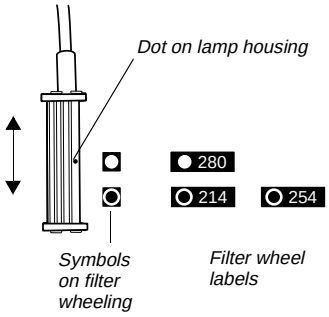


Changing wavelength

Change the wavelength as follows:

1 The Hg lamp housing has two positions, one for 280 nm, marked by on the filter housing, and the other one marked by for all other wavelengths. The Zn lamp housing has only one position, for 214 nm. There will be a faint click when the housing is correctly slide into either position.

2 Set the wavelength to be used by selecting lamp position (indicated by a dot on the lamp housing) in combination with the appropriate filter, i.e. the dot on the lamp housing should be adjacent to the symbol on the filter housing corresponding to the symbol on the filter wheel for the filter to be used. A click will indicate that the filter is in position.



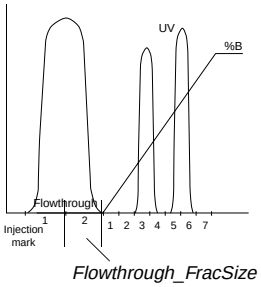
Note: *The new wavelength is not automatically registered in UNICORN. This must be entered by the user. When starting a template method, a question appears in which the user is prompted to state the wavelength to be used in the run.*

2.6 Collecting fractions

Fractions are collected with the fraction collector included in the system (Frac-900 or Frac-950). The template methods make it possible to fractionate in different ways:

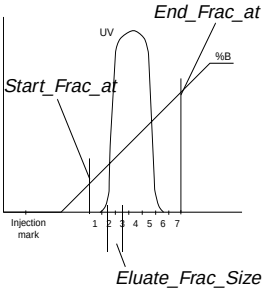
- Flowthrough fractionation.
- Eluate and peak fractionation.

Fraction collection is described in detail in *ÄKTAdesign Optional Configurations User Manual*.



Flowthrough fractionation

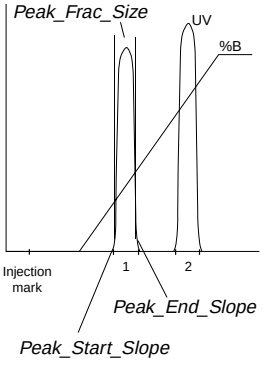
Flowthrough fractionation means that fixed volumes are collected before elution fractionation starts. This fractionation method is available in all method templates. The fractionation volume size is set in the Variables page in the block **Flowthrough_Fractionation** with variable **Flowthrough_FracSize**.



Eluate and/or peak fractionation

Eluate fractionation allows you to collect fixed volumes during elution within a set interval of %B. The eluate fractionation volume is set on the Variables page in block **Eluate_and_Peak_Fractionation** with variable **Eluate_Frac_Size**. The start of the fractionation interval is set with variable **Start_Frac_at** and the end of the interval is set with variable **End_Frac_at.**

Eluate 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 (**Peak_Frac_Size**) is set to a volume which is larger than the actual peak volumes.
- Peaks are collected in several fractions. The peak size (**Peak_Frac_Size**) is set to a volume which is smaller than the actual peak volume.

Select a method template with ”FP” as middle letters (Type of injection FP Type of gradient). The properties for the peak slopes are set in block **Eluate_and_Peak_Fractionation**. Variables **Peak_Start_Slope** and **Peak_End_Slope** controls the start and end points for the peak fractions to be collected. **Minimum_Peak_Width** controls the minimum peak width to be collected. **Peak_FracSize** sets the peak volume sizes during the fractionation slope interval.

2.7 Before a run

Selecting a method

UNICORN is supplied with a set of method templates which can be used as the basis for creating customised methods.

The basic steps required to create a method are:

3 Choose **New:Method** in the Main menu or the method editor and select system, technique, method template and column. Read the method notes to select a suitable method template.

4 Click the **OK** button.

5 Adjust the values for the method variables, e.g. Flow, in the **Variables** page.

6 Read the method notes to check that your system is configured to the requirements of the selected method template.

7 Save the method.

Calibrations

The table below lists the type and frequency of calibrations that can be done on ÄKTA*FPLC*. Refer to UNICORN User Manual section 6.6.1 and to the individual component User Manuals and Instructions for descriptions of how to perform these calibrations. The calibrations are performed from UNICORN by selecting **System:Calibrate** in **System Control**.

Component	*How often*
pH electrode (optional)	Every day.
Pressure reading	Once a year or when required.
Conductivity Flow Cell	
Cell constant	Only necessary if specific conductivity with high accuracy is measured (Cond_calib).
Temperature	Must be done when changing the Flow Cell (Temp).
Entering a new cell constant	Must be done when changing the Flow Cell (Cond_cell).
Sample pump (optional)	Whenever the running conditions are changed.

General preparation

Before starting any method, we recommend you make certain checks to ensure 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 the pump as described in the P-920 User Manual.

5 Check that the correct wavelength is set on the optical unit and that the correct UV flow cell is installed.

Note: *In UNICORN, the wavelength used is stated by answering a question on the Questions page before starting a run.*

6 Calibrate the pH electrode if required (optional). Refer to Monitor UPC–900 User Manual.

7 Check that the correct mixer chamber and tubings are installed for the method selected.

8 Check that the fraction collector has sufficient tubes fitted and is connected to the ÄKTA*FPLC* outlet.

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

2.8 During a run

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 ÄKTA*FPLC* and can display up to four panels for monitoring different aspects of the run. Click on the **Windows** toolbar button or choose **View:Windows** from the menu to select which panels to display.

Run data

The run data panel displays the current values for selected run parameters. Select **View:Run data contents** from the system control menu. Select the run data items to be displayed and click **OK.**

Curves

The curves panel displays the monitor signal values graphically. Position the cursor in the **Curves** panel and click with the right mouse button. Select **Properties...** or choose **View:Curve Properties...** to select the curves to display. All curves are always stored in the result file.

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.

Front panel displays

The front panel displays of Monitor UPC-900 and Pump P-920 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-920 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.

AU	Cond	%Tc	pH
0.00002	015.0		12.50

The main operating menu 1 of Monitor UPC-900 shows the absorbancy value with 6 digits for the selected wavelength, the conductivity as a percentage of full scale and the pH value (optional).

pH 12.50	Tc	22.4°C
735.8mS/cm	Tc	78.8%

By turning the dial one click, an alternative display of the conductivity is shown (main menu 2). This display shows pH, temperature and the actual conductivity value in mS/cm or µS/cm, together with the percentage value.

Changing parameters

From UNICORN

ÄKTA*FPLC* can be controlled with manual instructions issued from the **Manual** menu under **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 under **System Control** opens a dialogue 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 on **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 all that may be printed. Make sure that the **Instruction Set** box is checked and deselect any unwanted items. Click on **OK** to print out the instructions.

From the modules

Manual changes can also be performed on Pump P-920 and on Monitor UPC-900 using the selection dial.

Manual changes in UNICORN or on the modules are equivalent. Manual changes are normally recorded in the log book. However, manually entered changes carried out from a module after having entered changes in UNICORN are not logged.

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 setting** under **System Control** then **Special:Keyboard**. Select **Open Keylocked** or **KeyandDiallocked**.

2.9 Completion of a run and storage

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

Between runs

If a buffer containing salt has been run, it is very important to wash Pump P-920, the system and the column with distilled water, especially if organic solvent, e.g. ethanol, is to be used in the next run. Perform a **PumpWash** with distilled water to wash P-920.

Storage

Overnight

The system can be left filled with a buffer overnight.

Weekend and long term storage

If you are not using the separation unit for a few days or longer, perform a **PumpWash** 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% and store it in 20% ethanol (not the pH electrode, see separate instructions overleaf). Make sure that all tubing and all flow paths used are rinsed.

pH electrode (optional)

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

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

2.10 Cold room operation

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

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.

Running

Before starting a run, check the following:

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

2 Calibrate the pH electrode (optional).

3 Check the pH of the buffers.

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.

2.11 Scouting

Scouting can be used to automatically repeat a run when systematically varying one or more parameters. Some typical situations where scouting can be useful are:

- Finding the optimal flow rate.
- Optimising gradient length and slope.
- Optimising a step gradient.

Any parameters can be scouted, provided that they can be defined as variables in the method used. Scouting schemes are a part of the Run setup in the method editor of UNICORN. Refer to chapter 8 in the *Making Your First Run* booklet for a brief description, and to chapter 7 in the *UNICORN User Manual* for specific instructions on how to set up a scouting run.

3 Maintenance

3.1 Periodic maintenance

Regular maintenance will help to keep your ÄKTA*FPLC* 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 of 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, ensure that the entire system has been flushed thoroughly with distilled water before service and maintenance.

WARNING! NaOH is injurious to health. Avoid spillage.

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

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

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.

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.
pH electrode (optional)	• Calibrate the pH electrode according to Monitor UPC-900 User Manual
Pump P-920	• Check for leakage. If there are signs of liquid leaking out from the cylinder assemblies, the on-line filter may require replacement more often.
Every week	
On-line filter	• Replace the on-line filter. If Pump P-920 is used for sample application, the on-line filter may require replacement more often.
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. An increase in the volume of rinsing solution behind the pistons indicates internal pump leakage. Replace the piston seals according to Pump P-920 User Manual.

Interval	*Action*
Every month	
Flow restrictor	• Check that the flow restrictor generates the following back-pressure: 0.2 ± 0.05 MPa. Check the back-pressure as follows: 1 Disconnect the flow restrictor. 2 Connect a capillary to port 1 of the injection valve. Put the open end in the waste container. 3 Run the pump manually at 10 ml/min with water. Note the back-pressure on the pump display or in the RUN DATA window. 4 Connect the flow restrictor to the open end of the capillary (note the IN marking). 5 Run the pump at 10 ml/min with water. Note the pump display or in the RUN DATA window. 6 Calculate the back-pressure generated by the flow restrictor. Replace it if it is not within limit.
Every 6 months	
Monitor UPC-900	• Clean the flow cells according to Monitor UPC-900 User Manual.
Fraction collector	• Refer to the User Manual for your fraction collector.
Yearly	
Valve INV-907	• Check for external and/or internal leakage. Replace the distribution plate yearly or when required.
When required	
Pump P-920	• Replace piston seals. Refer to Pump P-920 User Manual. • Replace piston. Refer to the User Manual. • Clean or replace the pump valves. Refer to the User Manual.
Monitor UPC-900	• Clean the flow cells according to Monitor UPC-900 User Manual.

3.2 Cleaning the system

The procedures described below are for system cleaning. To bypass the column, use a piece of 0.5 mm i.d. PEEK tubing supplied with ÄKTA*FPLC*. 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 respective column in the Adviser-Media and Adviser-Column Healer section in UNICORN or to the Instructions supplied with the column.

At the end of the day

The system can be left filled with a buffer overnight.

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

1 Submerge the inlet tubings in distilled water.

2 Run the **PumpWash** instruction.

Leaving the system for a few days

Perform a **PumpWash** with distilled water. Repeat with a bacteriostatic solution such as 20% ethanol (not the pH electrode, see separate instructions below).

pH electrode (optional)

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

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

Monthly cleaning

WARNING! NaOH is injurious to health. Avoid spillage.

Clean the system every month or when problems such as ghost peaks occur. The system is cleaned as follows:

1 Disconnect the column and replace it with a suitable bypass capillary.

2 Place all the inlet tubings in 1 M NaOH.

3 Manually, perform **PumpWash** for all inlet tubings.

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

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

Other cleaning considerations

After repeated separation cycles, contaminating material may progressively build up in the system and on the columns. This material may not be removed by the cleaning step described above. The nature and degree of contamination depends on the sample and the chromatographic conditions employed.

3.3 Moving the system

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

CAUTION! Never lift the separation unit by the components attached to the mounting rails of the main components or to the system rack.

The base platform rests on low friction pads, which makes it easy to turn around to access the sides and rear of the separation unit.

Two persons are recommended to lift the separation unit. Before moving the system, ensure that:

- All cables and capillaries connected to peripheral equipment and liquid containers are disconnected.
- All items on top of the separation unit are removed.
- The extension mounting frame, if used, is removed and the rack rails are lowered to resting position.
- The system rack is locked in closed position.

Lift the separation unit by placing your fingers in the gap between the base platform and the work bench surface, grasping firmly and lifting.

4 Trouble-shooting

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

Type	*Page*
UV curve	33
Conductivity curve	34
Mixer M-925	35
Pressure curve	36
Monitor UPC-900	36
Pump P-920	37
INV-907	38
Fraction collector	38

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

4.1 UV curve

Fault	*Action*
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 a back-pressure of 0.2 ± 0.05 MPa. Check the back-pressure as follows: 1 Disconnect the flow restrictor. 2 Connect a capillary to port 1 of the injection valve. Put the open end in the waste container. 3 Run the pump manually at 10 ml/min with water. Note the back-pressure on the pump display or in the RUN DATA window. 4 Connect the flow restrictor to the open end of the capillary (note the IN marking). 5 Run the pump at 10 ml/min with water. Note the pump display or in the RUN DATA window. 6 Calculate the back-pressure generated by the flow restrictor. Replace it if it is not within limit. 3 Degas the buffer before use. 4 Check the connections of the optical unit and filter setting. 5 Clean the UV flow cell, see *Monitor UPC-900 User Manual.*

Fault	*Action*
Ghost peaks	1 Check that there is no air in the eluent. 2 Clean the system in accordance with section 3.2. 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. The mixer function is checked by placing a stirrer bar on top of the mixer housing. The stirrer bar should rotate when the system is in **RUN** mode. The mixer function can also be checked by running the installation test. 5 Unless you are using a low pressure column, try using a Flow restrictor FR-904 instead of FR-902. This generates a higher back-pressure (0.4 MPa instead of 0.2 MPa).

4.2 Conductivity curve

Fault	*Action*
Conductivity measurement with the same buffer appears to change over time	1 Clean the flow cell according to *Monitor UPC-900 User Manual.* 2 The ambient temperature may have decreased. Use a temperature compensation factor, see *Reference information B* of *Monitor UPC-900 User Manual.*
Ghost peaks appear in the gradient profile	1 A charged sample has been detected (e.g. protein). 2 Air bubbles are passing through the flow cell. Check for loose tubing connections. Use a flow restrictor.
Baseline drift or noisy signal	1 There may be air in the flow cell. 2 Check for leaking tubing connections. 3 Check that the column is equilibrated. If necessary, clean the column. 4 Check the operation of the mixer and the system pump. The mixer function is checked by placing a stirrer bar on top of the mixer housing. The stirrer bar should rotate when the system is in **RUN** mode. The pump and mixer functions can also be checked by running the installation test. 5 Clean the flow cell according to *Monitor UPC-900 User Manual.*

Fault	*Action*
Waves on the gradient	1 Check that the pump and the valves are operating properly and are programmed correctly. 2 Change to a larger mixing volume if necessary. 3 Check the operation of the mixer. The mixer function is checked by placing a stirrer bar on top of the mixer housing. The stirrer bar should rotate when the system is in **RUN** mode. The mixer function can also be checked by running the installation test.
Non-linear gradients or slow response to %B changes	1 Check that the tubing has been washed properly and that the pump is operating. 2 If a larger mixer than the standard 0.6 ml chamber is used, try changing to a smaller mixer volume.
Incorrect or unstable reading	1 Check that the conductivity flow cell cable is connected properly to the rear of the monitor. 2 Check that the pump operates correctly. 3 If temperature compensation is used, check that the temperature sensor is calibrated and that the correct temperature compensation factor is used. 4 Check that the column is equilibrated. If necessary, clean the column. 5 Check the operation of the mixer. The mixer function is checked by placing a stirrer bar on top of the mixer housing. The stirrer bar should rotate when the system is in **RUN** mode. The mixer function can also be checked by running the installation test.

4.3 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.

4.4 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 in the reservoirs. 2 Check all connections for leakage. 3 Follow the instructions in *Pump P-920 User Manual*.
Pump valves not functioning correctly	1 Clean the valves according to *Pump P-920 User Manual.*
Piston seal leaking	1 Replace the piston seal according to the instructions in *Pump P-920 User Manual.*
Blockage or partial blockage of the flow path	1 Flush the flow path to clear the blockage. 2 If necessary, replace the tubing. 3 Check the inlet tubing filter. It can get clogged if unfiltered buffers or samples are applied. See the instructions for flushing at the end of the run in section 2.9.

4.5 Monitor UPC-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 UV baseline	1 Try using a larger mixer chamber instead of the standard 0.6 ml mixer chamber.
Absolute conductivity value wrong	1 Turn the flow cell so that the end with the screws is facing the Flow restrictor FR-902. 2 Recalibrate the conductivity cell. 3 Calibration solution, 1.00 M NaCl, not prepared correctly. Prepare a new calibration solution and recalibrate the conductivity cell.
Unstable conductivity curve	1 Try using a larger mixer chamber instead of the standard 0.6 ml mixer chamber.

4.6 Pump P-920

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 from the pump cylinder assembly	Piston seal or end piece incorrectly fitted or gasket worn. 1 Replace or re-install the seal or gasket. 2 Run-in carefully, see *Pump P-920 User Manual.*
Low eluent flow and noise as the piston moves	1 Disassemble pump cylinder and examine the piston seal according to *Pump P-920 User Manual.* Replace if necessary. 2 Check the piston for damage. If damaged, replace the piston according to *Pump P-920 User Manual.* 3 Remember to replace the piston seal with new parts. 4 Ensure that the piston rinsing system is always used when working with aqueous buffers with high salt concentration.
Leaking connection and/or crystallized 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. Then tighten an extra 1/4 turn using a wrench.
Erratic pump pressure	1 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 *Pump P-920 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 For more details, refer to the *Pump P-920 User Manual*.

4.7 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, i.e. 1 for the injection valve. 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 **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 *INV-907 Instruction.*
High back-pressure	1 Perform cleaning-in-place according to *INV-907 Instruction*. 2 Change channel plate and distribution plate according to *INV-907 Instruction*.

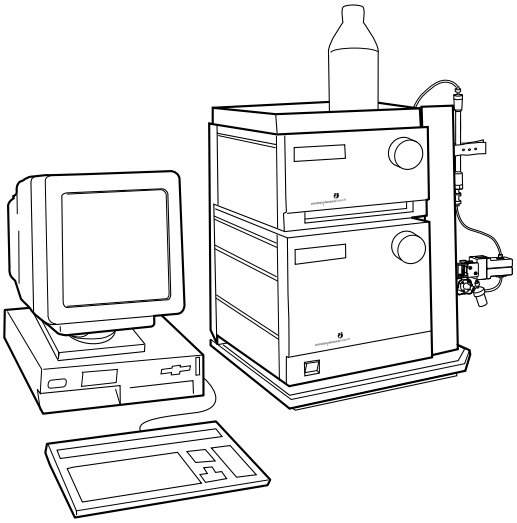
4.8 Fraction collector

Refer to the user manual for the fraction collector installed.

Reference information

A System description

A.1 The system



ÄKTA*FPLC* consists of a compact separation unit comprising modules and components, and a personal computer running UNICORN software version 3.2 or higher to control the separation unit. A fraction collector is included in the standard configuration.

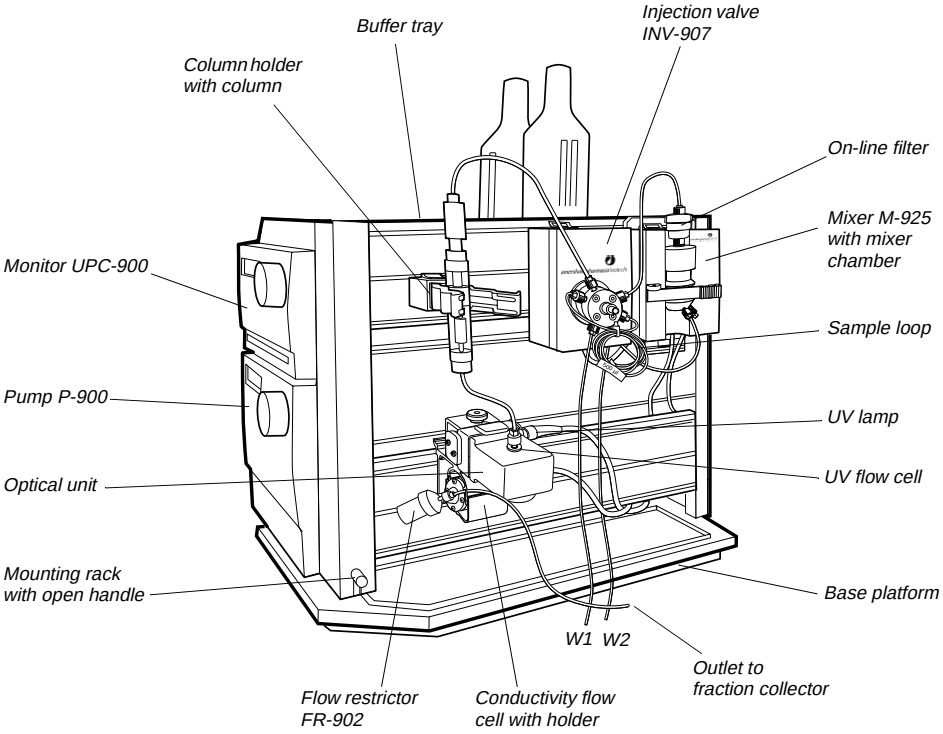
Communication between the computer and the various modules and components of ÄKTA*FPLC* is achieved via high speed data network connections referred to as UniNet 1 and UniNet 2. On UniNet 1, the more complex data signals between modules and the computer are run, whereas UniNet 2 carries the less complex data signals between modules and components.

The fluid handling equipment of ÄKTA*FPLC* is mounted on one side of the separation unit. This allows easy access to all the components, tubings and other fluid handling items located on the modules.

For optional configurations, the hinged system rack can be used to house further components. The rack can be both swung out and its upper part raised to allow optional components to be attached to the standard system configuration.

A.2 Modules and components

The following illustration shows the location of the modules and components of the separation unit.

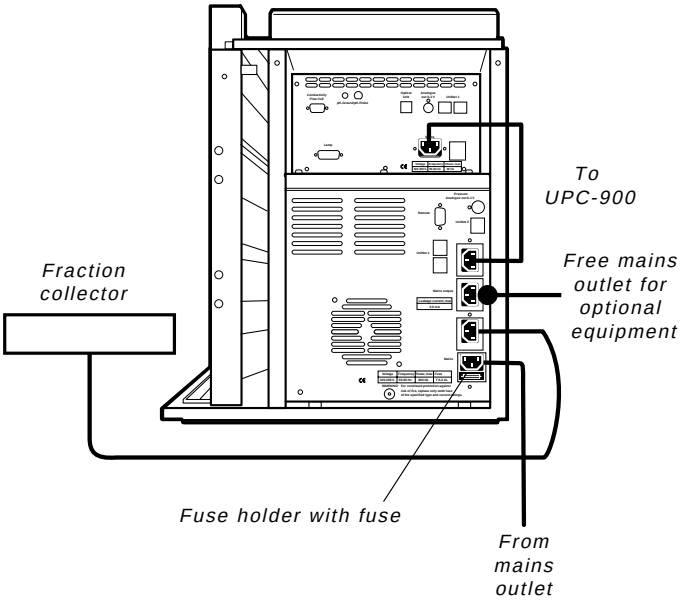


A.3 Electrical connections

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 ÄKTA*FPLC* are located at the rear of the system. The system is mounted on a base platform allowing easy access to the fluid handling components and the electrical connections.

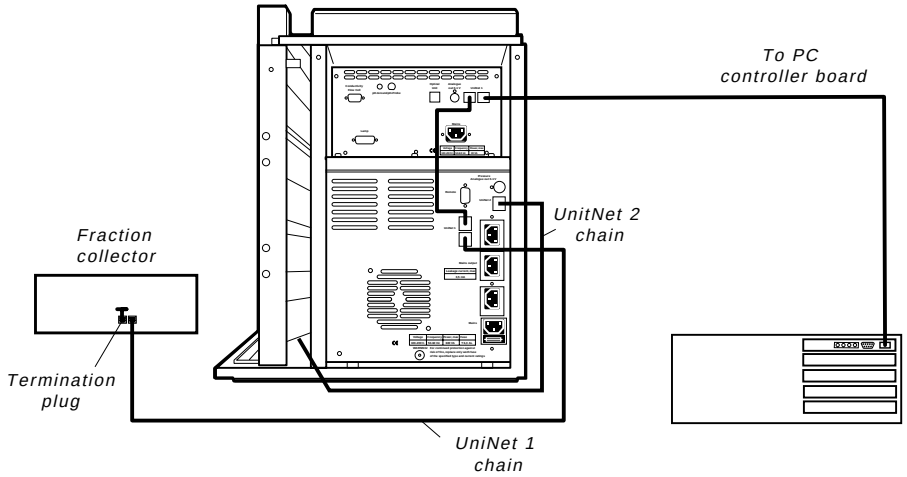
Mains cables



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 base of the system. The mains input fuse holder is located below the mains input.

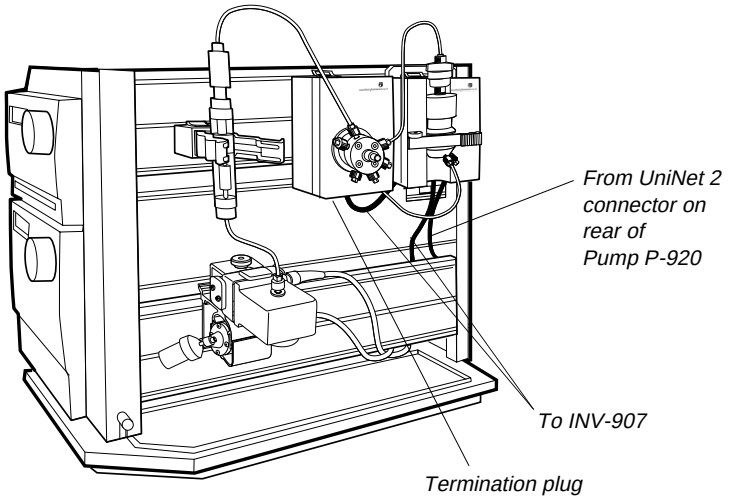
To open the fuse holder, remove the mains inlet cable to the system. Use a small-bladed screwdriver to lever the holder outwards.

UniNet 1 cables



The UniNet 1 data communication chain is routed from the computer via UPC-900 and P-920 to the fraction collector. The chain is terminated at the fraction collector with a termination plug.

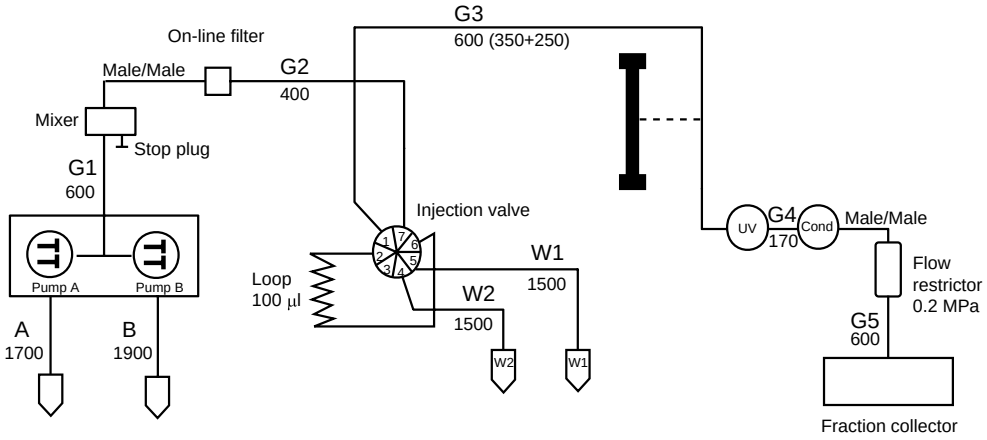
UniNet 2 cables



The UniNet 2 data communication chain, which controls the valve(s) and the mixer comes from the rear of Pump P-920. The chain is terminated at the injection valve with a termination plug.

A.4 Fluid handling path

The following flow diagram shows the positions of the modules, components and tubings in ÄKTA*FPLC* standard configuration. Refer to the flow diagram for their locations in the fluid handling path.



The G3 capillary is mounted at the factory as a column bypass. It is used initially during the installation test. When the test is complete and a column is to be fitted, the G3 capillary can be cut and used to connect the column. The figures state the length in millimetres of the pre-fabricated capillaries.

The table below shows the different tubings mounted from the factory on ÄKTA*FPLC*. At delivery, 0.5 mm i.d. PEEK tubing is installed from the Pump P-920 outlet to the outlet of the Flow restrictor FR-902 and onwards to the fraction collector. Columns are installed either using the tubing supplied with the columns, or with pieces of 0.50 mm PEEK tubing cut by the user to suitable lengths (0.50 mm i.d. PEEK tubing, orange, is supplied with ÄKTA*FPLC* system).

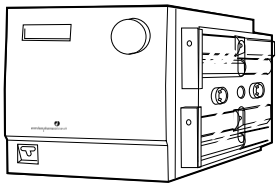
Tubings supplied with the fraction collector installed are described in *ÄKTAdesign Optional Configurations User Manual*.

Tubing i.d.	*Tubing o.d.*	*Material*	*Colour*	*Max. pressure*	*Volume of Connected 10 cm*	
1.6 mm (A, B)	1/8"	Teflon	Clear	2 MPa	201.1 µl	Inlet tubing
0.50 mm (G1–G5)	1/16"	PEEK	Orange	25 MPa	19.6 µl	From Pump P-920 to fraction collector
Union, m/m	1/16"	PEEK	Black	25 MPa	–	Between mixer/on-line filter and conductivity flow cell/ flow restrictor
0.75 mm (W1–W2)	1/16"	Tefzel	Clear	2 MPa	44.2 µl	Waste tubing.

B Modules and components description

A complete description of each module and component can be found in their respective manuals and instructions. Optional components for the ÄKTA*FPLC* system are described in *ÄKTAdesign Optional Configurations User Manual*.

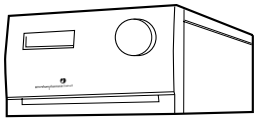
B.1 Pump P-920



Pump P-920 is a high precision laboratory pump for use in liquid chromatography and other applications where constant flow is required. The performance of Pump P-920 is accurate and reproducible from low to high flow rates over the whole pressure range. The chemical resistance of the pump makes it possible to use with corrosive liquids, such as organic solvents, as well as with high salt aqueous solutions.

The wide flow range makes it suitable both for analytical and preparative chromatography. Pump P-920 is designed to work with a wide range of columns and gels supplied by Amersham Pharmacia Biotech.

B.2 Monitor UPC-900

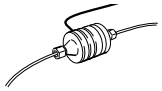


Monitor UPC-900 is a high precision on-line combined monitor for measurement of UV absorption, pH and conductivity in liquid chromatography. The UPC-900 offers fixed wavelengths of 214 (Zn lamp), 254 or 280 nm (Hg lamp), fast response, high accuracy and reproducibility and flow cells with low dead volumes. Additional wavelengths are obtainable using optical filters (accessories).

Monitor UPC-900 consists of a control unit, an optical unit with lamp assembly and a choice of two flow cells (optical path length 2 mm or 5 mm), a conductivity flow cell with integrated temperature sensor and a pH flow cell with pH electrode (optional).

B.3 UV and conductivity flow cells

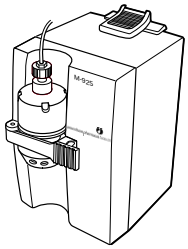
The type of UV flow cell used depends on the sample amount applied and the size of the column. The system is delivered with the 5 mm cell fitted. A 2 mm cell is available as an accessory. If a lower detection sensitivity is desired due to output signal limitation, the 2 mm cell should be used.



Conductivity flow cell with integrated temperature sensor

UV flow cells with 2 and 5 mm path lengths

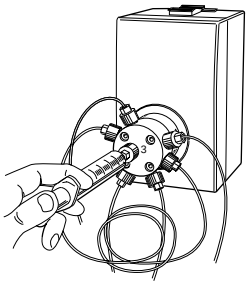
B.4 Mixer M-925



Mixer M-925 is a dynamic, single chamber mixer powered and controlled from Pump P-920. 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-920 in ÄKTA*FPLC*.

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

B.5 Injection 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 100 µl to 2 ml 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.

B.6 Fraction collector

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

In ÄKTA*FPLC*, the fraction collector allows fixed volume fractionation, eluate fractionation or automatic peak fractionation. The latter function is based on peak detection using slope 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.

B.7 Flow Restrictor FR-902

The flow restrictor generates a steady back-pressure to prevent air bubbles being formed after the column in the flow cells. FR-902 is set at the factory to 0.2 MPa.

B.8 On-line filter

The on-line filter is fitted between the output of Mixer M-925 and position 7 of the injection valve. Arrows on the on-line filter indicates the flow direction. It generates a back-pressure of maximum 0.5 MPa.

The filter is a depth type filter made of polypropene. It has a pore size of 2 µm.

The filter should be replaced every week. When changing the filter, use a tool to unscrew the filter body if it cannot be unscrewed by hand. When assembling the on-line filter, tighten the filter body by hand only. Never use a tool.

C Technical specifications

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

The relevant system specifications are listed below.

C.1 Operating data

Pump P-920

Flow rate range	
isocratic mode	0.05–20 ml/min in steps of 10 µl/min
gradient mode	0.1–20 ml/min in steps of 10 µl/min
Pressure range	0–5 MPa (50 bar, 725 psi)
Pressure pulsation	Max. 6% (dP/P) during pump stroke
pH stability range	1–13 (1–14 < 1 day exposure)
Viscosity	
< 10 ml/min	Max. 10cP
> 10 ml/min	Max. 5 cP
Flow rate reproducibility	
flow rate 0.5–10 ml/min	rsd < 0.2%
flow rate 10–20 ml/min	rsd < 0.5%
Gradient composition	
accuracy between turnings	± 2% at 0.5–5 ml/min and < 5 MPa
accuracy during turnings	± 2% at 0.5–5 ml/min and 0.5–2.0 MPa
reproducibility	rsd < 0.5% at 0.5–20 ml/min and < 5 MPa
Leakage	< 0.5 µl/min (pump module A and B each)

Monitor UPC-900

UV measurement

Absorbance range	0.01–5.0 AU (full scale)
Autozero range	-0.2–2.0 AU
Baseline adjust	Adjustable 0–100% of full scale
Wavelengths	
Hg lamp, fixed	254 and 280 nm
by changing filter	313, 365, 405, 436 and 546 nm
Zn lamp	214 nm

UV flow cell, 2 mm

Flow rate	0–100 ml/min
Max. pressure	4.0 MPa (40 bar, 580 psi)
Max. back-pressure	0.05 MPa at 100 ml/min
Liquid temperature range	+4 to +60 °C
Optical path length	2 mm
Cell volume	2 µl (30 µl detector volume)

UV flow cell, 5 mm

Flow rate	0–20 ml/min
Max. pressure	4.0 MPa (40 bar, 580 psi)
Max. back-pressure	0.02 MPa at 20 ml/min
Optical path length	5 mm
Cell volume	6 µl (10 µl detector volume)

Conductivity measurement

Conductivity range	1 µS/cm to 999.9 mS/cm

Conductivity flow cell

Flow rate	0–100 ml/min
Max. pressure	5 MPa (50 bar, 725 psi)
Max. back-pressure	0.01 MPa at 100 ml/min

pH measurement

pH range	0 to 14

Fraction collector

Refer to the User Manual of the fraction collector used.

C.2 Physical data

Degree of protection	IP 20
Power requirement	100–120/220–240 V ~, 50–60 Hz
Power consumption	900 VA
Fuse specification	T 6.3 AL
Dimensions, H x W x D	380 x 480 x 470 mm
Weight	50 kg
EMC standards	This product meets the requirements of the EMC Directive 89/336/EEC through the harmonized standard EN 61326-1 (emission and immunity). Note: The declaration of conformity is valid for the instrument if it is: • used in laboratory locations • used in the same state as it was delivered from Amersham Pharmacia Biotech except for alterations described in the User Manual • used as a “stand alone” unit or connected to other CE labelled Amersham Pharmacia Biotech modules or other products as recommended.
Safety standards	This product meets the requirement of the Low Voltage Directive (LVD) 73/23/EEC through the harmonized standard EN 61010-1.
Environment	+4 to +40 °C, 10–95% relative humidity (non-condensing), 84–106 kPa (840–1060 mbar atmospheric pressure).

C.3 Hardware requirements

- Compaq™ PC, Pentium II/333 MHz or later
  (minimum Pentium/90 MHz
- 64 Mb RAM (minimum 32 Mb) for one system
  128 Mb RAM (minimum 64 Mb) for two or more systems
- 1 Gb of available hard disk space, NTFS file system (minimum 150 Mb)
- Colour monitor, 1024 x 768 pixels (minimum 800 x 600), small fonts, 64k colours
- 1 ISA slot per connected system
- CD-ROM drive
- 1.44 Mb (3.5") diskette drive
- Mouse
- Supported printers:
  HP DeskJet 660C
  HP DeskJet 690C
  HP DeskJet 870Cxi
  HP DeskJet 895 C
  HP DeskJet 2500 C
  HP LaserJet 4M
  HP LaserJet 5MP
  HP LaserJet 4000 N

C.4 Software requirements

Microsoft Windows NT Workstation 4.0 (with Service Pack 4 or later)

C.5 Network requirements

These are the recommended network requirements for running UNICORN in a network installation.

- Supported network cards:
  3COM Etherlink III
  Compaq Netelligent 10/100 TX Embedded UTP Controller
  Compaq Integrated NetFlex-3 Controller
  AMD PCNET PCI Ethernet Adapter (Integrated)
- Novell™ NetWare™ version 4.50.189 or later, or
  Microsoft Windows NT Server 4.0.
  The UNICORN software works on earlier versions as well even though some versions of the Novell NetWare driver have known problems.
- A valid network connection

C.6 ÄKTA*FPLC* component materials

The wetted materials of ÄKTA*FPLC* are listed below:

	FFKM	PEEK	PTFE	FEP	ETFE	ECTFE	PP	PVDF	PE	Titanium alloy	Quartz	Gold	Alum. oxide	Stainl. st. (Elgiloy)	Ruby/ sapphire
Pump P-920			X			X		X	X	X			X	X	X
Monitor UPC-900		X	X							X	X				
Fraction collector	Refer to respective user manual														
Mixer M-925	X	X	X												
INV-907		X													
On-line filter		X					X								
Flow restrictor		X	X		X							X			
Tubing		X		X											
Inlet filters							X			X					
Unions/ Connectors		X			X										

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

D Chemical resistance guide and chemical compatibility

The chemical resistance of ÄKTA*FPLC* 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§	
Ethyl acetate	OK	Avoid	Silicone not resistant. Pressure limit for PEEK decreases.
Ethylene glycol	OK	OK	
Formic acid	OK	OK	Silicone not resistant
Glycerol	OK	OK	
Guanidinium hydrochloride	OK	OK	

Chemical	*Exposure*		*Comments*
	< 1 day	*up to 2 months*	
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	
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

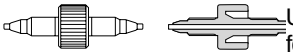
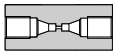
E Accessories and consumables

Item	*Quant./pack*	*A/C**	*Code no.*
Pump P-920			
Sealing kit containing two sealings, two gaskets and two wipers	1	C	18-1032-16
Rinsing tubing, 1.1 m i.d., 3.1 mm o.d.	2 m	A	18-1032-11
Monitor UPC-900			
HG lamp & housing complete	1	C	18-1128-22
Zn lamp & housing complete	1	C	18-1128-23
UV flow cell 5 mm	1	C	18-1128-24
UV flow cell 2 mm	1	C	18-1128-25
Filter 214 nm	1	C	18-0622-01
Filter 254 nm	1	C	18-0620-01
Filter 280 nm	1	C	18-0621-01
Filter 313 nm	1	C	18-0623-01
Filter 365 nm	1	C	18-0624-01
Filter 405 nm	1	C	18-0625-01
Filter 436 nm	1	C	18-0626-01
Filter 546 nm	1	C	18-0627-01
Filter wheel complete	1	A	18-0647-01
Conductivity cell	1	C	18-1111-05
Mixer M-925			
MIxer M-925 including one UniNet cable	1	A	18-1118-89
Mixing chambers:			
0.6 ml	1	A	18-1118-90
2 ml	1	A	18-1118-91
5 ml	1	A	18-1118-92
Valve INV-907			
Valve INV-907 including one UniNet cable (fill port, needle and syringe holder are not included)	1	A	18-1108-40
Injection fill port 0.7 mm	1	C	18-1127-37
Sample loops:			
10 µl	1	C	18-1120-39
100 µl	1	C	18-1113-98
500 µl	1	C	18-1113-99
1 ml	1	C	18-1114-01
2 ml	1	C	18-1114-02

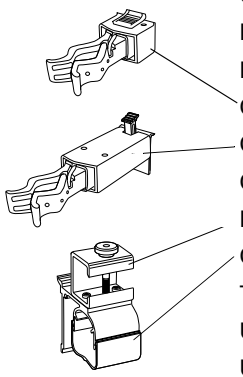
*) A = accessory, C = consumable

Item	Quant./pack	A/C*	Code no.
Fraction Collector Frac-950			
Fraction Collector Frac-950, complete with rack A (18 mm + 30 mm tubes)	1	A	18-6083-00
Rack A, complete with bowl, tube support and tube holder	1	A	18-6083-11
Rack B, complete with bowl, tube support and tube holder	1	A	18-6083-12
Rack C, complete with bowl, tube support and tube holder	1	A	18-6083-13
Rack D, complete with bowl, tube support and tube holder	1	A	18-6083-14
Rack E, complete with tube holder	1	A	18-6083-15
Rack F, complete with tube holder	1	A	18-6083-16
Rack G, complete with tube holder	1	A	18-6083-17
Safety bar with screws	1	A	18-6083-22
Dropsync assembly, complete	1	A	18-6083-23
Fraction Collector Frac-900			
Fraction Collector Frac-90, complete with 18 mm tube rack	1	A	18-1118-97
Tube racks, complete with bowl, tube support, holder and guide:			
12 mm	1	A	19-8684-03
18 mm	1	A	18-3050-03
30 mm	1	A	18-1124-67
Tube support	1	A	18-3054-02
Tube holder and guide:			
12 mm	1	A	19-7242-02
18 mm	1	A	19-8689-02
30 mm	1	A	18-1124-68
Eppendorf tube holder for 12 mm rack	100	A	18-8522-01
Flow Diversion Valve, FV-903 incl. mounting bracket	1	A	18-1114-50
Tubing holder	1	A	18-6464-01
Drive sleeve	5	C	19-6067-02

*) A = accessory, C = consumable

Item	Quant./pack	A/C*	Code no.
Cables			
UniNet, 0.18 m	1	A	18-1109-72
UniNet, 0.3 m	1	A	18-1109-73
UniNet, 0.7 m	1	A	18-1109-74
UniNet, 1.5 m	1	A	18-1117-75
UniNet, 3.0 m	1	A	18-1109-75
UniNet, 15.0 m	1	A	18-1117-74
Mains cable, US standard	1	A	19-2447-01
Mains cable, EU standard	1	A	19-2448-01
Mains distribution cable 0.3 m	1	A	18-1119-05
Mains distribution cable 1 m	1	A	18-1132-08
Signal Cable, 6 pin miniDIN-open	1	A	18-1110-64
Connectors and unions			
 Tubing connector, inlet nut for o.d. 1/8”, PEEK	10	A	18-1121-17
Ferrule, for inlet nut 1/8” o.d., PEEK	10	A	18-1121-18
 Union, 1/16” female/M6 male, PEEK	6	A	18-1112-57
 Union, luer female/1/16” male, PEEK	2	A	18-1112-51
 Union, M6 female/1/16” male, PEEK	8	A	18-1112-58
 Union, 1/16” male/1/16” male, for 1/16” o.d. tubing, PEEK	10	A	18-1120-92
 Union, 1/16” female/1/16” female, for 1/16” o.d. tubing, titanium	1	A	18-3855-01
 Fingertight connector 1/16”, for PEEK tubing o.d. 1/16”	10	A	18-1112-55
 Stop plug, 1/16”, PEEK	5	A	18-1112-52
 Stop plug, 5/16”, PEEK	5	A	18-1112-50
Tubing			
Teflon tubing, i.d. 1.6 mm, o.d. 1/8” (IN)	3 m	A	18-1121-16
PEEK tubing, i.d. 0.50 mm, o.d. 1/16” (G)	2 m	A	18-1113-68
PEEK tubing, i.d. 0.75 mm, o.d. 1/16”	2 m	A	18-1112-53
Tefzel tubing, i.d. 0.75 mm, o.d. 1/16” (W)	2 m	A	18-1119-74

*) A = accessory, C = consumable



Item	Quant./pack	A/C*	Code no.
Miscellaneous			
Inlet filter assembly	2	A	18-1113-15
Inlet filter set	10	C	18-1114-42
On-line filter	1	A	18-1112-44
On-line filter kit	10	C	18-1027-11
Flow restrictor, FR-902	1	A	18-1121-35
Flow restrictor, FR-904	1	A	18-1119-63
Column holder, for one column, short	1	A	18-1113-17
Column holder, for one column, long	1	A	18-1126-32
Column holder, for up to six columns	1	A	18-1113-18
Flow cell holder UPC-900	1	A	18-3055-87
Clamp, Conductivity flow cell	1	A	18-1111-14
Tubing cutter	1	A	18-1112-46
U-wrench, M6	1	A	19-7481-01
U-wrench, 1/4”	1	A	18-1112-45
Allen key, 2.5 mm	1	A	19-4442-01
Chart recorder REC 111, 1 channel	1	A	18-1132-32
Chart recorder REC 112, 2 channel	1	A	18-1132-33

*) A = accessory, C = consumable

Item	Quant./pack	A/C*	Code no.
User Manuals			
ÄKTA*FPLC* Manual Box complete, containing all User Manuals and Instructions for ÄKTA*FPLC*	1	A	18-1140-98
Making your first run	1	A	18-1140-48
UNICORN version 3.10 User Manual	1	A	18-1134-58
UNICORN version 3.20 Frac-950 module for UNICORN applications			18-1138-53
System Manual	1	A	18-1140-45
Method Handbook	1	A	18-1125-58
ÄKTAdesign Optional Configurations User Manual	1	A	18-1139-57
Short Instruction Pump P-920	1	A	18-1125-50
Short Instruction Monitor UPC-900	1	A	18-1125-52
File containing module User Manuals and component Instructions for ÄKTA*FPLC* equipment	1	A	18-1125-59
Pump P-920 User Manual	1	A	18-1125-54
Monitor UPC-900 User Manual	1	A	18-1125-55
Installation Guide	1	A	18-1140-46
Fraction Collector Frac-900 User Manual	1	A	18-1115-93
Fraction Collector Frac-950 User Manual	1	A	18-1139-56

*) A = accessory, C = consumable

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