ÄKTA design

# ÄKTA FPLC

## System Manual

18-1140-45

Amersham Biosciences

### Important user information

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

**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. 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:

- 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 the ÄKTAFPLC™ 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 *ÄKTAFPLC 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 runs* 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 *ÄKTAFPLC Optional Configurations User Manual* which describes the extended functions of ÄKTAFPLC.

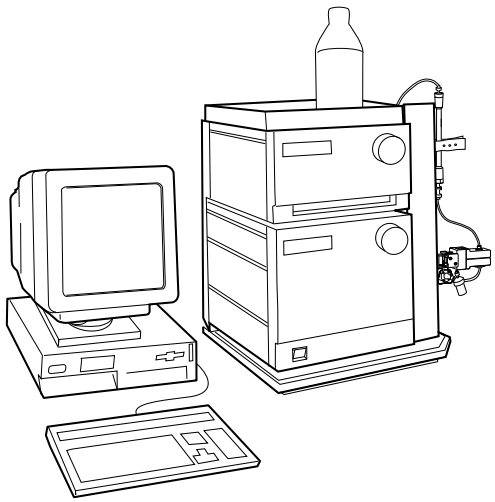
# 1 Introduction

## 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.

ÄKTAFPLC™ 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.



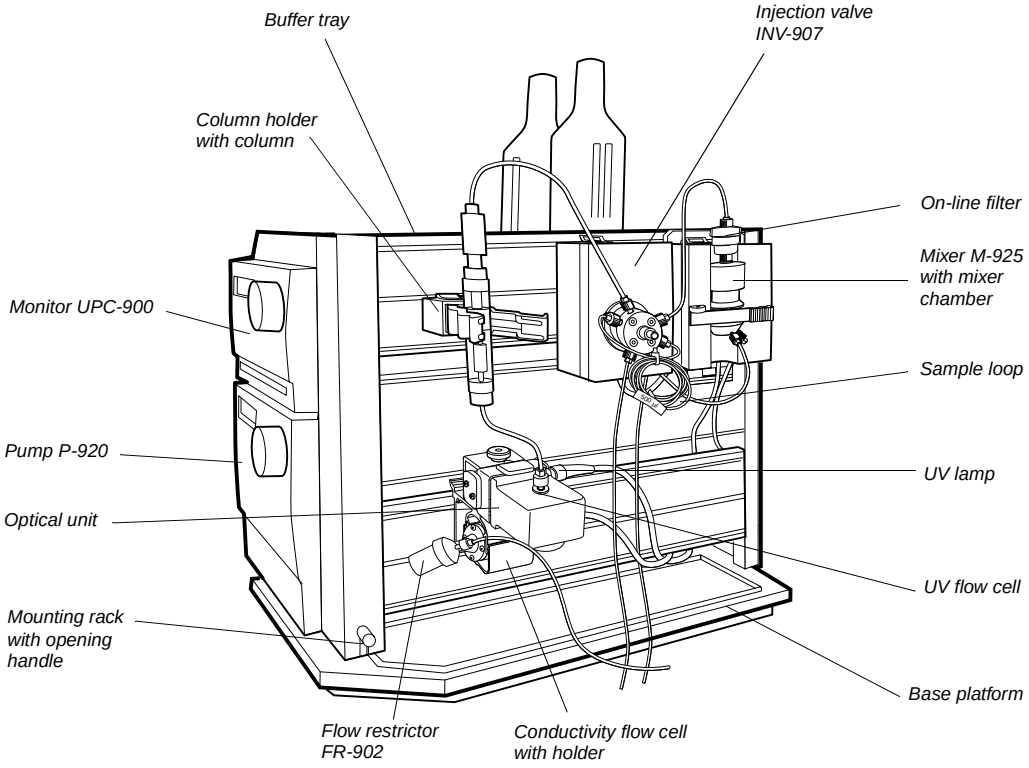
In its standard configuration, ÄKTAFPLC consists of a compact separation unit including modules and components, and a personal computer running UNICORN™ software version 4.12 or higher to control the separation unit. A fraction collector, Frac-900 or Frac-950, is also included. A hinged rack allows easy attachment of optional equipment when expanding the standard configuration.

ÄKTAFPLC is described in detail in section *5.1* of *Reference information* in this manual and brief descriptions of the individual modules and components are given in section *5.2* 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 Manuals.

UNICORN provides a Method Wizard as a basis for creating customised methods. Instructions for the Wizard are available in UNICORN User Manuals and *ÄKTAFPLC Making your first runs*.

*ÄKTAFPLC 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 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!** 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 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!** Make sure that the locking screws holding the upper part of the system rack are tightened sufficiently when it is raised to upper position.

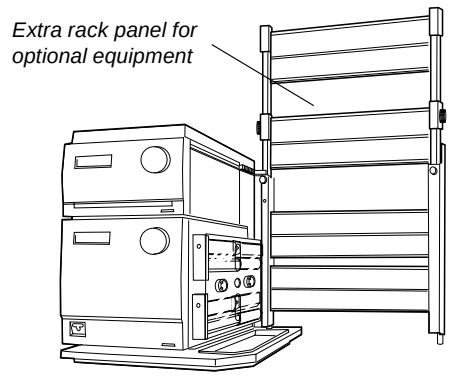
**WARNING!** There must always be a sample loop or Superloop connected to ports 2 and 6 of the injection valve. This is to prevent liquid spraying out of the ports when switching the valve. This is especially dangerous if hazardous chemicals are used.
If the system is configured for sample application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

## 1.3 Optional configurations

The ÄKTAFPLC standard system configuration can easily be changed to optional configurations. This built-in flexibility in the standard ÄKTAFPLC 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 *ÄKTAFPLC Optional Configurations User Manual*.




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

Optional configurations supported by ÄKTAFPLC 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 four air sensors.
- Connection of AD converter.

# 2 Operation

This chapter describes how to optimise and operate ÄKTAFPLC 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), cold room operation (section 2.10), and feedback tuning (section 2.12).

## 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 ÄKTAFPLC. A comprehensive list of the recommended pre-packed columns is given overleaf.

On delivery, the system is equipped with i.d. 0.50 mm 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 ÄKTAFPLC 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 *ÄKTAFPLC Optional Configurations User Manual* for further details.

### 2.1.1 Recommended columns

The tables below list recommended columns.

***Ion Exchange Columns***

| *Code no* | *Column name* |
|---|---|
| 17-6002-33 | HiTrap IEX Selection Kit (7 x 1 ml) |
| 17-1153-01 | HiTrap™ Q HP, (5 x 1 ml) |
| 17-1154-01 | HiTrap Q HP, (5 x 5 ml) |
| 17-1151-01 | HiTrap SP HP, (5 x 1 ml) |
| 17-1152-01 | HiTrap SP HP, (5 x 5 ml) |
| 17-5158-01 | HiTrap Q XL, (5 x 1 ml) |
| 17-5159-01 | HiTrap Q XL, (5 x 5 ml) |
| 17-5160-01 | HiTrap SP XL, (5 x 1 ml) |
| 17-5161-01 | HiTrap SP XL, (5 x 5 ml) |
| 17-5053-01 | HiTrap Q FF, (5 x 1 ml) |
| 17-5156-01 | HiTrap Q FF, (5 x 5 ml) |
| 17-5054-01 | HiTrap SP FF, (5 x 1 ml) |
| 17-5157-01 | HiTrap SP FF, (5 x 5 ml) |
| 17-5055-01 | HiTrap DEAE FF, (5 x 1 ml) |
| 17-5154-01 | HiTrap DEAE FF, (5 x 5 ml) |
| 17-5056-01 | HiTrap CM FF, (5 x 1 ml) |
| 17-5155-01 | HiTrap CM FF, (5 x 5 ml) |
| 17-5162-01 | HiTrap ANX FF (high sub), (5 x 1 ml) |
| 17-5163-01 | HiTrap ANX FF (high sub), (5 x 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 FF |
| 17-5090-01 | HiPrep 16/10 DEAE FF |
| 17-5190-01 | HiPrep 16/10 Q FF |
| 17-5192-01 | HiPrep 16/10 SP FF |
| 17-5191-01 | HiPrep 16/10 ANX FF (high sub) |
| 17-1064-01 | HiLoad™ 16/10 Q Sepharose HP |
| 17-1066-01 | HiLoad 26/10 Q Sepharose HP |
| 17-1137-01 | HiLoad 16/10 SP Sepharose HP |

| *Code no* | *Column name* |
|---|---|
| 17-1138-01 | HiLoad 26/10 SP Sepharose HP |
| 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 |
| 17-5181-01 | SOURCE™ 15 Q 4.6/100 PE |
| 17-5182-01 | SOURCE 15 S 4.6/100 PE |
| 17-5166-01 | Mono Q™ 5/50 GL |
| 17-5167-01 | Mono Q 10/100 GL |
| 17-0506-01 | Mono Q HR, 16/10 |
| 17-5168-01 | Mono S™ 5/50 GL |
| 17-5169-01 | Mono S 10/100 GL |
| 17-0507-01 | Mono S HR, 16/10 |

***Gel filtration (Size exclusion) Columns***

| *Code no* | *Column name* |
|---|---|
| 17-1165-01 | HiPrep 16/60 Sephacryl S-100 HR |
| 17-1194-01 | HiPrep 26/60 Sephacryl S-100 HR |
| 17-1166-01 | HiPrep 16/60 Sephacryl S-200 HR |
| 17-1195-01 | HiPrep 26/60 Sephacryl S-200 HR |
| 17-1167-01 | HiPrep 16/60 Sephacryl S-300 HR |
| 17-1196-01 | HiPrep 26/60 Sephacryl S-300 HR |
| 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 |
| 17-5174-01 | Superdex™ 75 10/300 GL |
| 17-5175-01 | Superdex 200 10/300 GL |
| 17-5176-01 | Superdex Peptide 10/300 GL |

***Hydrophobic Interaction Columns***

| *Code no* | *Column name* |
|---|---|
| 17-1349-01 | HiTrap HIC Selection Kit (5 x 1 ml) |
| 17-1355-01 | HiTrap Phenyl FF (high sub), (5 x 1 ml) |
| 17-5193-01 | HiTrap Phenyl FF (high sub), (5 x 5 ml) |
| 17-1353-01 | HiTrap Phenyl FF (low sub), (5 x 1 ml) |
| 17-5194-01 | HiTrap Phenyl FF (low sub), (5 x 5 ml) |
| 17-1351-01 | HiTrap Phenyl HP, (5 x 1 ml) |
| 17-5195-01 | HiTrap Phenyl HP, (5 x 5 ml) |
| 17-1357-01 | HiTrap Butyl FF, (5 x 1 ml) |
| 17-5197-01 | HiTrap Butyl FF, (5 x 5 ml) |
| 17-1359-01 | HiTrap Octyl FF, (5 x 1 ml) |
| 17-5196-01 | HiTrap Octyl FF, (5 x 5 ml) |
| 17-5095-01 | HiPrep 16/60 Phenyl FF (high sub) |
| 17-5094-01 | HiPrep 16/60 Phenyl FF (low sub) |
| 17-5097-01 | HiPrep 16/60 Octyl FF |
| 17-5096-01 | HiPrep 16/60 Butyl FF |
| 17-1085-01 | HiLoad 16/60 Phenyl Sepharose HP |
| 17-1086-01 | HiLoad 26/60 Phenyl Sepharose HP |
| 17-1184-01 | RESOURCE ETH 1 ml |
| 17-1185-01 | RESOURCE ISO 1 ml |
| 17-1186-01 | RESOURCE PHE 1 ml |
| 17-5186-01 | SOURCE 15 PHE 4.6/100 PE |

***Affinity Columns***

| *Code no.* | *Column name* |
|---|---|
| 17-0402-01 | HiTrap Protein A HP, (5 x 1 ml) |
| 17-0402-03 | HiTrap Protein A HP, (2 x 1 ml) |
| 17-0403-01 | HiTrap Protein A HP, (1 x 5 ml) |
| 17-0404-01 | HiTrap Protein G HP, (5 x 1 ml) |
| 17-0404-03 | HiTrap Protein G HP, (2 x 1 ml) |
| 17-0405-01 | HiTrap Protein G HP, (1 x 5 ml) |
| 17-0406-01 | HiTrap Heparin HP, (5 x 1 ml) |
| 17-0407-01 | HiTrap Heparin HP, (1 x 5 ml) |

| Code no. | Column name |
|---|---|
| 17-5079-01 | HiTrap rProtein A FF, (5 x 1 ml) |
| 17-5079-02 | HiTrap rProtein A FF, (2 x 1 ml) |
| 17-5080-01 | HiTrap rProtein A FF, (1 x 5 ml) |
| 17-0412-01 | HiTrap Blue HP, (5 x 1 ml) |
| 17-0413-01 | HiTrap Blue HP, (1 x 5 ml) |
| 17-0716-01 | HiTrap NHS-activated HP, (5 x 1 ml) |
| 17-0717-01 | HiTrap NHS-activated HP, (1 x 5 ml) |
| 17-5110-01 | HiTrap IgM Purification HP, (5 x 1 ml) |
| 17-5111-01 | HiTrap IgY Purification HP, (1 x 5 ml) |
| 17-5112-01 | HiTrap Streptavidin HP, (5 x 1 ml) |
| 17-5130-01 | GSTrap™ FF, (5 x 1 ml) |
| 17-5130-02 | GSTrap FF, (2 x 1 ml) |
| 17-5131-01 | GSTrap FF, (1 x 5 ml) |
| 17-5143-01 | HiTrap Benzamidine FF (high sub), (5 x 1 ml) |
| 17-5143-02 | HiTrap Benzamidine FF (high sub), (2 x 1 ml) |
| 17-5144-01 | HiTrap Benzamidine FF (high sub), (1 x 5 ml) |
| 17-5189-01 | HiPrep 16/10 Heparin FF |
| 17-5234-01 | GSTPrep FF 16/10 |
| 17-0408-01 | HiTrap Chelating HP, (5 x 1) |
| 17-0409-01 | HiTrap Chelating HP, (5 x 5) |

***Chromatofocusing Columns***

| Code no. | Column name |
|---|---|
| 17-5171-01 | Mono P™ 5/200 GL |
| 17-5170-01 | Mono P 5/50 GL |

***Buffer Exchange/Desalting Columns***

| Code no. | Column name |
|---|---|
| 17-1408-01 | HiTrap Desalting, (5 x 5 ml) |
| 17-5087-01 | HiPrep 26/10 Desalting |

### 2.1.2 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** 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 entered into the UNICORN column list and 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**.

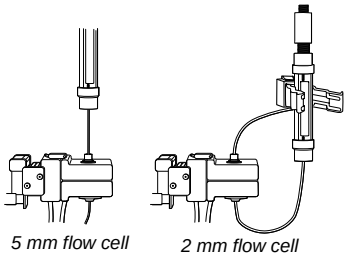
Click **Save as** again to add the updated column parameters to the column list.

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

### 2.1.3 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 ÄKTAFPLC 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 options. These are described in *ÄKTAFPLC Optional Configurations User Manual.*

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

| ***Sample application technique*** | ***Volume to inject*** |
|---|---|
| Sample loop, manual filling | 0–2 ml |
| Superloop[1] | 1 ml - 150 ml |
| Sample pump[2] | >1 ml |
| Autosampler[3] | 1-500 µl |

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

[2] How to use the sample pump and the method modifications required is described in the ÄKTAFPLC Optional Configurations User Manual. Refer also to the User Manual for the sample pump.

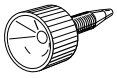
[3] Depending on autosampler model and tubing selection. Refer to the *A-900 or A-905 User Manual.*

## 2.3 Manual filling of sample loops

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

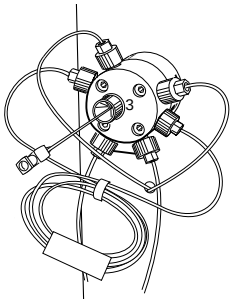
### 2.3.1 Preparation

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.

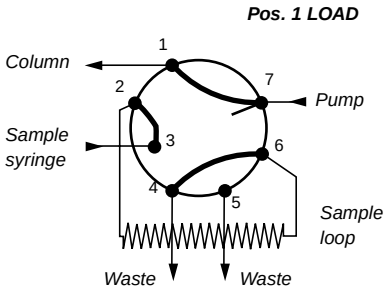



3 Tighten the fill port until the nozzle has formed a seal around the needle's tip, i.e. when it feels as if you are penetrating a septum at the end of the injection fill port. The seal should allow easy insertion and removal of the needle.

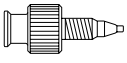
4 Mount the syringe holder on the fill port.

5 Make sure 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 ÄKTAFPLC 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 |

### 2.3.2 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:

1 Set the injection valve to position INJECT.

2 Disconnect the tubing from the column inlet and immerse it in a Waste bottle.

3 Run the pump with buffer at low speed.

4 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.*

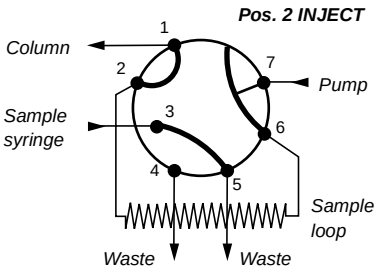
5 Insert the syringe into the injection fill port on the injection valve. Set the injection valve to position LOAD.

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

6 Reconnect the column tubing.

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 when the injection valve is in position LOAD, self drainage will occur and air will enter the sample loop.*

### 2.3.3 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.*

### 2.3.4 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.

The volume for emptying the sample loop is set in the **Sample Injection** dialog in the Method Wizard.

## 2.4 Mixing gradients

### 2.4.1 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.

### 2.4.2 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.

Other mixer chambers with 2 ml and 5 ml mixer volumes are available as accessories. See section *5.5* 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.*

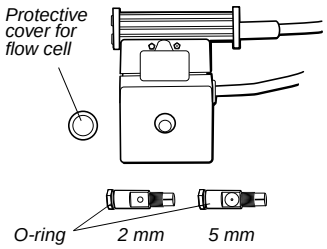
## 2.5 Changing UV flow cell and wavelength

### 2.5.1 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:*** *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.*

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

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

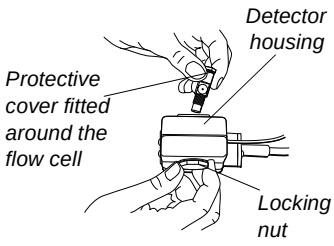
3 Remove the protective cover from the old flow cell and transfer it to the new flow cell.

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.

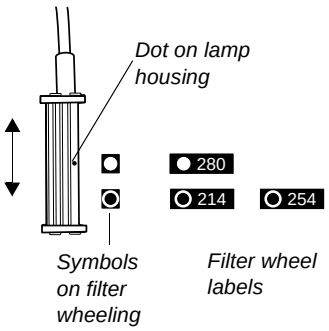


### 2.5.2 Changing wavelength

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 method, a question appears in which the user is prompted to state the wavelength to be used in the run.*

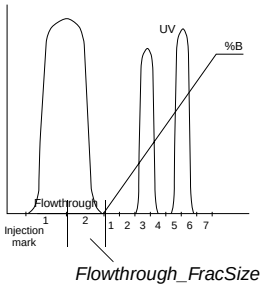
## 2.6 Collecting fractions

Fractions are collected with the fraction collector included in the system (Frac-900 or Frac-950). The software provides fractionation in two different ways:

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

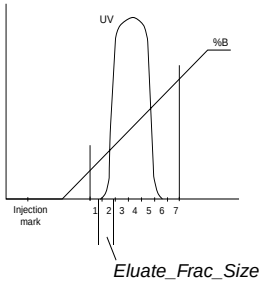
Fractionation is selected and specified in the fractionation dialogs in the Method Wizard.

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




### 2.6.1 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.

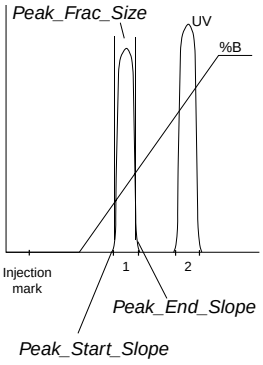



### 2.6.2 Fixed volume and/or peak fractionation

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 or Cond, 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.

## 2.7 Before a run

### 2.7.1 Selecting a method

Use the UNICORN Method Wizard for creating of customised methods.

The basic steps required to create a method are:

1 In the **Method Editor**, select **File:Method Wizard**. Select system if required.

2 Select the appropriate parameter values in the subsequent pages. Click **OK** to go to the next page.

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

4 Check and fine-tune the values for the method variables, e.g. for the flow, in the **Variables** page.

5 Read the method notes to check that your system is configured to the requirements of the method.

6 Save the method.

***Note:*** *Before starting the run, check that the system is in End mode, and that the valves are in their initial positions.*

### 2.7.2 Calibrations

The table below lists the type and frequency of calibrations that can be done on ÄKTAFPLC. Refer to the *UNICORN User Manuals* 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<br>Cell constant<br>Temperature<br>Entering a new cell constant<br>Sample pump (optional) | <br>Only necessary if specific conductivity with high accuracy is measured (Cond_Calib).<br>Must be done when changing the conductivity flow cell (Temp).<br>Must be done when changing the conductivity flow cell (Cond_Cell).<br>Whenever the running conditions are changed. |

### 2.7.3 General preparation

Before starting any method, we recommend you make certain 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 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 ÄKTAFPLC outlet.

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

## 2.8 During a run

### 2.8.1 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 ÄKTAFPLC 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**. 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** 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      13.40ml/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).

```
pH12.50    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.

### 2.8.2 Changing parameters

***From UNICORN***

ÄKTAFPLC 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 all that may be printed. Make sure that the **Instruction Set** box is checked and clear any unwanted items. Click **OK** to print 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. 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**.

## 2.9 Completion of a run and storage

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

### 2.9.1 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.

### 2.9.2 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% ethanol 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.

### 2.9.3 *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 might 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) shall 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.

### 2.10.1 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.

### 2.10.2 Running

Before starting a run, check the following:

1 Make sure that the temperature of the buffers has reached the ambient temperature.

2 Calibrate the pH electrode (optional).

3 Check the pH of the buffers.

### 2.10.3 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.

## 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 Runs* 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.

## 2.12 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 viscosity.

The feedback tuning is set in the Method Wizard and it is 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.

### 2.12.1 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 (slightly below the column pressure limit) and the minimum allowed flow.

The PID parameter values can later be changed separately in **System:Settings** in the **System Control** module.

***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 added manually to a 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 2-1.

| *Instruction* | *Parameter description* |
|---|---|
| SystemPumpControlMode | To activate feedback tuning, select **PressFlowControl**.<br>**PressLevel** is the pressure control set point<br>**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.<br>**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 2-1.** 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.<br>*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.<br>*Note*: Most often, a simple PI-regulator is preferable for control of flow rate, and ÄKTAFPLC is therefore configured by default with the D parameter set to zero. |

**Table 2-2.** 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.

### 2.12.2 Feedback tuning of the sample pump

***Tuning principle***

The feedback tuning of the sample pump is simpler than the feedback 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:

*Alternative 1*

1 Select **View:Run Setup**. Select the **Variables** page.

2 Tick **Show details**.

3 Change the variable under block **Alarm_Sample_PressureLimit.**

*Alternative 2*

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:

*Alternative 1*

1 Select **View:Run Setup.** Select the **Variables** page.

2 Select **Show details.**

3 Change the variable under block **PressureReg_Sample_Pump.**

*Alternative 2*

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:Alarms&Mon.**

2 Select **Alarm_SamplePressure_960.**

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

4 Select **Pump** to switch to the **Pump Instructions** dialog. Select **SamplePumpControlMode_960.**

5 Select **PressFlowControl** and set the **MinFlow** value. Click **Execute** to start feedback tuning.

***Note:*** *Start the system pump with a low flow rate after running the sample pump with feedback tuning.*

# 3 Maintenance

## 3.1 Periodic maintenance

Regular maintenance will help to keep your ÄKTAFPLC 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 might 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!** NaOH is injurious to health. Avoid spillage.

**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!** 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.

The following table lists the maintenance operations that should be performed by the user at regular intervals.

| *Interval* | *Action* |
|---|---|
| ***Every day*** | |
| System | • Inspect the complete system for eluent leakage.<br>• 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*** | |
| 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:<br>1 Disconnect the flow restrictor.<br>2 Connect a capillary to port 1 of the injection valve. Put the open end in the waste container.<br>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.<br>4 Connect the flow restrictor to the open end of the capillary (note the IN marking).<br>5 Run the pump at 10 ml/min with water. Note the pump display or in the **Run Data** window.<br>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*** | |

| *Interval* | *Action* |
|---|---|
| Valve INV-907 | • Check for external and/or internal leakage. Replace the distribution plate yearly or when required. Refer to *Valve INV-907 instructions*. |
| ***When required*** | |
| Pump P-920 | • Replace piston seals. Refer to *Pump P-920 User Manual*.<br>• Replace piston. Refer to the User Manual.<br>• 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*. |
| 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. |

## 3.2 Cleaning the system

The procedures described below are for system cleaning. To bypass the column, use a piece of i.d. 0.5 mm. PEEK tubing supplied with ÄKTAFPLC. 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 Instructions supplied with the column.

### 3.2.1 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.

### 3.2.2 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 might 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.

### 3.2.3 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.

### 3.2.4 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, make sure 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:


| *Monitor curve/component* | *Page* |
|---|---|
| UV curve | 52 |
| Conductivity curve | 53 |
| Mixer M-925 | 55 |
| Pressure curve | 56 |
| Monitor UPC-900 | 57 |
| Pump P-920 | 58 |
| INV-907 | 59 |
| Fraction collector | 60 |


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

## 4.1 UV curve

| *Error symptom* | *Possible cause/Action* |
|---|---|
| **Noisy UV-signal, signal drift or instability** | 1 The buffer may be impure. Check if the signal is still noisy with water.<br>2 There may be air in the flow cell. Check that the flow restrictor generates a back-pressure of 0.2 ± 0.05 MPa.<br><br>Check the back-pressure as follows:<br>1 Disconnect the flow restrictor.<br>2 Connect a capillary to port 1 of the injection valve. Put the open end in the waste container.<br>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.<br>4 Connect the flow restrictor to the open end of the capillary (note the IN marking).<br>5 Run the pump at 10 ml/min with water. Note the pump display or in the **Run Data** window.<br>6 Calculate the back-pressure generated by the flow restrictor. Replace it, if it is not within limit.<br>3 Degas the buffer before use.<br>4 Check the connections of the optical unit and filter setting.<br>5 Clean the UV flow cell, see *Monitor UPC-900 User Manual*. |

| *Error symptom* | *Possible cause/Action* |
|---|---|
| **Ghost peaks** | 1 Remove any air in the eluent by degassing.<br>2 Clean the system in accordance with chapter 3.<br>3 Clean the column in accordance with the column instructions.<br>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.<br>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

| *Error symptom* | *Possible cause/Action* |
|---|---|
| **Conductivity measurement with the same buffer appears to change over time** | 1 Clean the flow cell according to *Monitor UPC-900 User Manual*.<br>2 The ambient temperature may have decreased. Use a temperature compensation factor, see Reference information in *Monitor UPC-900 User Manual*. |
| **Ghost peaks appear in the gradient profile** | 1 A charged sample has been detected (e.g. protein).<br>2 Air bubbles are passing through the flow cell. Check for loose tubing connections. Use a flow restrictor. |

| *Error symptom* | *Possible cause/Action* |
|---|---|
| **Baseline drift or noisy signal** | 1 There may be air in the flow cell.<br>2 Check for leaking tubing connections.<br>3 Check that the column is equilibrated. If necessary, clean the column.<br>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.<br>5 Clean the flow cell according to *Monitor UPC-900 User Manual*. |
| **Waves on the gradient** | 1 Check that the pump and the valves are operating properly and are programmed correctly.<br>2 Change to a larger mixing volume if necessary.<br>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.<br>2 If a larger mixer than the standard 0.6 ml chamber is used, try changing to a smaller mixer volume |

| *Error symptom* | *Possible cause/Action* |
|---|---|
| **Incorrect or unstable reading** | 1 Check that the conductivity flow cell cable is connected properly to the rear of the monitor.<br>2 Check that the pump operates correctly.<br>3 If temperature compensation is used, check that the temperature sensor is calibrated and that the correct temperature compensation factor is used.<br>4 Check that the column is equilibrated. If necessary, clean the column.<br>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

| *Error symptom* | *Possible cause/Action* |
|---|---|
| **Leakage** | 1 Check the tubing connections. Retighten or replace if necessary.<br>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.<br>2 The mixer function can also be checked by running the installation test. |

## 4.4 Pressure curve

| *Error symptom* | *Possible cause/Action* |
|---|---|
| **Erratic flow, noisy baseline signal, irregular pressure trace** | Air bubbles passing through or trapped in the pump:<br>1 Check that there is sufficient eluent in the reservoirs.<br>2 Check all connections for leakage.<br>3 Follow the instructions in *Pump P-920 User Manual*. |
| | Pump valves not functioning correctly:<br>1 Clean the valves according to *Pump P-920 User Manual*. |
| | Piston seal leaking:<br>1 Replace the piston seal according to the instructions in *Pump P-920 User Manual*. |
| | Blockage or partial blockage of the flow path:<br>1 Flush the flow path to clear the blockage.<br>2 If necessary, replace the tubing.<br>3 Check the inlet tubing filter. It can get clogged if unfiltered buffers or samples are applied.<br>See the instructions for flushing at the end of the run in section 2.9. |

## 4.5 Monitor UPC-900

| *Error symptom* | *Possible cause/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.<br>2 Recalibrate the conductivity cell.<br>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

| *Error symptom* | *Possible cause/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.<br>1 Replace or re-install the seal or gasket.<br>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.<br>2 Check the piston for damage. If damaged, replace the piston according to *Pump P-920 User Manual*.<br>3 Remember to replace the piston seal with new parts.<br>4 Make sure 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.<br>2 Tighten the connector with your fingers. Then tighten an extra 1/4 turn using a wrench. |

| *Error symptom* | *Possible cause/Action* |
|---|---|
| **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*.<br><br>There can be several causes of an abnormal pressure recording, for example:<br>• air trapped in the pump cylinders<br>• partially blocked solvent filters<br>• leaking connections<br>• piston seal leakage<br>• pump valve malfunction<br>• piston damaged<br><br>For more details, refer to the *Pump P-920 User Manual*. |

## 4.7 INV-907

| *Error symptom* | *Possible cause/Action* |
|---|---|
| **The valve is not switching** | 1 Check the connection to the pump. The valve should be connected to the UniNet-2 socket.<br>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.<br>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.<br>1 Check that the distribution plate marking **3** is horizontal. |
| **External leakage** | 1 Check the tubing connections. Tighten or replace if required. |

| *Error symptom* | *Possible cause/Action* |
|---|---|
| **Internal leakage** | Internal leakage can be detected at the small hole on the underside of the valve body.<br>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 I*NV-907 Instruction*.<br>2 Change channel plate and distribution plate according to *INV-907 Instruction*. |

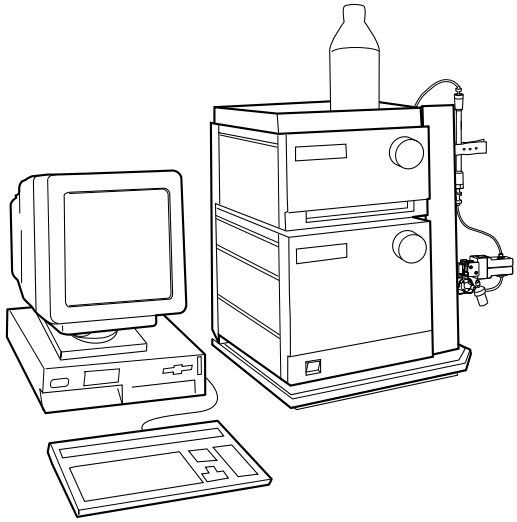
## 4.8 Fraction collector

Refer to the user manual for the fraction collector installed.

# 5 Reference information

## 5.1 System description

### 5.1.1 The system



ÄKTAFPLC consists of a compact separation unit comprising modules and components, and a personal computer running UNICORN software version 4.12 or higher to control the separation unit. A fraction collector is included in the standard configuration.

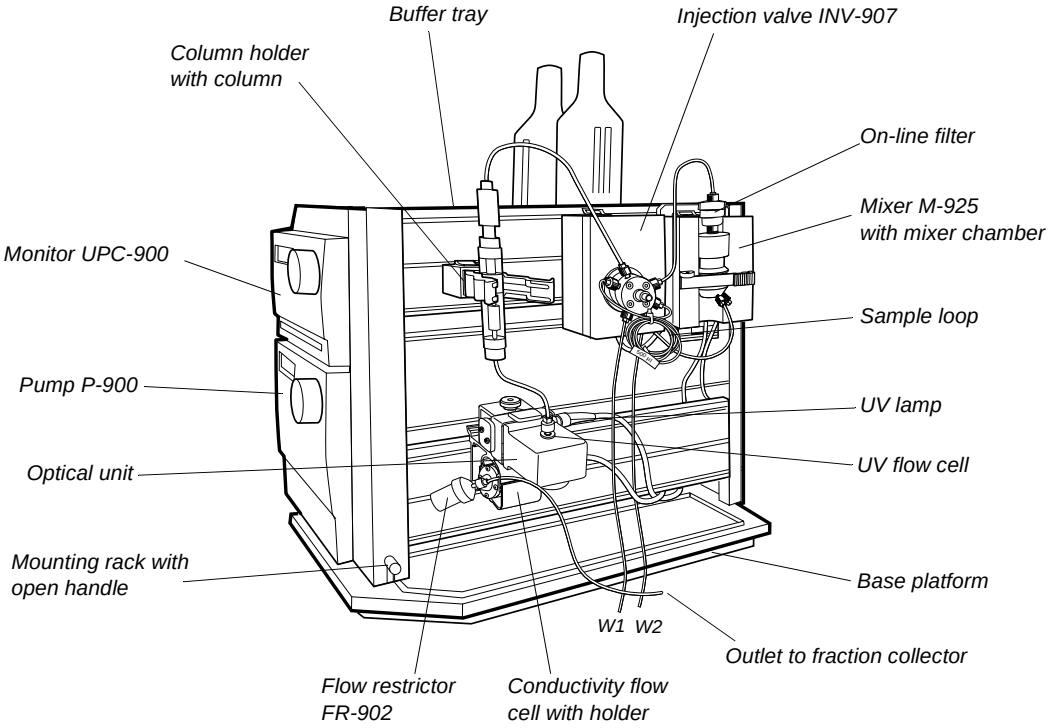
Communication between the computer and the various modules and components of ÄKTAFPLC is achieved via high speed data network connections referred to as UniNet-1 and UniNet-2. On UniNet-1, the more complex data signals between modules and the computer are run, whereas UniNet-2 carries the less complex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

The fluid handling equipment of ÄKTAFPLC 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.

### 5.1.2 Modules and components

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



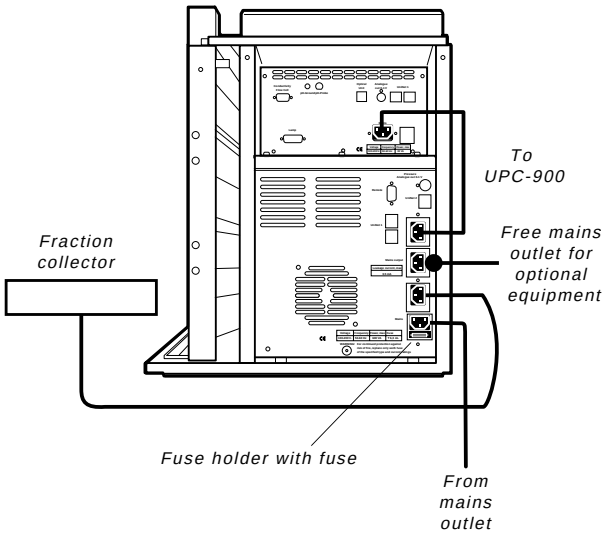
### 5.1.3 Electrical connections

**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 ÄKTAFPLC 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.

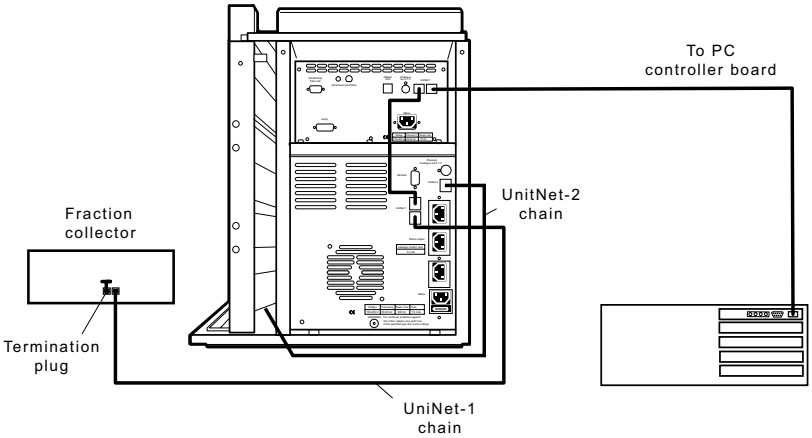
***Mains cables***

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.



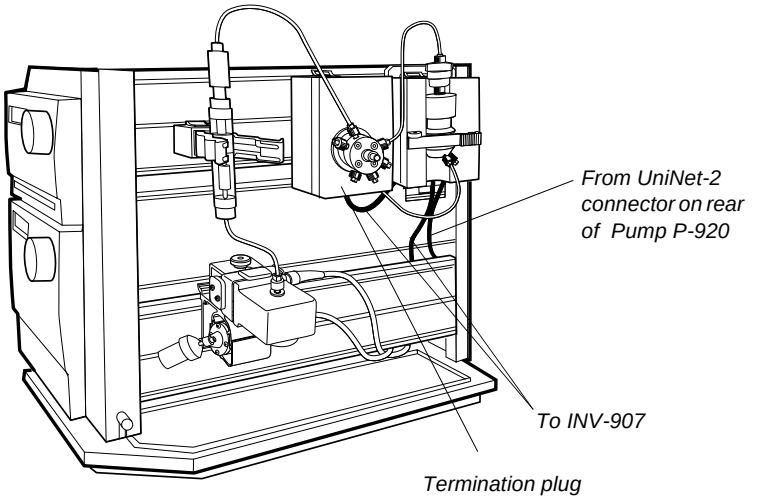

To open the fuse holder, remove the mains inlet cable to the system. Use a small-bladed screwdriver to lever the holder outwards.

***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***

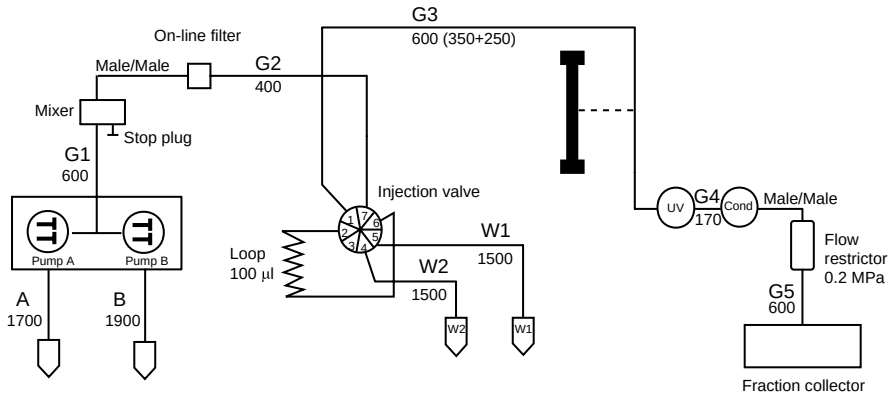



The UniNet-2 data communication chain, which controls the valve(s) and the mixer comes from the rear of Pump P-920. The chain is terminated at the injection valve with a termination plug.

If using sample pump P-960, it will be the last component in the UniNet-2 chain. P-960 has an internal termination.

### 5.1.4 Fluid handling path

The following flow diagram shows the positions of the modules, components and tubings in ÄKTAFPLC 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 initially used 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 ÄKTAFPLC. At delivery, i.d. 0.5 mm 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 (i.d. 0.50 mm PEEK tubing, orange, is supplied with ÄKTAFPLC system).

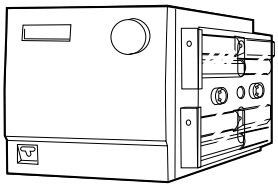
Tubings supplied with the fraction collector installed are described in *ÄKTAFPLC Optional Configurations User Manual*.

| *Tubing i.d.* | *Tubing o.d.* | *Material* | *Color* | *Max. pressure* | *Volume of 10 cm* | *Connected from/to* |
|---|---|---|---|---|---|---|
| 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 | Pump P-920 to fraction collector |
| Union, mm | 1/16" | PEEK | Black | 25 MPa | – | Mixer/on-line filter, conductivity cell/ flow restrictor |
| 0.75 mm (W1–W2) | 1/16" | Tefzel | Clear | 2 MPa | 44.2 µl | Waste tubing. |

## 5.2 Modules and components description

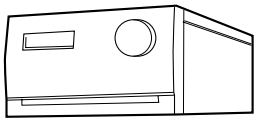
A complete description of each module and component can be found in their respective manuals and instructions. Optional components for the ÄKTAFPLC system are described in *ÄKTAFPLC Optional Configurations User Manual*.

### 5.2.1 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.

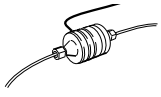
The wide flow range makes it suitable both for analytical and preparative chromatography. Pump P-920 is designed to work with a wide range of columns and gels supplied by Amersham Biosciences
Monitor UPC-900



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).

### 5.2.2 UV and conductivity flow cells

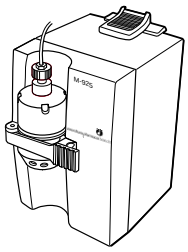


*Conductivity flow cell with integrated temperature sensor*

*UV flow cells with 2 and 5 mm path lengths*

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.

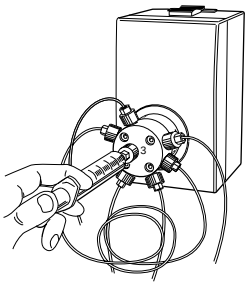
### 5.2.3 Mixer M-925



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 ÄKTAFPLC.

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

### 5.2.4 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.

### 5.2.5 Fraction collector

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

In ÄKTAFPLC, the fraction collector allows fixed volume fractionation, eluate fractionation or automatic peak fractionation. The latter function is based on UV 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.

### 5.2.6 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.


### 5.2.7 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.

## 5.3 Technical specifications

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

The relevant system specifications are listed below.

### 5.3.1 Operating data

| | |
|---|---|
| ***Pump P-920*** | |
| Flow rate range<br>isocratic mode<br>gradient mode | <br>0.05–20 ml/min in steps of 10 µl/min<br>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<br>< 10 ml/min<br>> 10 ml/min | <br>Max. 10cP<br>Max. 5 cP |
| Flow rate reproducibility<br>flow rate 0.5–10 ml/min<br>flow rate 10–20 ml/min | <br>rsd < 0.2%<br>rsd < 0.5% |
| Gradient composition<br>accuracy between turnings<br>accuracy during turnings<br>reproducibility | <br>± 2% at 0.5–5 ml/min and < 5 MPa<br>± 2% at 0.5–5 ml/min and 0.5–2.0 MPa<br>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***<br>***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<br>Hg lamp, fixed<br>by changing filter<br>Zn lamp | <br>254 and 280 nm<br>313, 365, 405, 436 and 546 nm<br>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. | |

### 5.3.2 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 |
| Environment | +4 to +40 °C, 10–95% relative humidity (non-condensing),<br>84–106 kPa (840–1060 mbar atmospheric pressure). |

### 5.3.3 Hardware requirements

Refer to *UNICORN Administration and Technical Manual*.

### 5.3.4 Software requirements

Refer to *UNICORN Administration and Technical Manual*.

### 5.3.5 Network requirements

Refer to *UNICORN Administration and Technical Manual*.

### 5.3.6 ÄKTAFPLC component materials

The wetted materials of ÄKTAFPLC 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

## 5.4 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 |

| *Chemical* | *Exposure* | | *Comments* |
|---|---|---|---|
| | *< 1 day* | *up to 2 months* | |
| 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.<br>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<br>p-Xylene | OK | Avoid | Silicone, PP and PE are affected by long term use |

## 5.5 Accessories and consumables

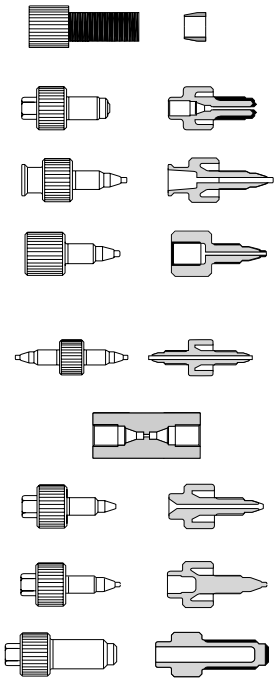
| *Item* | *Quant./ pack* | *A/C** | *Code no.* |
|---|---|---|---|
| ***Pump P-920*** | | | |
| Sealing kit containing two sealings, two gaskets and two wipers | 1 | C | 18-1032-16 |
| Rinsing tubing, i.d. 1.1 mm, o.d. 3.1 mm | 2 m | A | 18-1032-11 |
| ***Monitor UPC-900*** | | | |
| HG lamp & housing 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:<br>0.6 ml<br>2 ml<br>5 ml | <br>1<br>1<br>1 | <br>A<br>A<br>A | <br>18-1118-90<br>18-1118-91<br>18-1118-92 |

*) A = accessory, C = consumable

| *Item* | *Quant./ pack* | *A/C** | *Code no.* |
|---|---|---|---|
| ***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 | 1 | C | 18-1127-66 |
| 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 |
| ***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 |
| *) A = accessory, C = consumable | | | |
| ***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 |

| *Item* | *Quant./ pack* | *A/C** | *Code no.* |
|---|---|---|---|
| Tube support | 1 | A | 18-3054-02 |
| Tube holder and guide:<br>12 mm<br>18 mm<br>30 mm | <br>1<br>1<br>1 | <br>A<br>A<br>A | <br>19-7242-02<br>19-8689-02<br>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 |
| ***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 |
| Signal cable, 6 pin miniDIN-open | 1 | A | 18-1110-64 |

*) A = accessory, C = consumable

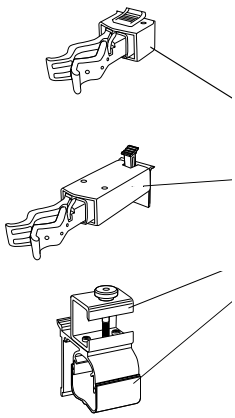


| *Item* | *Quant./ pack* | *A/C** | *Code no.* |
|---|---|---|---|
| ***Connectors and unions*** | | | |
| Tubing connector, inlet nut for o.d. 1/8", PEEK | 10 | A | 18-1121-17 |
| Ferrule, for inlet nut 1/8" o.d., PEEK | 10 | A | 18-1121-18 |
| Union, 1/16" female/M6 male, PEEK | 6 | A | 18-1112-57 |
| 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.* |
|---|---|---|---|
| ***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 |
| Tefzel tubing, i.d. 1.0 mm, o.d. 1/16" | 3 m | A | 18-1142-38 |
| ***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 one small column | 1 | A | 18-1149-98 |
| 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 |

*) A = accessory, C = consumable



| *Item* | *Quant./ pack* | *A/C** | *Code no.* |
|---|---|---|---|
| Chart recorder REC 111, 1 channel | 1 | A | 18-1132-32 |
| Chart recorder REC 112, 2 channel | 1 | A | 18-1132-33 |
| ***User Manuals*** | | | |
| ÄKTAFPLC Manual Box complete, containing all User Manuals and Instructions for ÄKTAFPLC | 1 | A | 18-1140-80 |
| Making your first run | 1 | A | 18-1140-48 |
| UNICORN Getting started | 1 | A | 56-3207-99 |
| UNICORN version 4.10 manuals | | | 18-1164-09 |
| System Manual | 1 | A | 18-1140-45 |
| Method Handbook | 1 | A | 18-1125-58 |
| ÄKTAFPLC Optional Configurations User Manual | 1 | A | 18-1174-46 |
| Short Instruction Pump P-920 | 1 | A | 18-1125-50 |
| Short Instruction Monitor UPC-900 | 1 | A | 18-1125-52 |
| File containing<br>module User Manuals and component Instructions for ÄKTAFPLC equipment<br>Pump P-920 User Manual<br>Monitor UPC-900 User Manual | <br><br><br>1<br>1<br>1 | <br><br><br>A<br>A<br>A | <br><br><br>18-1125-59<br>18-1125-54<br>18-1125-55 |
| Installation Guide | 1 | A | 18-1140-46 |
| Fraction collector Frac-900 User Manual | 1 | A | 18-1120-11 |
| Fraction collector Frac-950 User Manual | 1 | A | 18-1139-56 |
| *) A = accessory, C = consumable | | | |

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