ÄKTA*design*

ÄKTAexplorer

System Manual

amersham pharmacia biotech

Important user information

⚠️

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

WARNING!
The Warning sign is used to call attention to the necessity to follow an instruction in detail to avoid personal injury. Be sure not to proceed until the instructions are clearly understood and all stated conditions are met.

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

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

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

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Sweden

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About this manual

This manual describes the operation of ÄKTAexplorer.

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

The installation of the chromatography system is described in a separate Installation Guide.

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

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

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1 Introduction

1.1 General

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

ÄKTAexplorer features:

- Flow rates and pressures up to:

  - 100 ml/min and 10 MPa in ÄKTAexplorer 100 (P-901)

  - 10 ml/min and 25 MPa in ÄKTAexplorer 10 (P-903)

- BufferPrep for fast pH optimization.

- One working platform for all liquid chromatography techniques suitable for protein purification, from micro-gram to gram scale.
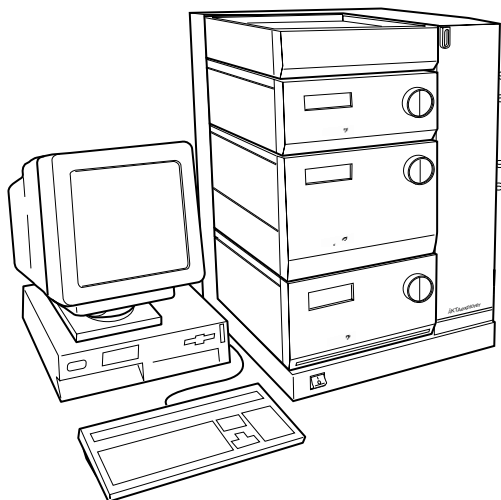
ÄKTAexplorer consists of a compact separation unit and a personal computer running UNICORN™ control system version 3.2 or higher. Fraction collectors are available as accessories.

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

1 Introduction _____

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

Column Selection Valve, V2 (PV-908)

Injection Valve, V1 (INV-907)

Column

Door button

Column Holders

Outlet Valve, V4 (PV-908)

Sample Valve, V5* (PV-908)

pH electrode (standard component only in ÄKTAexplorer 100 and ÄKTAexplorer 100 Air)

Flow restrictor

To fraction collector (optional)

Sample Tubing Holder*

Column Selection Valve, V3 (PV-908)

Sample Tube Holder*

Sample Tray*

*) standard component only in ÄKTAexplorer 100, ÄKTAexplorer 100 Air and ÄKTAexplorer 10 S

Outlet Valve, V4 (PV-908)

Flow Direction Valve, V7 (INV-907), (standard component only in ÄKTAexplorer 100 and ÄKTAexplorer 100 Air

Buffer Valve, V6 (IV-908)

Column Selection Valve, V2 (PV-908)

Box-900

Injection Valve, V1 (INV-907)

Monitor pH/C-900

Monitor UV-900

Pump P-900 (P-901 alt. P-903)

Sample Valve, V5* (PV-908)

Column Selection Valve, V3 (PV-908)

Valve door

Mains switch

Mixer M-925

Sample Pump P-950*

Switch Valve (SV-903)

UV cell

Conductivity cell

*) standard component only in ÄKTAexplorer 100, ÄKTAexplorer 100 Air and ÄKTAexplorer 10 S

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

1 *Introduction* _____
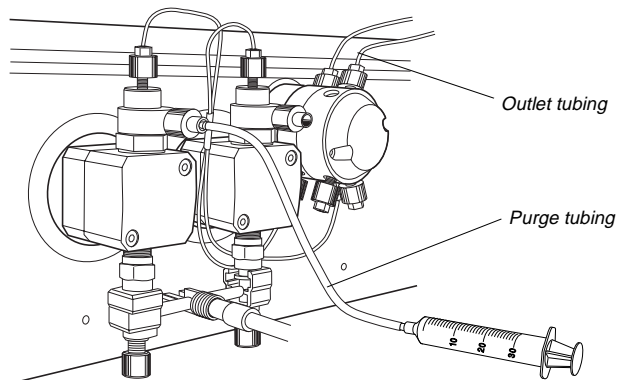
1.2 Safety

- The system is designed for indoor use only.

- Do not use in a dusty atmosphere or close to spraying water.

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

> **WARNING!** The instruments must not be opened by the user. They contain high voltage circuits which can give a lethal electric shock.

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

> **WARNING!** In ÄKTAexplorer 100, never use 0.5 or 0.75 mm i.d. tubing with columns that can only withstand a low maximum pressure and that allow high flow rates, as the columns may rupture, resulting in injury.

> **WARNING!** In ÄKTAexplorer 10, never use 0.25 mm i.d. tubing with columns that can only withstand a low maximum pressure and that allow high flow rates, as the columns may rupture, resulting in injury.

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

> **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 being used.
> If using a sample pump for sample application directly onto the column, a tubing must be connected between ports 3 and 6.

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

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

> **WARNING!** If the door is quickly pulled open to its full extent, the internal capillary tubing may be pulled from their connections causing leakage.

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

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

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

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

WARNING! If heavier instrument components such as the pump are removed from the rack and the door is then opened fully, the shift in the centre of gravity of the system may cause it to tip over.

WARNING! Never place buffer containers on the top of the valve door. If this is done, the containers may fall down when opening the valve door. Place the buffer containers on the buffer tray, above Box-900.

WARNING! Never block the ports on the Outlet valve with stop plugs, since this will create over-pressure and may result in injury.

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

WARNING! The maximum allowed pressure for the tubing in the Tubing kit 1.0 is 3.4 MPa (34 bar, 493 psi). Set a pressure limit in UNICORN that is less than this value. If higher pressures are used, the tubing may break, releasing a jet of liquid.

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

1.3   Optional configurations

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

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

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

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

Optional configurations supported by ÄKTAexplorer are:

- Connection of up to 9 motorised multi-port valves. These valves can be used to accomplish the following functions:

    - Buffer selection.

    - Sample selection[1]

    - Optional functions.

- On-line pH measurement[2].

- Connection of a dedicated sample pump[1].

- Sample application using a Superloop™.

- Connection of a fraction collector.

- Connection of an auto sampler.

- Connection of up to three air sensors[3].

- Connection of external equipment using digital input/output signals through the system pump P-901/903 REMOTE connector.

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[1] Included in the standard configuration of ÄKTAexplorer 10 S, 100 and 100 Air.

[2] Included in the standard configuration of ÄKTAexplorer 100 and 100 Air.

[3] Included in the standard configuration of ÄKTAexplorer 100 Air.

2 Operation

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

- Columns and tubing (section 2.1).

- Sample application techniques (sections 2.2 - 2.4).

- Gradient forming techniques (section 2.5).

- BufferPrep (section 2.6)

- Collecting fractions (section 2.7).

The chapter also discusses how methods are selected and system handling while preparing a run (section 2.8), during runs (section 2.9), after runs (section 2.10), and cold room operation (section 2.11).

Please note that there are two Flow restrictors available in the system:

- FR-904
  Mounted at factory, and pre-adjusted to give a back-pressure of 0.4 MPa over the nominal flow range. Normally, this Flow restrictor should be used.

- FR-902
  Supplied separately with the system, and pre-adjusted to give a back-pressure of 0.2 MPa over the nominal flow range. This Flow restrictor is used when a low pressure column, such as HiLoad or HiTrap, should be used.

2.1 Columns and tubing

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

It is very important to use the correct tubing kit taking into consideration the maximum allowed pressure for the column and the size of the column.

ÄKTAexplorer 10 tubing

On delivery, the system is equipped with 0.50 mm i.d. tubing
(marked G, PEEK tubing, orange) from the pump to the Injection valve
and 0.25 mm i.d. tubing (marked G, PEEK tubing, blue) from the
Injection valve to the fraction collector (optional).

There is also a Tubing kit 0.50 available. It should be used with
columns that have a low maximum pressure and allow high flow rates.
If tubing with smaller inner diameter is used, the back-pressure will be
too high and the columns may rupture.

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

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

ÄKTAexplorer 100 tubing

On delivery, the system is equipped with 0.75 mm i.d. tubing
(marked G, PEEK tubing, green) from the pump to the fraction
collector (optional). Tubing kit 0.5 (i.d. 0.5 mm, marked L, PEEK
tubing, orange) and Tubing kit 1.0 (i.d. 1.0 mm, marked H, PEEK
tubing, grey) are also available:

> **WARNING!** Never use 0.5 or 0.75 mm i.d. tubing with columns
> that only can withstand a low pressure and that allow high flow
> rates, as the column may rupture, resulting in injury.

• To decrease peak band broadening and increase resolution, Tubing
  kit 0.5 should be used for columns giving peak volumes less than
  1 ml.

• Tubing kit 1.0 should be used with columns that have a low
  maximum pressure and allow high flow rates. If tubing with
  smaller inner diameter is used, the back-pressure will be too high
  and the columns may rupture.

Recommended tubing and columns – ÄKTAexplorer 10

The tables below shows which tubing kit should be used for each column. It is important that the recommendations in these tables are followed. The tubing to be changed is described in *Reference Information*, section *A.4.*

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

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

Ion Exchange Columns

Code no	Column name	0.25	0.50
17-5004-01	Mini Q™ PE 4.6/50	X, P	
17-0686-01	Mini Q PC 3.2/3	X, P	
17-0546-01	Mono Q™ HR 5/5	X	
17-0556-01	Mono Q HR 10/10	RF	X
17-0506-01	Mono Q HR 16/10	RF	X
17-5005-01	Mini S™ PE 4.6/50	X, P	
17-0687-01	Mini S PC 3.2/3	X, P	
17-0547-01	Mono S™ HR 5/5	X	
17-0557-01	Mono S HR 10/10	RF	X
17-0507-01	Mono S HR 16/10	RF	X
17-5065-01	SOURCE™ 15Q PE 4.6/100	RF	X
17-5067-01	SOURCE 15S PE 4.6/100	RF	X
17-1177-01	RESOURCE™ Q, 1 ml		X
17-1179-01	RESOURCE Q, 6 ml		X
17-1178-01	RESOURCE S, 1 ml		X
17-1180-01	RESOURCE S, 6 ml		X
17-1153-01	HiTrap™ Q High Performance, 1 ml		X
17-1154-01	HiTrap Q High Performance, 5 ml		RF
17-1151-01	HiTrap SP High Performance, 1 ml		X
17-1152-01	HiTrap SP High Performance, 5 ml		RF
17-6002-33	HiTrap IEX Selection kit		X
17-5053-01	HiTrap Q Sepharose Fast Flow, 1 ml		X
17-5156-01	HiTrap Q Sepharose Fast Flow, 5 ml		RF
17-5054-01	HiTrap SP Sepharose Fast Flow, 1 ml		X
17-5157-01	HiTrap SP Sepharose Fast Flow, 5 ml		RF
17-5055-01	HiTrap DEAE Sepharose Fast Flow, 1 ml		X
17-5154-01	HiTrap DEAE Sepharose Fast Flow, 5 ml		RF
17-5056-01	HiTrap CM Sepharose Fast Flow, 1 ml		X

Code no	Column name	0.25	0.50
17-5155-01	HiTrap CM Sepharose Fast Flow, 5 ml		RF
17-5162-01	HiTrap ANX (high sub), 1 ml		X
17-5163-01	HiTrap ANX (high sub), 5 ml		RF
17-5158-01	HiTrap Q XL, 1 ml		X
17-5159-01	HiTrap Q XL, 5 ml		RF
17-5160-01	HiTrap SP XL, 1 ml		X
17-5161-01	HiTrap SP XL, 5 ml		RF
17-5092-01	HiPrep 16/10 Q XL		RF
17-5093-01	HiPrep 16/10 SP XL		RF
17-5090-01	HiPrep 16/10 DEAE		RF
17-5091-01	HiPrep 16/10 CM		RF
17-1064-01	HiLoad™ 16/10 Q Sepharose High Perf.		X
17-1066-01	HiLoad 26/10 Q Sepharose High Perf.		RF
17-1137-01	HiLoad 16/10 SP Sepharose High Perf.		X
17-1138-01	HiLoad 26/10 SP Sepharose High Perf.		RF
17-1060-01	HiLoad 16/10 Q Sepharose Fast Flow		X
17-1062-01	HiLoad 26/10 Q Sepharose Fast Flow		RF
17-1135-01	HiLoad 16/10 SP Sepharose Fast Flow		X
17-1136-01	HiLoad 26/10 SP Sepharose Fast Flow		RF

Size Exclusion Columns

Code no	Column name	0.25	0.50
17-5003-01	Superdex™ Peptide PE 7.5/300	X	
17-1458-01	Superdex Peptide PC 3.2/30	X, P	
17-1453-01	Superdex Peptide HR 10/30	X	
17-0771-01	Superdex 75 PC 3.2/30	X, P	
17-1047-01	Superdex 75 HR 10/30	X	
17-1089-01	Superdex 200 PC 3.2/30	X, P	
17-1088-01	Superdex 200 HR 10/30	X	
17-0774-01	Fast Desalting PC 3.2/10	X	
17-1408-01	HiTrap Desalting 5 ml		RF
17-5087-01	HiPrep 26/10 Desalting		RF
17-1139-01	HiLoad 16/60 Superdex 30 prep. grade	X	
17-1140-01	HiLoad 26/60 Superdex 30 prep. grade	X	
17-1068-01	HiLoad 16/60 Superdex 75 prep. grade	X	
17-1070-01	HiLoad 26/60 Superdex 75 prep. grade	X	
17-1069-01	HiLoad 16/60 Superdex 200 prep. grade	X	
17-1071-01	HiLoad 26/60 Superdex 200 prep. grade	X	
17-1165-01	HiPrep 16/60 Sephacryl S-100 HR		RF
17-1194-01	HiPrep 26/60 Sephacryl S-100 HR		RF
17-1166-01	HiPrep 16/60 Sephacryl S-200 HR		RF
17-1195-01	HiPrep 26/60 Sephacryl S-200 HR		RF
17-1167-01	HiPrep 16/60 Sephacryl S-300 HR		RF
17-1196-01	HiPrep 26/60 Sephacryl S-300 HR		RF

Hydrophobic Interaction Columns

Code no	Column name	0.25	0.50
17-5071-01	SOURCE 15PHE PE 4.6/100		X
17-1184-01	RESOURCE ETH 1 ml		X
17-1185-01	RESOURCE ISO 1 ml		X
17-1186-01	RESOURCE PHE 1 ml		X
17-1349-01	HiTrap HIC Selection kit		X
17-1085-01	HiLoad 16/10 Phenyl Sepharose HP		X
17-1086-01	HiLoad 26/10 Phenyl Sepharose HP		RF
17-5095-01	HiPrep 16/10 Phenyl (high sub)		RF
17-5094-01	HiPrep 16/10 Phenyl (low sub)		RF
17-5096-01	HiPrep 16/10 Butyl		RF
17-5097-01	HiPrep 16/10 Octyl		RF

Reversed Phase Columns

Code no	Column name	0.25	0.50
17-5116-01	SOURCE 5RPC ST 4.6/150	X	
17-5068-01	SOURCE 15RPC ST 4.6/100	X	
17-1181-01	RESOURCE RPC 1 ml	RF	X
17-1182-01	RESOURCE RPC 3 ml	RF	X
17-0704-01	µRPC C2/C18 SC 2.1/10	X, P	
17-5057-01	µRPC C2/C18 SC 4.6/100	X, P	
17-6000-24	Sephasil™ Protein C4 5µm ST 4.6/100	X	
17-6000-21	Sephasil Protein C4 5µm ST 4.6/250	X	
17-6000-25	Sephasil Peptide C8 5µm ST 4.6/100	X	
17-6000-22	Sephasil Peptide C8 5µm ST 4.6/250	X	
17-6000-26	Sephasil Peptide C18 5µm ST 4.6/100	X	
17-6000-23	Sephasil Peptide C18 5µm ST 4.6/250	X	
17-6000-27	Sephasil Protein C4 12µm ST 4.6/250	X	
17-6000-28	Sephasil Peptide C8 12µm ST 4.6/250	X	
17-6000-29	Sephasil Peptide C18 12µm ST 4.6/250	X	
17-0532-01	PepRPC 5µm HR 5/5	X	

Affinity Columns

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

Code no	Column name	0.25	0.50
17-5080-01	HiTrap rProtein A, 5 ml		RF
17-0402-03	HiTrap Protein A, 1 ml (2 pcs)		X
17-0402-01	HiTrap Protein A, 1 ml (5 pcs)		X
17-0403-01	HiTrap Protein A, 5 ml (5 pcs)		RF
17-0404-03	HiTrap Protein G, 1 ml (2 pcs)		X
17-0404-01	HiTrap Protein G, 1 ml (5 pcs)		X
17-0405-01	HiTrap Protein G, 5 ml		RF
17-0406-01	HiTrap Heparin, 1 ml		X
17-0407-01	HiTrap Heparin, 5 ml		RF
17-0412-01	HiTrap Blue, 1 ml		X
17-0413-01	HiTrap Blue, 5 ml		RF
17-5105-01	HiTrap Con A, 1 ml		X
17-5106-01	HiTrap Lentil Lectin, 1 ml		X
17-5107-01	HiTrap Wheat Germ Lectin, 1 ml		X
17-5108-01	HiTrap Peanut Lectin, 1 ml		X
17-5109-01	HiTrap Lectin Test kit, 1 ml		X
17-5110-01	HiTrap IgM Purification, 1 ml		X
17-5111-01	HiTrap IgY Purification, 5 ml		RF
17-5112-01	HiTrap Streptavidin, 1 ml		X

Recommended tubing and columns – ÄKTAexplorer 100

The tables below shows which tubing kit should be used for each column. It is important that the recommendations in the table is followed. The tubing to be changed is described in *Reference Information*, section *A.4*.

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

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

Ion Exchange Columns

Code no	Column name	0.5	0.75	1.0
17-0546-01	Mono Q™ HR 5/5	(+)	X	
17-0556-01	Mono Q HR 10/10		X	
17-0547-01	Mono S™ HR 5/5	(+)	X	
17-0557-01	Mono S HR 10/10		X	
17-1177-01	RESOURCE™ Q, 1 ml	(+)	X	

Code no	Column name	0.5	0.75	1.0
17-1179-01	RESOURCE Q, 6 ml		X	(o)
17-1178-01	RESOURCE S, 1 ml	(+)	X	
17-1180-01	RESOURCE S, 6 ml		X	(o)
17-1153-01	HiTrap™ Q High Performance, 1 ml	(+)	X	
17-1154-01	HiTrap Q High Performance, 5 ml		X	
17-1151-01	HiTrap SP High Performance, 1 ml	(+)	X	
17-1152-01	HiTrap SP High Performance, 5 ml		X	
17-6002-33	HiTrap IEX Selection kit	(+)	X	
17-5053-01	HiTrap Q Sepharose Fast Flow, 1 ml	(+)	X	
17-5156-01	HiTrap Q Sepharose Fast Flow, 5 ml		X	
17-5054-01	HiTrap SP Sepharose Fast Flow, 1 ml	(+)	X	
17-5157-01	HiTrap SP Sepharose Fast Flow, 5 ml		X	
17-5055-01	HiTrap DEAE Sepharose Fast Flow, 1 ml	(+)	X	
17-5154-01	HiTrap DEAE Sepharose Fast Flow, 5 ml		X	
17-5056-01	HiTrap CM Sepharose Fast Flow, 1 ml	(+)	X	
17-5155-01	HiTrap CM Sepharose Fast Flow, 5 ml		X	
17-5162-01	HiTrap ANX (high sub), 1 ml	(+)	X	
17-5163-01	HiTrap ANX (high sub), 5 ml		X	
17-5158-01	HiTrap Q XL, 1 ml	(+)	X	
17-5159-01	HiTrap Q XL, 5 ml		X	
17-5160-01	HiTrap SP XL, 1 ml	(+)	X	
17-5161-01	HiTrap SP XL, 5 ml		X	
17-5092-01	HiPrep 16/10 Q XL		X	
17-5093-01	HiPrep 16/10 SP XL		X	
17-5090-01	HiPrep 16/10 DEAE		X	
17-5091-01	HiPrep 16/10 CM		X	
17-5065-01	SOURCE™ 15Q PE 4.6/100		X	
17-5067-01	SOURCE 15S PE 4.6/100		X	
17-1064-01	HiLoad™ 16/10 Q Sepharose High Perf.		X	
17-1066-01	HiLoad 26/10 Q Sepharose High Perf.		X	
17-1137-01	HiLoad 16/10 SP Sepharose High Perf.		X	
17-1138-01	HiLoad 26/10 SP Sepharose High Perf.		X	
17-1060-01	HiLoad 16/10 Q Sepharose Fast Flow		X	
17-1062-01	HiLoad 26/10 Q Sepharose Fast Flow		X	
17-1135-01	HiLoad 16/10 SP Sepharose Fast Flow		X	
17-1136-01	HiLoad 26/10 SP Sepharose Fast Flow		X	

Size Exclusion Columns

Code no	Column name	0.5	0.75	1.0
17-1453-01	Superdex Peptide HR 10/30	(+)	X	
17-1047-01	Superdex 75 HR 10/30	(+)	X	
17-1088-01	Superdex 200 HR 10/30	(+)	X	
17-0591-01	Fast Desalting Column HR 10/10		X	
17-1408-01	HiTrap Desalting 5 ml		X	
17-5087-01	HiPrep 26/10 Desalting		X	
17-1139-01	HiLoad 16/60 Superdex 30 prep. grade		X	
17-1140-01	HiLoad 26/60 Superdex 30 prep. grade		X	
17-1068-01	HiLoad 16/60 Superdex 75 prep. grade		X	
17-1070-01	HiLoad 26/60 Superdex 75 prep. grade		X	
17-1069-01	HiLoad 16/60 Superdex 200 prep. grade		X	
17-1071-01	HiLoad 26/60 Superdex 200 prep. grade		X	
17-1165-01	HiPrep 16/60 Sephacryl S-100 HR		X	
17-1194-01	HiPrep 26/60 Sephacryl S-100 HR		X	
17-1166-01	HiPrep 16/60 Sephacryl S-200 HR		X	
17-1195-01	HiPrep 26/60 Sephacryl S-200 HR		X	
17-1167-01	HiPrep 16/60 Sephacryl S-300 HR		X	
17-1196-01	HiPrep 26/60 Sephacryl S-300 HR		X	

Hydrophobic Interaction Columns

Code no	Column name	0.5	0.75	1.0
17-1184-01	RESOURCE ETH 1 ml		X	
17-1185-01	RESOURCE ISO 1 ml		X	
17-1186-01	RESOURCE PHE 1 ml		X	
17-1187-01	RESOURCE HIC Test kit, 3x1 ml	(+)	X	
17-1349-01	HiTrap HIC Selection kit, 5x1 ml	(+)	X	
17-1085-01	HiLoad 16/10 Phenyl Sepharose HP		(x)	X
17-1086-01	HiLoad 26/10 Phenyl Sepharose HP		(x)	X
17-5095-01	HiPrep 16/10 Phenyl (high sub)		X	
17-5094-01	HiPrep 16/10 Phenyl (low sub)		X	
17-5096-01	HiPrep 16/10 Butyl		X	
17-5097-01	HiPrep 16/10 Octyl		X	

Reversed Phase Columns

Code no	Column name	0.5	0.75	1.0
17-5116-01	SOURCE 5RPC ST 4.6/150	X		
17-5068-01	SOURCE 15RPC ST 4.6/100	X		
17-1181-01	RESOURCE RPC 1 ml	(+)	X	
17-1182-01	RESOURCE RPC 3 ml		X	
17-0704-01	µRPC C2/C18 SC 2.1/10	X, P		
17-5057-01	µRPC C2/C18 SC 4.6/100	X, P		
17-6000-24	Sephasil™ Protein C4 5µm ST 4.6/100	X		

Code no	Column name	0.5	0.75	1.0
17-6000-21	Sephasil Protein C4 5µm ST 4.6/250	X		
17-6000-25	Sephasil Peptide C8 5µm ST 4.6/100	X		
17-6000-22	Sephasil Peptide C8 5µm ST 4.6/250	X		
17-6000-26	Sephasil Peptide C18 5µm ST 4.6/100	X		
17-6000-23	Sephasil Peptide C18 5µm ST 4.6/250	X		
17-6000-27	Sephasil Protein C4 12µm ST 4.6/250	X		
17-6000-28	Sephasil Peptide C8 12µm ST 4.6/250	X		
17-6000-29	Sephasil Peptide C18 12µm ST 4.6/250	X		
17-0532-01	PepRPC 5µm HR 5/5	X		

Affinity Columns

Code no	Column name	0.5	0.75	1.0
17-0408-01	HiTrap Chelating 1 ml	(+)	X	
17-0409-01	HiTrap Chelating 5 ml		X	
17-0716-01	HiTrap NHS-activated 1 ml	(+)	X	
17-0717-01	HiTrap NHS-activated 5 ml		X	
17-5130-01	GSTrap™, 1 ml (5 pcs)	(+)	X	
17-5130-02	GSTrap, 1 ml (2 pcs)	(+)	X	
17-5131-01	GSTrap, 5 ml		X	
17-5079-02	HiTrap rProtein A, 1 ml (2 pcs)	(+)	X	
17-5079-01	HiTrap rProtein A, 1 ml (5 pcs)	(+)	X	
17-5080-01	HiTrap rProtein A, 5 ml		X	
17-0402-03	HiTrap Protein A, 1 ml (2 pcs)	(+)	X	
17-0402-01	HiTrap Protein A, 1 ml (5 pcs)	(+)	X	
17-0403-01	HiTrap Protein A, 5 ml (5 pcs)		X	
17-0404-03	HiTrap Protein G, 1 ml (2 pcs)	(+)	X	
17-0404-01	HiTrap Protein G, 1 ml (5 pcs)	(+)	X	
17-0405-01	HiTrap Protein G, 5 ml		X	
17-0406-01	HiTrap Heparin, 1 ml	(+)	X	
17-0407-01	HiTrap Heparin, 5 ml		RF	
17-0412-01	HiTrap Blue, 1 ml	(+)	X	
17-0413-01	HiTrap Blue, 5 ml		RF	
17-5105-01	HiTrap Con A, 1 ml	(+)	X	
17-5106-01	HiTrap Lentil Lectin, 1 ml	(+)	X	
17-5107-01	HiTrap Wheat Germ Lectin, 1 ml	(+)	X	
17-5108-01	HiTrap Peanut Lectin, 1 ml	(+)	X	
17-5109-01	HiTrap Lectin Test kit, 1 ml	(+)	X	
17-5110-01	HiTrap IgM Purification, 1 ml	(+)	X	
17-5111-01	HiTrap IgY Purification, 5 ml		X	
17-5112-01	HiTrap Streptavidin, 1 ml	(+)	X	

Selecting tubing kit for other columns – ÄKTAexplorer 100

For other columns, select the tubing kit as described below.

Note: Before starting to perform the described method below, ensure that the On-line filter does not generate too high a back-pressure. A blocked On-line filter will affect the choice of tubing kit. Replace the On-line filter if necessary. Also, ensure that the column itself is clean and does not generate too high a back-pressure.

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

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

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

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

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

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

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

Extra system pressure measurement

Sometimes an extra system pressure measurement must be performed, in order to use low pressure columns such as HiTrap and HiLoad. This is to compensate for the pre-column pressure so that the complete pressure range up to 0.5 MPa can be utilised across the column. Use Flow restrictor FR–902 for these columns.

It is necessary to account for the pre-column pressure by measuring the pressure in the absence of the column. This is achieved as follows:

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

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

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

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

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

1 In the **Method Editor**, select **File:Column list** to open the Columns dialogue box. Selection of a column in the list will display its parameters in the field to the right of the box.

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

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

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

For further information, refer to section *5.11.2 Editing column parameters*, in the *UNICORN 3.1 User Manual.*

If any of the above mentioned actions do not succeed, decrease the flow rate.

Connecting the column

The column is connected between the two column valves. Connect the columns to the valve positions set in the method. Position 1 is for bypass and no column should be connected to this position.
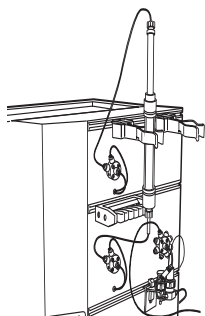
2.2 Sample application overview

With ÄKTAexplorer, the sample can be applied in a number of different ways to suit the application, sample volume and the degree of automation required.

The sample can be applied as follows:

- Using a sample loop, filled manually with a syringe or automatically with Pump P-950.

- Directly onto the column using Pump P-950.

- Superloop™, filled manually with a syringe.

The following table shows which technique is recommended for different sample volumes, and which method template to use.

For a description of the available method templates, and their contents, please refer to the method notes in UNICORN.

Sample application technique	Volume to inject	Template name begins with
Sample loop		
manual filling	0–2[1] ml	Manual injection
automated filling	0.1–2[1]	Sample Pump Loop Injection
Directly onto the column using Pump P-950[2]	> 1 ml	Direct Injection
Superloop[3]	1 ml - 150 ml	Manual injection

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

[2] How to apply the sample directly onto the column using the Sample Pump is described in the ÄKTAdesign Optional Configurations User Manual.

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

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

- Automated partial filling of the sample loop using Pump P-950 (0.2–1 ml).

- Applying the sample directly onto the column with the Pump P-950 (> 1 ml).

- Using a Superloop (1–150 ml).

Section 2.3 describes manual filling of sample loops and section 2.4 automated filling of sample loops. The other sample application techniques are described in the *ÄKTAdesign Optional Configurations User Manual*.

2.3   Manual filling of sample loops

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

Preparation

Prepare the Injection Valve as follows:

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

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

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

4   Mount the syringe holder on the fill port.

5   Ensure that waste tubing is connected to port 4 of the Injection Valve.

6   Mount the sample loop between ports 2 and 6 of the Injection Valve.
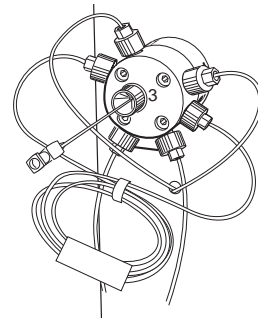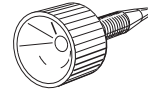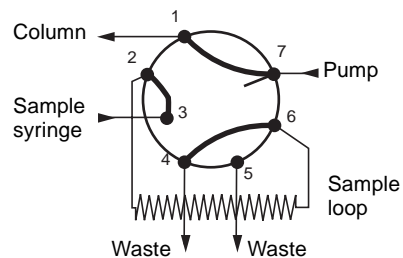
   Note: If the syringe is removed before the sample is injected onto the column, self-drainage can occur and the loop will be emptied.

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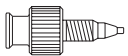
A Union Luer female/1/16" male connector is supplied with ÄKTAexplorer and is an alternative to the injection fill port. If used, the Union Luer connector replaces the injection fill port in port 3 of the Injection Valve.

Four sizes of sample loop are available:

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

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

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

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

Partial filling

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

Partial filling is achieved as follows:

Note: The flow must be off.

1   Set the Injection Valve to position LOAD.

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

3   Fill the sample loop carefully with buffer.
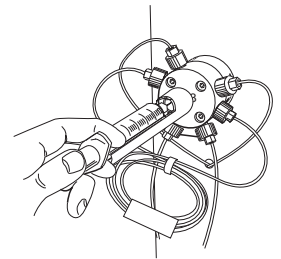
4   Set the Injection Valve to position INJECT before taking out the syringe.

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

5   Load the syringe with the required volume of sample.

  Note: No more than half (50%) a loop volume of sample should be loaded into the loop.
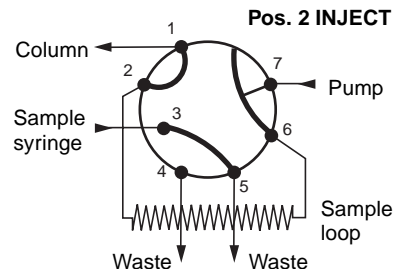
6   Insert the syringe into the injection fill port on the Injection Valve. Set the Injection Valve to position LOAD.

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

7   Gently load the syringe contents into the sample loop.

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

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

Complete filling

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

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

Complete filling is achieved as follows:

1   Set the Injection Valve to position LOAD.

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

3   Gently load the syringe contents into the loop.

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

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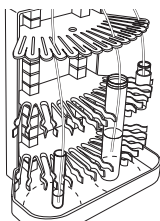
Emptying the sample loop

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

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

2.4   Automated filling of sample loops using Pump P-950

Pump P-950 is a standard component in ÄKTAexplorer 10 S, 100 and 100 Air. It is also available as an accessory to other ÄKTAexplorer systems.

The sample loop can be filled automatically, which can be useful in, for example, scouting runs where samples must be applied repeatedly. Using sample loops supplied by Amersham Pharmacia Biotech, volumes between 0.1–2.0 ml can be applied. The sample is drawn into the sample loop via tubing connected to the Sample Valve with the aid of a Sample Pump, Pump P-950.

Use a *Sample Pump Loop Injection Y Type of gradient* method template when filling sample loops automatically.

General preparation

Prepare the system as follows:

1   Connect the Sample Pump inlet tubing to port 3 on the Injection Valve.

2   Ensure that the tubing from the central port of the Sample Valve is connected to port 4 on the Injection Valve.

3   Connect the sample loop between ports 2 and 6 on the Injection Valve.

4   Ensure that the Sample Pump has been calibrated recently (see Pump P-950 User Manual for details).
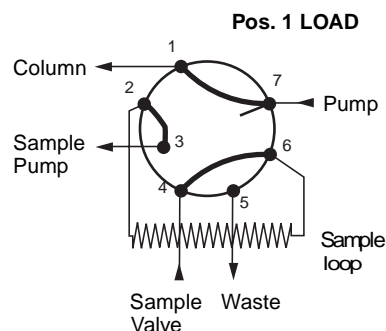
Note: Pump P-950 must be calibrated whenever the running conditions are changed, e.g. viscosity of sample or buffer, temperature, back-pressure etc. If the Sample Pump is not used frequently it should be calibrated before use.

5   Place the inlet tubing from port 8 of the Sample Valve into a small bottle of starting buffer, buffer A. This solution should be used to rinse the tubing between the Sample Valve and the Injection Valve to minimize carry-over when changing from one sample to another.

6   Place the inlet tubing from the Sample Valve into test tubes containing the samples.

7   Set the Injection Valve to position LOAD, position 1.

8   Set the Sample Valve in the position corresponding to the first sample, position S1 (sample tube one).

9   Start the Sample Pump manually from UNICORN. Set the flow rate to 0.5 ml/min and let the pump run for approximately 40 seconds (= 0.35 ml) to completely fill the inlet tubing to the Sample Valve with sample.

10 Stop the Sample Pump.

11 Set the Sample Valve in the position for the next sample.

12 Repeat step 9 - 11 until all inlet tubing to the Sample Valve have been filled with samples.

13 Set the Sample Valve manually from UNICORN in position S8. Start the Sample Pump from UNICORN. Set the flow rate to 0.5 ml/min and let the pump run for approximately 4 minutes (= 2 ml) to completely fill the tubing from the test tube with starting buffer to the Injection Valve with starting buffer.

14 Stop the Sample Pump.

15 Set the Injection Valve to position INJECT, pos. 2.

16 Flush the sample loop by starting Pump P-950. Let a volume of 5 times the volume of the sample loop pass through.

17 Stop Pump P-950 and set the Injection Valve to LOAD, pos.1., by clicking on **End**. The system is now ready to start a run.

Filling the loop

Prepare the system as described above and proceed as described below.

1   Use a *Sample Pump Loop Injection Y Type of gradient* method template. In the method, enter 5 times the volume of the sample loop for the variable **Rinse_loop_with.** This will rinse the tubing between the Sample Valve and the Injection Valve as well as the sample loop before the sample is loaded into the sample loop.

Note: When the same sample is used for repeatedly application, the tubing between the Sample Valve and the Injection Valve as well as the sample loop do not need to be rinsed with buffer between runs. Enter zero for the variable **Rinse_loop_with.**
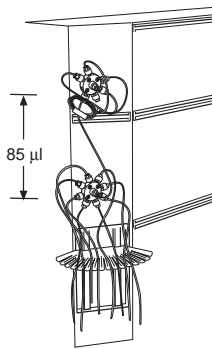
85 µl

2   Enter the sample volume in the variable **Fill_loop_with**. To compensate for the volume between the Sample Valve and the Injection Valve, 85 µl should be added to the sample volume entered in the variable in UNICORN. For complete filling an overfill of 2-5 times the loop volume is needed for maximal reproducibility between the runs.

Note: When only one sample is used, it is only necessary to compensate for the volume between the Sample Valve and the Injection Valve the first time the sample is applied.

3   Selection of the different samples is specified in the **Sample_inlet** variable. The samples will be applied automatically. In scouting runs you can enter different sample volumes with the variable **Fill_loop_with.**

Emptying the sample loop

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

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

2.5   Mixing gradients

Gradients

There are two different techniques available for mixing gradients. The standard technique using two separate buffers, one to each pump module, and the BufferPrep technique using four solutions, two to each pump module, generating the buffer on-line with a switch valve before each pump module. The minimum flow rate for BufferPrep is 1.0 ml/min. The outputs of the pump modules are routed to a mixer.

The BufferPrep method is described in section *2.6 BufferPrep*.

Mixer

> **WARNING!** When using hazardous chemicals, ensure that the mixer chamber has been flushed thoroughly with distilled water before removing the chamber.

ÄKTAexplorer 10
The mixer is delivered with two different mixer chambers, 0.6 and 2 ml. At delivery the 0.6 ml mixer chamber is installed and can be used at gradient volumes up to 5 ml/min (binary gradients).

ÄKTAexplorer 100
The mixer is delivered with a 2 ml chamber installed. Two separate chambers, 5 and 12 ml, are also supplied.

The recommended minimum gradient volume for each mixing chamber is specified in the table below.

	Recommended minimum gradient volume	
Mixing chamber volume	*Binary gradient*	*BufferPrep gradient*
0.6 ml	5 ml	–
2 ml	16 ml	60 ml
5 ml	38 ml	80 ml
12 ml	90 ml	113 ml

When using eluents that are more difficult to mix such as isopropanol and water, a larger mixer volume can be used to get optimum mixing.

Note: If the pH and conductivity curves indicate uneven mixing of your buffers (unstable curves), change to a larger mixer chamber.

Note: Always place the buffer bottles lower than the mixer when changing chambers, to prevent draining

2.6   BufferPrep

What is BufferPrep?

BufferPrep eliminates the time consuming manual buffer preparation and titration usually needed for every pH change in chromatography. For any pH and salt concentration entered, BufferPrep automatically calculates and prepares the composition of the buffer on-line, from four stock solutions. Linear and step salt gradients can be run and pH can be used as a variable scouting parameter.

BufferPrep is optimized for use in ion exchange chromatography method development, but can also be used for size exclusion chromatography. BufferPrep should not be used for other chromatographic techniques.

The four stock solutions consist of:

1   A mix of buffering components. Up to five different buffering components with up to three pKa's each enabling a broad pH range to be covered.

2   An acid (HCl) or base (NaOH) for on-line titration of pH.

3   Distilled water.

4   An inert salt (e.g. NaCl or KCl) for salt gradient formation.

BufferPrep compensates for the pKa drift with changed ionic strength and temperature. A number of pre-defined recipes are available. New recipes can also be created, see *Reference Information,* section C.

The pH is generated from pump module A and the salt concentration from pump module B. The stock solutions containing the buffering component mix and the HCl or NaOH are connected to pump module A. The stock solutions containing salt and water are connected to pump module B. A switch valve on each pump module, together with the BufferPrep algorithms, generates the correct mixing ratios for the set pH. Each pump module delivers 50% of the set flow.

A2: Acid/Base
A11-18: Buffer stock
B1: Water
B2: Salt stock
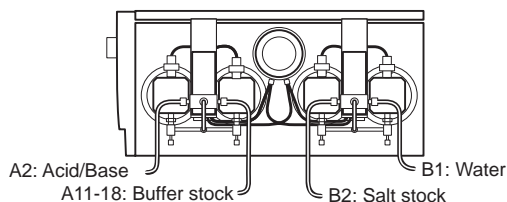
Pump module A

To achieve a set pH, the buffering stock solution and the acid/base stock solution are mixed via the switch valve in a ratio that depends on the buffering substance characteristics at the set pH. An example of the mixing ratios between the two stock solutions is shown below.

Mixing ratios in Pump module A at different pH

%Acid

%Buffer

pH
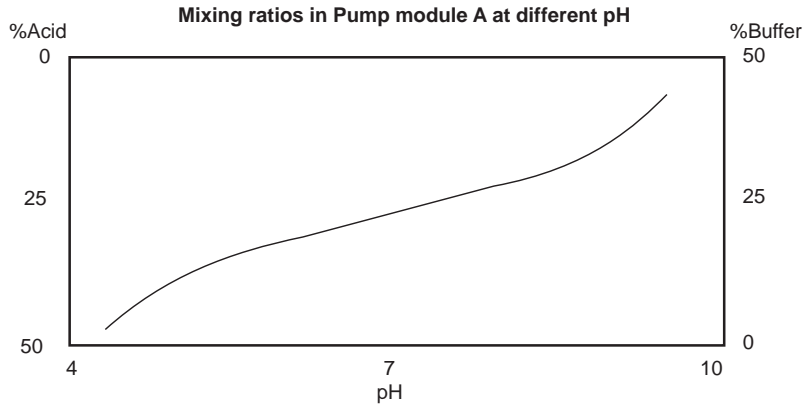
Note that the shape of the mixing ratio curve will differ for different buffering substances.

Pump module B

To achieve the set salt concentration, the salt stock solution and water are mixed via the switch valve in a ratio that depends on the programmed salt concentration variation during elution.

Mixing ratios in Pump module B during a salt gradient

% H_2O

% Salt

% B
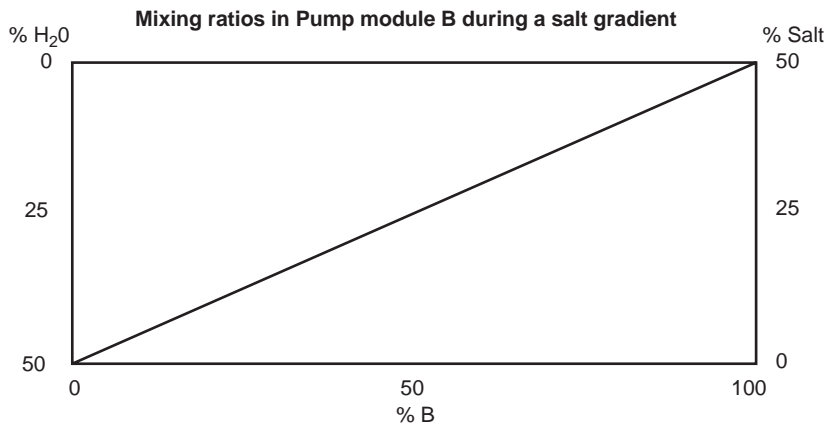
Strategy for using BufferPrep

BufferPrep should be used to quickly find the optimal pH for your ion exchange separations. Use it as follows:

1   Start with a recipe covering a broad pH range and scout for pH in steps of, for example, 0.5–1 pH units.

2   Select the pH that gives the best result.

3   Either continue with the broad pH range recipe or change to a single buffer recipe within the pH range that gave the best result.

4   Scout for pH in steps of, for example, 0.2 pH units, to locate the optimal pH.

5   When the optimal pH is found either continue to run BufferPrep at the desired pH (fine tune the recipe for the highest accuracy) or, prepare the buffer manually to obtain the highest possible reproducibility when optimizing other parameters.

Creating a method for pH scouting

1   Select a Type of injection Y BufferPrep method template.

2   Open the **BufferPrep** page in **Run setup** of the Method Editor and select the **BufferPrep ON** button.

3   Select the recipe that corresponds to the technique and pH range required. The broad pH range recipes are:

  • CIEX: (for cation exchange chromatography) pH 3 to 7.5.

  • AIEX: (for anion exchange chromatography) pH 5 to 9.5.

  • AIEX: (for anion exchange chromatography) pH 6 to 9.0.

    A number of single buffer recipes, each covering narrower pH ranges, are also available (see *Reference information C6)*.

4   Prepare the required solutions. Details about preparation can be found in the **Note** field in the **BufferPrep** page The required solutions and the inlets to which they should be connected, are displayed on the right in the **BufferPrep** page. Accuracy of preparation is essential. Use ampoules of exact concentrations for HCl and NaOH if available. If not available, the correction factors may need to be adjusted each time a new stock solution is prepare.

Inlet A11-A18: Buffer mix
Inlet A2:   HCl or NaOH
Inlet B1: H_2O
Inlet B2:   Salt

5   Open the **Scouting** page and select **BufferPrep_pH** from the list
     displayed. Enter the required pH for each run.

     *Note: To keep the equilibration volumes to a minimum between
     runs during pH scouting, start with the lowest pH and increase the
     pH for each run when titrating with an acid (as in the AIEX and
     CIEX recipes). When titrating with a base start with the highest
     pH.*

6   Click on the **Variables** page.

     To obtain a stable pH, ensure that the equilibration volume is at
     least:

     In ÄKTAexplorer 10: 9 ml with 0.6 ml mixer, or
                          14 ml with 2 ml mixer.

     In ÄKTAexplorer 100:30 ml with 2 ml mixer,
                          50 ml with 5 ml mixer, or
                          100 ml with 12 ml mixer.

     Up to 20 column volumes of equilibration may be required to
     obtain a stable pH.

7   Save the method.

Preparing the system for a BufferPrep run

1   Calibrate the pH monitor. Refer to Monitor pH/C-900 User
     Manual. For high accuracy measurements, calibration should be
     performed with the pH electrode fitted in the flow cell at the flow
     rate to be used in the scouting run.

2   Manually fill the inlet tubing with the stock solution by using
     the **PumpWash** instruction in UNICORN.

3   If equilibration is not programmed into the method, equilibrate the
     system manually without a column with the set pH before starting
     the run. Select **System Control:Manual:Other** and click on **Recipe.**
     Select the same recipe as you have in your method. Click on
     Execute. Select **Pump** and select **BufferPrep_pH** and enter
     the same pH as set in the method. Click on **Execute.** Set a flow rate
     and click on **Execute.**

     Use the following equilibration volumes (at minimum) to obtain a
     steady pH reading:

     In ÄKTAexplorer 10:   9 ml with 0.6 ml mixer, or
                              14 ml with 2 ml mixer.

     In ÄKTAexplorer 100:   30 ml with 2 ml mixer,
                              50 ml with 5 ml mixer, or
                              100 ml with 12 ml mixer.

4   When running pH scouting, the sample should, if possible, have a pH close to the highest pH in the scouting run for AIEX and close to the lowest pH for CIEX. The conductivity of the sample should be below 5 mS/cm.

5   Start the run.

Fine tuning

To obtain higher pH accuracy, the recipe can be fine tuned around a specific pH. When scouting over a broad pH range fine tuning is less beneficial. Run BufferPrep manually at 0 and 100% B as follows:

1   From the **System Control:Manual** menu select the recipe under **Other, BufferPrep_pH** and **Flow** under **Pump**. Start a run manually at 0% B. Ensure the buffer valve is set correctly to the required inlet (A11 – A18).

2   Check the pH reading when stable.

3   Change to 100% B by setting the **Gradient** instruction in **Manual:Pump** to 100% B for **Target** and 0 for **Length**. Click on **Execute**.

4   Check the pH reading when stable at 100% B.

5   If the reading is acceptable at both 0 and 100% B, the run can be started.

6   If the pH reading is not acceptable, select Correction Factors in the BufferPrep page of the Method Editor. Enter the pH deviation at 0 and 100% B. (For example, if the pH has been set to 7 and the actual pH is 7.1 enter 0.1, or if the actual pH is 6.9, enter –0.1). If default correction factors exists, add the measured deviation to these factors.

7   Save the method.

   Note: The new correction factors will only apply while this method is used. When a new method is created and a recipe is selected, default correction factors will apply. To change default correction factors for a recipe see section C.4 in Reference information.

Creating your own recipes

Refer to section *C.1* in *Reference information* for details on how to create recipes or edit existing recipes.

2.7 Collecting fractions

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

- Flowthrough fractionation.

- Eluate and peak fractionation.

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

Flowthrough_FracSize
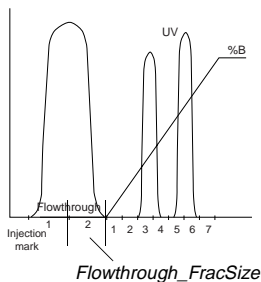
Flowthrough fractionation

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

Eluate_Frac_Size

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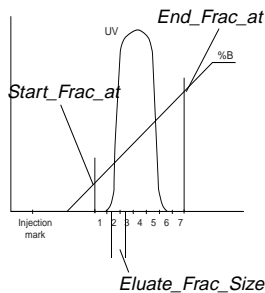
Eluate fractionation allows you to collect fixed volumes during elution within a set interval of %B. The eluate fractionation volume is set on the Variables page in block **Eluate_and_Peak_Fractionation** with variable **Eluate_Frac_Size**. The start of the fractionation interval is set with variable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

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

- Peaks are collected in one fraction each. The peak size (**Peak_Frac_Size**) is set to a volume which is larger than the actual peak volumes.

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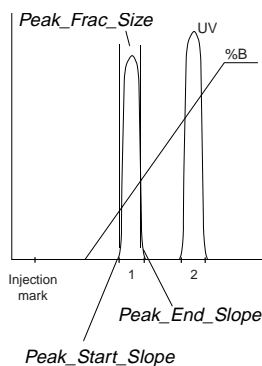
Select a method template with "FP" as middle letters (Type of injection FP Type of gradient). The properties for the peak slopes are set in block **Eluate_and_Peak_Fractionation**. Variables **Peak_Start_Slope** and **Peak_End_Slope** controls the start and end points for the peak fractions to be collected.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

Outlet valve

An outlet valve is included for directing the liquid flow to either waste or to fractionation.

- Port 1 (valve default position) should always be connected to a waste flask of suitable size (W3).

- A fraction collector can be connected to port 2 of the outlet valve (G11/H11 in ÄKTAexplorer 10 and G15/H15/L6 in ÄKTAexplorer 100).

- Port 3–8 of the outlet valve can be used for fractionation of up to 6 larger fractions. This is possible in ÄKTAexplorer 100 and 100 Air systems. Select a template with "V" as the middle letter (Type of injection V Type of gradient). In the block Valve_Fractionation on the Variables page, enter the number of fractions to collect, the fraction volume and in which port (F3 - F8) fractionation should start.

2.8　Before a run

Selecting a method

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

The basic steps required to create a method are:

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

2　Click the **OK** button.

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

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

5　Save the method.

Calibrations

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

| Component	How often
pH monitor	Every day.
Pump P-950 (if applicable)	Whenever the running conditions are changed, e.g. viscosity of sample or buffer, temperature, backpressure etc. If the Sample Pump is not used frequently it should be calibrated before use.
Pressure reading	Once a year or when required.
Conductivity Flow Cell	
Cell constant	Only necessary if specific conductivity with high accuracy is measured (Cond_calib).
Temperature	Must be done when changing the Flow Cell (Temp).
Entering a new cell constant	Must be done when changing the Flow Cell (Cond_cell).

Using the pH electrode

When using the pH electrode (if applicable), the flow restrictor FR-904, which is mounted from factory, must be replaced with the supplied flow restrictor FR-902. Otherwise, the long term stability and lifetime of the pH electrode will deteriorate.

General preparation

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

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

2 Check that there is sufficient eluent available.

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

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

5 Calibrate the pH electrode[1] if required. Refer to Monitor pH/C–900 User Manual. Remember to change the flow restrictor FR-904 to flow restrictor FR-902 when the pH electrode is mounted in the pH flow cell.

6 Calibrate the Sample Pump P-950[2] if it has not been used recently or if the running conditions have changed, e.g. sample viscosity, back pressure etc.

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

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

9 Use the **SystemWash** instruction to wash the system in bypass mode with the start buffer.

_____
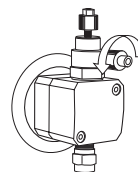
[1] Optional component in ÄKTAexplorer 10, 10 S and 10 XT.

[2] Optional component in ÄKTAexplorer 10 and 10 XT.

2.9 During a run

Viewing progress

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

The System Control window in UNICORN displays the current status
of ÄKTAexplorer and can display up to four panels for monitoring
different aspects of the run. Click on the **Windows** toolbar button or
choose **View:Windows** from the menu to select which panels to display.

Run data

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

Curves

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

Flow scheme

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

Logbook

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

Front panel displays

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

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

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

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

λ 1[215]	1.123	AU
λ 1[254]	0.02345	AU

λ 1[280]	0.1234	AU

The main operating menu of the Monitor UV-900 shows the absorbance values with 4 digits for up to 3 active wavelengths. The display for the third wavelength is reached by turning the dial clockwise. It is also possible to view all three wavelengths simultaneously by turning the dial one step further (only three digits).

Cond	**Temp**	**pH**
25.4%	**22.9ºC**	**11.5**

The main operating menu of the pH/C-900 Monitor shows the conductivity as a percentage of full scale together with the current temperature in the flow cell, and the pH value.

4.000 mS/cm	**22.9ºC**
- - -	**25.4%**

└── *Bar graph*

By turning the dial one click, an alternative display of the conductivity is shown. This display shows the temperature, and the actual conductivity value in mS/cm or µS/cm together with the percentage value and as a horizontal bar graph with 10% resolution.

Changing parameters

From UNICORN

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

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

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

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

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

From the modules

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

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

• **Open** – the dial on the module can be used for manual changes.

• **Keylocked** – the dial on the module can be used to select different menus, but cannot be used to change any parameters.

• **KeyandDiallocked** – Neither menu selection nor parameter changes can be performed.

To select access mode, select **System setting** under **System Control** then **Special:Keyboard**. Select **Open Keylocked** or **KeyandDiallocked**.

2.10 Completion of a run and storage

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

Storage

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

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

Weekend and long term storage
Flush the system with water and then fill it with 20% ethanol (not the pH electrode, see separate instructions below). To avoid deforming the pump tubing in the Sample pump during long time storage, remove and store the pump tubing outside the Sample pump.
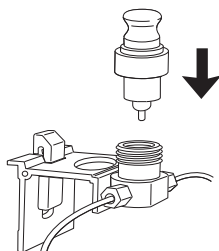
pH electrode
The pH electrode[1] should **always** be stored in a 1:1 mixture of pH 4 buffer and 2 M KNO_3 when not in use. When the pH electrode is removed from the flow cell, the dummy electrode (supplied) can be inserted in the flow path. Remember to change from the flow restrictor FR-902 to flow restrictor FR-904 when the pH electrode is no longer to be used in the flowpath.

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[1] Optional component in ÄKTAexplorer 10, 10 S and 10 XT.

2.11 Cold room operation

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

Preparation

1   Place the separation unit in the cold room.

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

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

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

5   Tighten any leaking connector.

Running

Before starting a run, check the following:

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

2   Calibrate the pH electrode.

3   Check the pH of the buffers.

Note: The measured temperature is the temperature in the conductivity flow cell, which can differ from the ambient temperature.

Removal from cold room

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

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

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

4   Tighten any leaking connector.

3 Maintenance

3.1 Periodic maintenance

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

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

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

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

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

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

> **WARNING!** NaOH is injurious to health. Avoid spillage.

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

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

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

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

Interval	Action
Every day	
System	• Inspect the complete system for eluent leakage.
	• The system can be left filled with buffer overnight. If you are not using the separation unit for a few days, wash the flow path with distilled water. Remove the column and the pH electrode (if applicable). Replace the column by a bypass capillary and fit the pH dummy electrode. 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	• Calibrate the pH electrode (if applicable) according to section 3.6 in the Monitor pH/C-900 User Manual.
Pump P-900	• Check for leakage. If there is a sign of liquid leaking between the pump head and the housing side panel or increased or decreased volume of rinsing solution, replace the piston seals, refer to section 4.4 in Pump P-900 User Manual.
	• When changing eluent, it is important to remove trapped air. Purge the pump according to section 2.8 in the Pump P-900 User Manual. If there is still air in the inlet tubing, stop and remove the air bubbles according to section 5.5 in the Pump P-900 User Manual. *Note: If air is allowed to enter the columns, their performance can be heavily altered or destroyed.*
Every week	
Inlet filters	• Check the inlet filters visually and replace them if necessary.
On-line filter	• Replace the on-line filter.

Interval	Action
Every week	
Pump rinsing solution	• Change rinsing solution. Always use 20% ethanol as rinsing solution.
	If the volume of rinsing solution in the storage bottle has increased, it can be an indication of internal pump leakage. Replace the piston seals according to section 4.4 in Pump P-900 User Manual.
	If the volume of rinsing solution in the storage bottle has decreased significantly, check if the rinsing system connectors are mounted properly.
	If the rinsing system connectors are not leaking, the rinsing membranes or piston seals may be leaking. Replace the membranes and piston seals according to section 4.4 in Pump P-900 User Manual.
Every month	
Flow restrictor	• Check that flow restrictor generates the following back-pressure: FR-904: 0.4 ±0.05 MPa FR-902: 0.2 ±0.05 MPa
	Check the back-pressure as follows: 1 Disconnect the flow restrictor 2 Connect a capillary (approx. 1 m, i.d. 1 mm) to a free port in valve V2. Set the valve manually to this port. Put the open end in a waste container. 3 Run the pump at 10 ml/min with water. Note the back-pressure (Bp1) on the pump display, or in the RUN DATA window. 4 Connect the flow restrictor to the open end of the capillary (observe the IN marking). Put the flow restrictor in the waste container. 5 Run the pump at 10 ml/min with water. Note the back-pressure (Bp2) on the pump display, or in the RUN DATA window. 6 Calculate the back-pressure generated by the flow restrictor. Replace it if it is not within limit.
Every 3 months	
Monitor UV-900	• Check the instrument according to section 4.3 in Monitor UV-900 User Manual.

Interval	*Action*
Every 6 months	
Monitor UV-900	• Clean the cell and optical fibre connectors according to section 4.3 in Monitor UV-900 User Manual.
Monitor pH/C-900	• Clean the UV flow cells according to section 4.2 in Monitor pH/C-900 User Manual. Cleaning the flow cells may be require more often if crude samples are regularly used.
	• Check the pH electrode according to section 3.6 in Monitor pH/C-900 User Manual. Replace the pH electrode if necessary.
Mixer M-925	• Check that the mixer chamber is clean and without damage. Check the tubing connectors. Replace if required.
Yearly	
Valve INV-907, IV-908 and PV-908	• Check for external or internal leakage. Replace channel plate and distribution plate yearly or when required.
Every 2 years	
Mixer M-925	• Replace the complete mixing chamber on a regular basis.
When required	
Pump P-900	• Replace piston seals. Refer to section 4.4 in Pump P-900 User Manual.
	• Replace piston. Refer to section 4.6 in Pump P-900 User Manual.
	• Clean or replace the inlet and outlet check valves. Refer to section 4.7 in Pump P-900 User Manual.
Monitor pH/C-900	• Clean the conductivity flow cell according to section 4.2 in Monitor pH/C-900 User Manual.
	• Clean the pH electrode flow cell (if applicable) according to section 4.3 in Monitor pH/C-900 User Manual.
Pump P-950	• Pump P-950 must be calibrated whenever the running conditions are changed, e.g. viscosity of sample or buffer, temperature, back-pressure etc.

3.2   Cleaning the system

The protocols described below are for system cleaning.

The column selection valves should be set to column bypass position. If the column is to be left in the flow path, please observe the rated maximum flow and pressure for the column.

For column cleaning procedures and column storage instructions, please refer to the respective column in the Adviser-Media Adviser-Column Healer section or to the Instruction supplied with the column.

At the end of the day

If the system will be used with the same buffers next day, let the system run isocratic at a low flow rate (10% of the used flow rate).

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

1   Submerge the inlet tubings in distilled water.

2   Run the **PumpWash** instruction.

Leaving for a few days

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

pH electrode (if applicable): 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 flowpath.

CAUTION! Never leave the pH electrode in the electrode holder for any period of time when the system is not used, since this may cause the glass membrane of the electrode to dry out. Remove 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 KNO3.
Do NOT store in water only.

Additional wash of Outlet valve and Sample valve inlet tubing.

A **SystemWash** does not include wash of the Outlet and Sample[1] valves. Wash the valves as follows:

Outlet Valve: In system control, start the system flow rate. Rinse the valve by switching between the ports manually from system control.

Sample Valve: Place all the sample inlet tubing in the washing solution. Start the Sample Pump and rinse the valve by switching between the ports manually from system control.

Monthly cleaning

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

Wash with 1M NaOH using the **SystemWash** instruction. Immediately wash the system with distilled water to rinse the system from NaOH.

Cleaning-in-place

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

A template for cleaning-in-place, CIP, is available in UNICORN and gives many possibilities to design a powerful cleaning protocol for individual problems.

3.3 Moving the system

Two persons are required to lift the system.

> **CAUTION!** Never lift the system by the valves or the valve door.

Before moving the system, disconnect all cables and tubing connected to peripheral components and liquid containers. Remove all items from the top of the system. Close the valve door completely. Grasp the system firmly by placing the fingers in the gap between the swivel platform and the base of the main unit and lift.

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[1] The Sample Valve is optional in ÄKTAexplorer 10 and 10 XT.

4 Trouble-shooting

4.1 Faults and actions

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

Type	Page
UV curve	48
Conductivity curve	49
pH curve	51
Pressure curve	52
Monitor UV-900	53
Monitor pH/C-900	53
Pump P-900	53
Mixer M-925	54
Pump P-950	55
Valve SV-903	55
Valve IV-908, PV-908, INV-907	55
BufferPrep	56

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

4.2 UV curve

Fault	Action
Ghost peaks	1 Check that there is no air in the eluents. Degas if necessary.
	2 Clean the system in accordance with section *3.2*.
	3 Clean the column in accordance with the column instructions.
	4 Check that the mixer is functioning correctly 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.

Fault	Action
Noisy UV-signal, signal drift or instability	1 The buffer may be impure. Check if the signal is still noisy with water.
	2 There may be air in the flow cell. Check that flow restrictor generates the following back-pressure: FR-904: 0.4 ±0.05 MPa FR-902: 0.2 ±0.05 MPa Check the back-pressure as follows: 1 Disconnect the flow restrictor. 2 Connect a capillary (approx. 1 m, i.d. 1 mm) to a free port in valve V2. Set the valve manually to this port. Put the open end in a waste container. 3 Run the pump at 10 ml/min with water. Note the back-pressure (Bp1) on the pump display, or in the RUN DATA window. 4 Connect the flow restrictor to the open end of the capillary (observe the IN marking). Put the flow restrictor in the waste container. 5 Run the pump at 10 ml/min with water. Note the back-pressure (Bp2) on the pump display, or in the RUN DATA window. 6 Calculate the back-pressure generated by the flow restrictor. Replace it if it is not within limit.
	3 Degas the buffer before use.
	4 Check the connections of the UV-cell optical fibres.
	5 Clean the UV-cell, see section 4.4 of Monitor UV-900 User Manual.

4.3   Conductivity curve

Fault	Action
Baseline drift or noisy signal	1 There may be air in the flow cell. Be sure to use a flow restrictor after the flow cell.
	2 Check for leaking tubing connections.
	3 Check that the column is equilibrated. If necessary clean the column.
	4 Check the operation of the mixer and the 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 mixer function can also be checked by running the installation test.
	5 Clean the flow cell according to procedure in section 4.5 of Monitor pH/C-900 User Manual.

Fault	Action
Conductivity measurement with the same buffer appears to decrease over time	1 Clean the flow cell according to procedure in section 4.5 of Monitor pH/C-900 User Manual.
	2 The ambient temperature may have decreased. Use a temperature compensation factor, see section B.2.1 in Reference information of Monitor pH/C User Manual.
Waves on the gradient	1 Check that the pump is operating and is programmed correctly.
	2 Check that the mixing chamber is free from dirt or particles.
	3 Change to a larger mixing chamber volume if necessary.
	4 Check the motor operation. Place a hand on the mixer and start it by starting the pump at a low flow rate. You should both hear and feel the mixer motor and stirrer when they are spinning.
Ghost peaks appear in the gradient profile	1 A charged sample has been detected (e.g. protein).
	2 Air bubbles are passing through the flow cell. Check for loose tubing connections. Use the flow restrictor.
Unlinear gradients or slow response to %B changes	1 Check that the tubing has been washed properly and that the pump is operating.
	2 Change to smaller mixer volume.
Incorrect or unstable reading	1 Check that the conductivity flow cell cable is connected properly to the rear of the instrument.
	2 Check that the pump and valves operate correctly.
	3 If temperature compensation is being used, check that the temperature sensor is calibrated, and that the correct temperature compensation factor is in use.
	4 Check that the column is equilibrated. If necessary clean the column.
	5 Check the operation of the mixer. The mixer function is checked by placing a stirrer bar on top of the mixer housing. The stirrer bar should rotate when the system is in **Run** mode. The mixer function can also be checked by running the installation test.

4.4   pH curve

Fault	*Action*
No response to pH changes	1 Check that the electrode cable is connected properly to the rear of the instrument.
	2 The electrode glass membrane may be cracked. Replace the electrode.
Small response to pH changes	1 Clean the pH electrode as detailed in section 4.3 of the Monitor pH/C-900 User Manual.
	2 If the problem remains, replace the pH electrode.
Slow pH response **or** **Calibration impossible**	1 Check the electrode glass membrane. If it is contaminated, clean the electrode following the instructions in section 4.3 of the Monitor pH/C-900 User Manual.
	2 If the membrane has dried out, the electrode may be restored by soaking it in buffer overnight.
Incorrect unstable pH reading	1 Check that the electrode cable is connected properly to the rear of the instrument.
	2 Check that the pump and valves operates correctly.
	3 Check that the electrode is correctly inserted in the flow cell and, if necessary, hand-tighten the nut.
	4 If air in the flow cell is suspected, tap the flow cell carefully or tilt it to remove the air. Alternatively, flush the cell with buffer at 20 ml/min for 1/2 min. Use the flow restrictor FR-902 after the pH electrode.
	5 Check that the pH electrode is not broken.
	6 Check that the pH electrode is calibrated.
	7 Check the slope (see section 3.6 of the Monitor pH/C-900 User Manual). If it is outside the range 80–105% or the asymmetry potential deviates more than 60 mV from 0 mV, clean the pH electrode. Recalibrate and if the problem persists, replace the pH electrode.
	8 Clean the pH electrode if required, see section 4.3 of the Monitor pH/C-900 User Manual
	9 Compare the response of the pH electrode with that of another pH electrode. If the response differ greatly, the electrode may require cleaning or replacement.
	10 There may be interference from static fields. Connect the pH flow cell and the rear panel of the monitor using a standard laboratory 4 mm "banana plug" cable.
	11 Check that the pH electrode has been calibrated at the correct temperature.

Fault	Action
	12 In organic solvents such as ethanol, methanol and acetonitrile, stable pH measurements are not possible since dehydration of the membrane will occur. It is recommended that the pH electrode is not used in applications using organic solvents. Mount the dummy electrode instead.
	13 Clogged liquid junction. Refer to section 4.3 of the Monitor pH/C-900 User Manual.
pH values vary with varied back pressure	Replace the pH electrode.

4.5   Pressure curve

Fault	Action
Erratic flow, noisy baseline signal, irregular pressure trace	
Possible causes are:	
Air bubbles passing through or trapped in the pump	1 Check that there is sufficient eluent present in the reservoirs.
	2 Check all connections for leaks
	3 Follow the instructions in section 5.5 of Pump P-900 User Manual.
Inlet or outlet check valves not functioning correctly	1 Clean the valves in according to section 4.7 of Pump P-900 User Manual.
Piston seal leaking	1 Replace the piston seal according to the instructions in section 4.4 of Pump P-900 User Manual.
Blockage or part blockage of flowpath	1 Flush through to clear blockage.
	2 If necessary, replace tubing.
	3 Check inlet tubing filter. It can become clogged if unfiltered buffers or samples are applied. See instructions for flushing through at the end of the run in section 3.8 of Pump P-900 User Manual.

4.6 Monitor UV-900

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

4.7 Monitor pH/C-900

Fault		Action
No text on the front display	1	Check that the mains cable is connected and the power switch is in ON-position 1.
Absolute conductivity value wrong	1	Turn the flow cell so that the end with the screws is facing the pH flow cell.
	2	Recalibrate the conductivity cell.
	3	Calibration solution, 1.00 M NaCl, not prepared correctly. Prepare a new calibration solution and recalibrate the conductivity cell.
Unstable conductivity curve	1	Try using a larger mixer chamber instead of the standard mixer chamber.

4.8 Pump P-900

Fault		Action
No text on the front display	1	Check that the mains cable is connected and the power switch is in ON-position 1.
Liquid leaking between the pump head and the side panel		Piston seal or rinsing membrane incorrectly fitted or worn.
	1	Replace or re-install the seal or the membrane.
	2	Run-in carefully, see section 4.4 of Pump P-900 User Manual.
Leaking connection and/or crystalized material around a connector	1	Unscrew the connector and check if it is worn or incorrectly fitted. If so replace the connector.
	2	Gently tighten the connector with your fingers.

Fault	Action
Low eluent flow and noise	1 Disassemble the pump head and examine the piston spring as the pistons move according to section 4.4 of Pump P-900 User Manual. Replace if necessary.
	2 If the spring is corroded, check piston seal and rinse membrane. Ensure that piston rinsing system is always used when working with aqueous buffers with high salt concentration.
	3 Check the piston for damage. If damaged, replace the piston according to section 4.5 of Pump P-900 User Manual.
	4 Remember to replace the piston seal and rinse membrane with new items.
Erratic pump pressure	To check the pump function, a recording of the pressure can be made, or by checking the pressure in UNICORN. By observing the piston stroke indicator in the Check menu together with the pressure trace, the pump head which is functioning abnormally can be identified (see section B.1.1 and B.2.2 in the Pump P-900 System Manual).
	There can be several causes of an abnormal pressure recording, for example:
	• air trapped in the pump heads
	• partially blocked solvent filters
	• leaking connections
	• piston seal leakage
	• check valve malfunction
	• piston damaged.
	Some examples of normal and abnormal pressure traces together with comments are shown in section 5.4 of Pump P-900 User Manual.

4.9   Mixer M-925

Fault	Action
Leakage	1 Check the tubing connections. Retighten or replace if necessary.
	2 Check the mixer chamber. Replace if liquid has penetrated the mixer chamber walls and sealings.

4.10 Pump P-950

Fault	*Action*	
Leakage	1	Check all tubing connections for leakage. Replace connectors or connection block if necessary.
	2	Check if there is damage to the inlet or outlet tubing. Replace if necessary.
Erratic flow or pressure pulsation	1	Check the tubing connectors.
	2	Check the solvent filter.
	3	Air bubbles may be trapped in the pump. Purge the pump according to section 2.6 in the Pump P-950 User Manual.
Not running	1	Check that the system power is on.
	2	Check the UniNet-2 connection (the indicator on the sample pump should have steady light).

4.11 Valve SV-903

Fault	*Action*	
The valve is not switching	1	Check the connections
	2	Check that the pump is operating and is programmed correctly.
External leakage	1	Check the tubing connections. Tighten or replace if necessary.
Internal leakage	1	Replace the valve.

4.12 Valve IV-908, PV-908, INV-907

Fault	*Action*	
The valve is not switching	1	Check the connections to the pump. The valve should be connected to the UniNet 2 socket, not the UniNet 1 socket.
	2	Check the ID-switch on the valve. The ID number should correspond to the number set in UNICORN.
	3	Check the UniNet cable and replace if required.
The valve is switching to the wrong position		The valve parts may have been incorrectly assembled after replacement.
	1	Check that the distribution plate marking **i/o** or **3** is horizontal.

Fault	Action
External leakage	1 Check the tubing connections. Tighten or replace if necessary.
Internal leakage	Internal leakage can be detected at the small hole on the underside of the valve body.
	1 Internal valve parts may be worn. Change channel plate and distribution plate according to section 4 of the relevant valve instruction.
High back-pressure	1 Do cleaning-in-place according to the instructions in section 4 of the relevant valve instruction.
	2 Change channel plate and distribution plate according to section 4 of the relevant valve instruction.

4.13 Bufferprep

Fault	Action
Unstable pH	1 Check the system by running the method without a column or sample.
	2 If still unstable:
	Check that the correct mixer volume is used.
	Check that the pump is operating correctly.
	Check for a dirty or broken pH electrode.
	Check that the valves are operating correctly.
	Check that the pH is not set too far from the pKa of the buffer components.
	3 If the pH is stable when running without column or sample:
	Check if there is too low concentration of buffer components causing low buffering capacity. See also section C.5.
	Check the pH of the sample.
Incorrect pH	1 Collect some eluent. Check the pH electrode calibration by measuring the pH of the collected eluent on a separate pH monitor.
	2 When using standard recipes, use the following mixture to measure the pH separately. Mix, using a 25 ml pipette, 25 ml of the buffer stock solution, 25 ml of the acid and 50 ml of water. Check the pH with a known good quality pH electrode. The reading should be close to: 5.0 - 9.5 pH AIEX pH 6.5 6.0 - 9.0 pH AIEX pH 6.1 3.0 - 7.5 pH CIEX pH 3.7 If not, prepare new buffers.

Fault	*Action*
	3 If mixture pH is correct:
	Check for a dirty or broken pH electrode.
	Check that the valves are operating correctly.
	4 If a non-standard recipe is used:
	Check that the pH is not set too far from the pKa of the buffer components.
	Check if there is too low concentration of buffer components causing low buffering capacity.
	Check that the correct pKa values are used and that all pKa values have been entered.
Drifting pH	1 Set the Injection Valve to position WASTE. Pump through at least 30 ml of buffer at the set pH to stabilize the pH. Switch the valve back to position LOAD. Equilibrate the column.

4 *Trouble-shooting* _____

Reference information

A   System description

A.1 The System
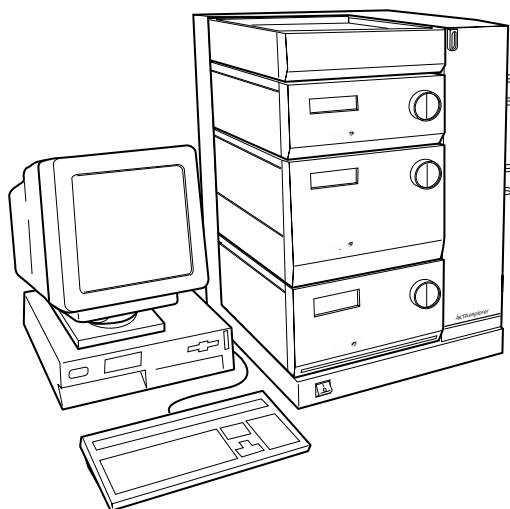
ÄKTAexplorer consists of a compact separation unit including modules and components, and a personal computer running UNICORN software version 3.2, or higher to control the separation unit. For fractionation, fraction collectors are available as an accessory.

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

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

Most of the fluid handling equipment of ÄKTAexplorer is mounted on the valve door, a fully opening section of the separation unit. This allows easy access to all components, tubing and other fluid items located on the modules.

A.2 Component locations

The following illustrations show the locations of the standard components of the separation unit.

Column Selection Valve, V2 (PV-908)

Injection Valve, V1
(INV-907)

Column

Door button

Column Holders

Outlet Valve, V4
(PV-908)

Sample Valve, V5*
(PV-908)

pH electrode (standard component only in
ÄKTAexplorer 100 and
ÄKTAexplorer 100 Air)

Flow restrictor

To
fraction
collector (optional)

Sample Tubing Holder*

Sample Tube Holder*

Column Selection Valve, V3
(PV-908)

Sample Tray*

*) standard component only in
ÄKTAexplorer 100,
ÄKTAexplorer 100 Air and
ÄKTAexplorer 10 S

Outlet Valve, V4
(PV-908)

Flow Direction Valve, V7
(INV-907), (standard component only in
ÄKTAexplorer 100 and ÄKTAexplorer 100 Air

Buffer Valve, V6
(IV-908)

Column Selection Valve, V2
(PV-908)

Box-900

Injection Valve, V1
(INV-907)

Monitor pH/C-900

Monitor UV-900

Pump P-900
(P-901 alt. P-903)

Sample Valve, V5*
(PV-908)

Column Selection Valve, V3
(PV-908)

Valve door

Mains switch

Mixer M-925

Sample Pump P-950*

Switch Valve
(SV-903)

UV cell

Conductivity cell

*) standard component only in
ÄKTAexplorer 100,
ÄKTAexplorer 100 Air and
ÄKTAexplorer 10 S

A.3 Electrical connections

All electrical connections for ÄKTAexplorer are located at the rear of
the system. The system is mounted on a swivel platform allowing easy
access to the fluid handling components and the electrical connections.

Mains cables

Swivel
platform

To
fraction
collector
(optional)

Fuseholder with fuse

To mains socket
(grounded)
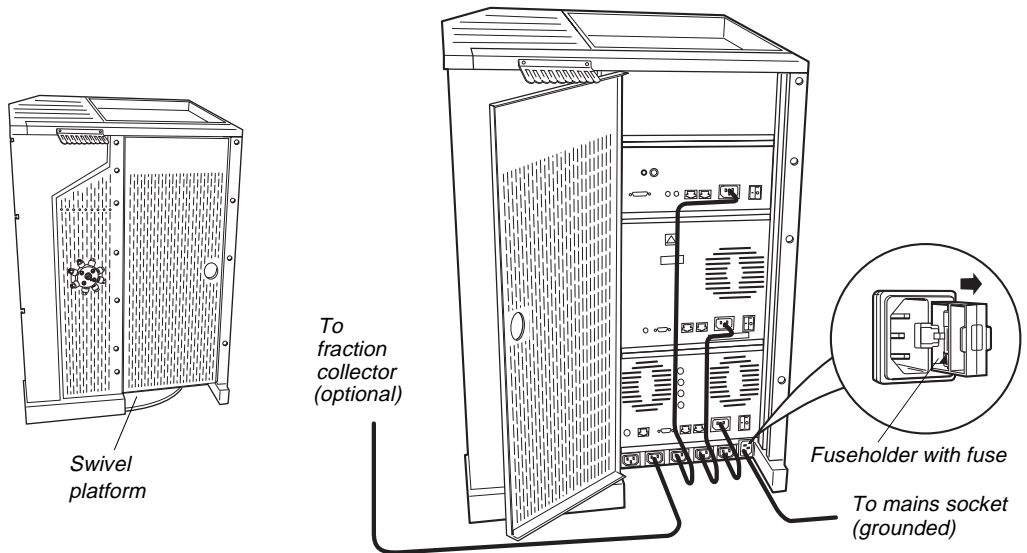
> **WARNING!** Never attempt to remove the mains fuse while mains
> voltage is applied to the system. For continued protection against
> risk of fire, replace only with fuse of the specified type and rating.
> Please, refer to the Technical specifications for fuse data.

Only one mains input is required for the complete system. The supply
voltage for the components in the system and the fraction collector
(optional) is distributed from the base of the system. The mains input
fuse is located to the right of the mains input. To open the fuse holder,
after first removing the power to the system, use a small bladed
screwdriver to lever the holder outwards.

UniNet 1 chain
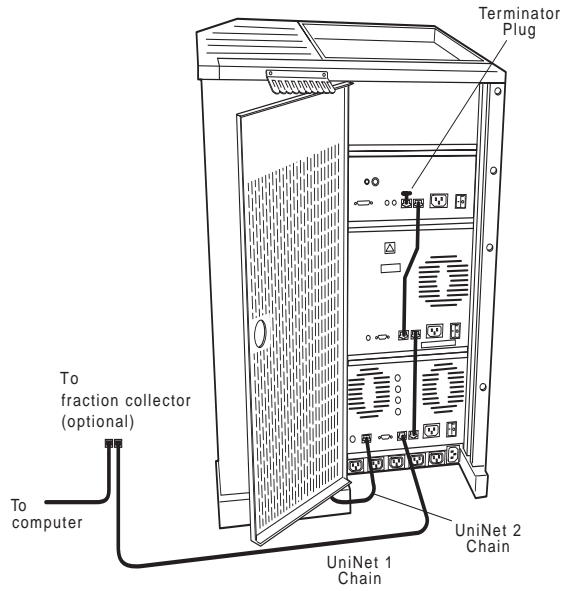
The UniNet 1 data communication chain comes from the computer via the fraction collector (optional) or to the rear of Pump P-900. The chain is terminated at Monitor pH/C-900 with a termination plug.

UniNet 2 chain in ÄKTAexplorer 10 and 10 XT

From UniNet 2
connector at rear of
Pump P-901

Termination plug

UniNet 2 chain in ÄKTAexplorer 10 S, 100 and 100 Air

From UniNet 2
connector at rear of
Pump P-901

Termination plug
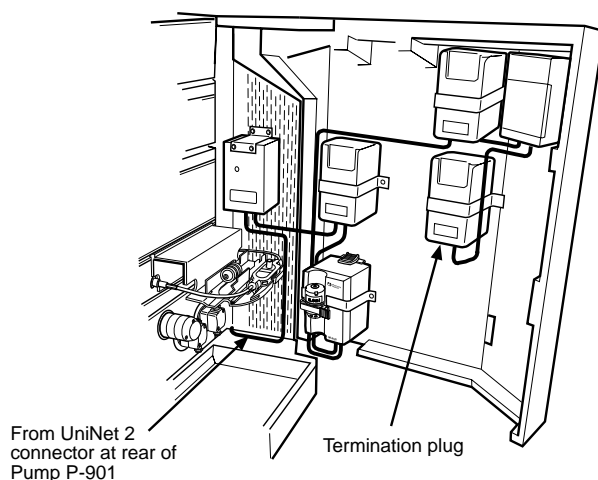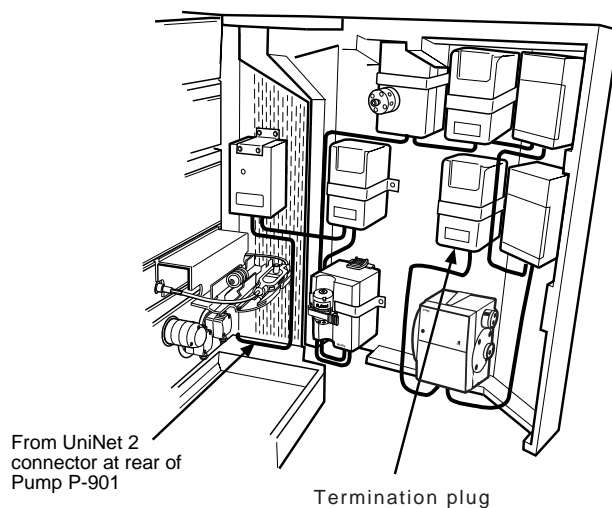
The UniNet 2 data communication chain, which controls the valves, the mixer and the sample pump (if applicable) in the system comes from the rear of Pump P-900 and links components inside the system. The chain is terminated at the Column Selection Valve, V3, with a termination plug.

A.4 Fluid Handling Path – ÄKTAexplorer 10

The following illustrations of the system show the positions of the components and tubing in ÄKTAexplorer 10. Refer to the flow diagram for their locations in the fluid handling path.

ÄKTAexplorer 10 S includes a sample pump and is therefore illustrated in the next section, *ÄKTAexplorer 100.   .*

Column Selection Valve, V2 (PV-908)

Injection Valve, V1 (INV-907)

Column

Column Holders

Outlet Valve, V4 (PV-908)

Flow restrictor

To fraction collector

Column Selection Valve, V3 (PV-908)

Buffer Valve V6

Outlet Valve V4

Column Selection Valve V2

Injection Valve V1

Mixer M-925

Column Selection Valve V3

A2   B1

A11   B2

Buffer Valve V6

Alternative placement for tubing holder
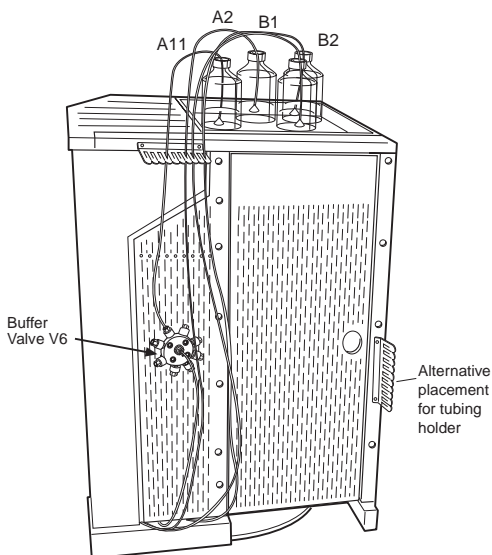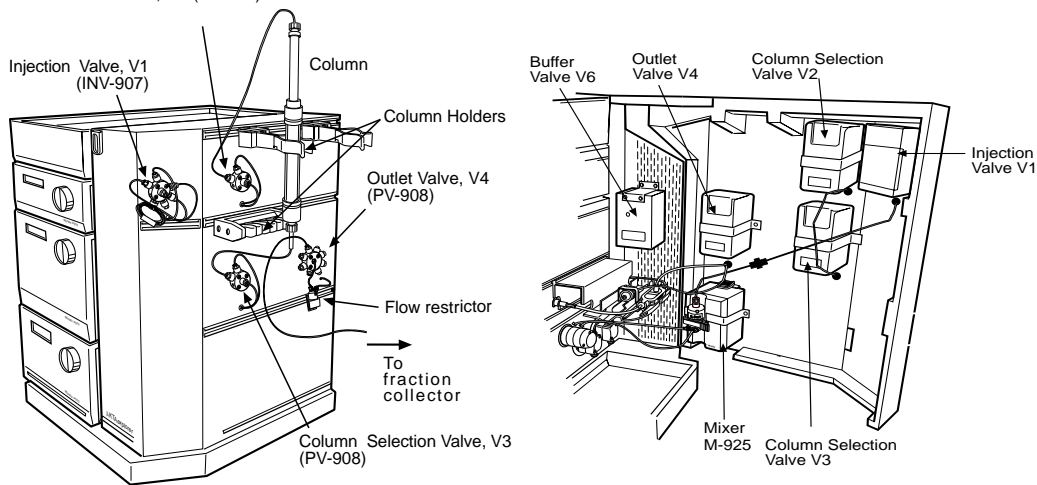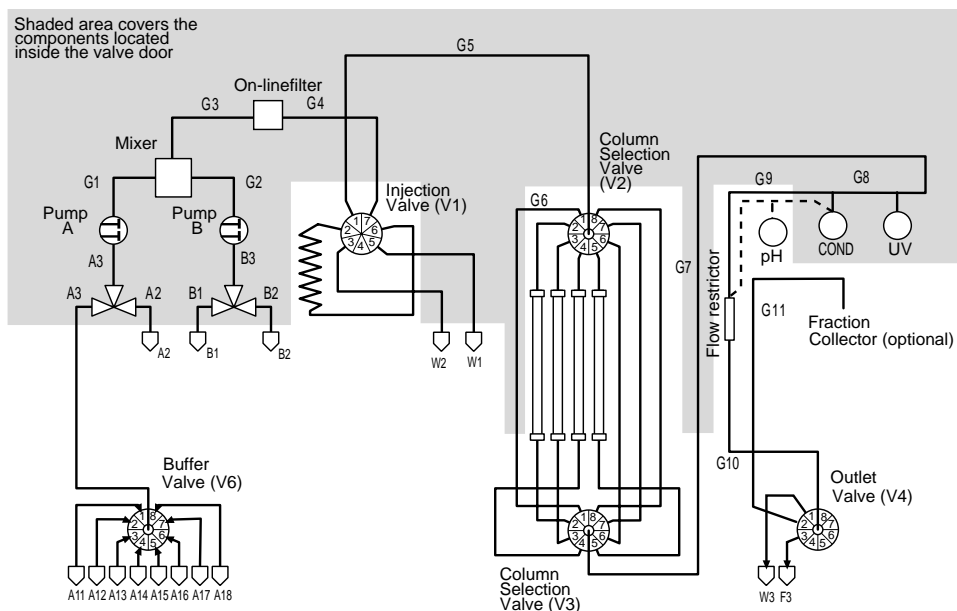
The table shows the tubing available for ÄKTAexplorer 10, and where they are located in the system. At delivery 0.5 mm i.d. PEEK tubing is installed from the pump to the Injection valve, and 0.25 mm i.d. PEEK tubing from the outlet of the injection valve to the fraction collector (if applicable). The column is installed either by using the tubing supplied with the column or by using a piece of PEEK tubing cut by the user to suitable length (0.25 mm and 0.50 mm PEEK tubing is supplied with the ÄKTAexplorer system).

Tubing i.d.	Tubing o.d.	Material	Colour	Max. pressure	Volume of 10 cm	Connected
0.25 mm	1/16"	PEEK	Blue	25 MPa	4.9 µl	From Injection Valve to fraction collector (G5 + G7–G11). (Tubing kit 0.25, installed at delivery)
0.50 mm	1/16"	PEEK	Orange	25 MPa	19.6 µl	From Pump P-900 to injection valve (G1–G4 + G6). G6 is installed from factory, and is connected between V2 and V3 to bypass the column.
0.50 mm	1/16"	PEEK	Orange	25 MPa	19.6 µl	From Injection valve to fraction collector (Tubing kit 0.50). F3=flowthrough (H5 + H7–H12, F3)
0.75 mm	1/16"	Tefzel	Clear	7 MPa	44.2 µl	Waste tubing (W1-W3)
1.6 mm	1/8"	Teflon	Clear	3.4 MPa	201.1 µl	Inlet tubing (A1-A3, B1-B3, A11-A18)

A.5 Fluid Handling Path – ÄKTAexplorer 100

The following illustrations of the system show the positions of the components and tubing in ÄKTAexplorer 100. Refer to the flow diagram for their location in the fluid handling path.

Note that the illustrations also apply to ÄKTAexplorer 10 S (components related to the sample pump are included).
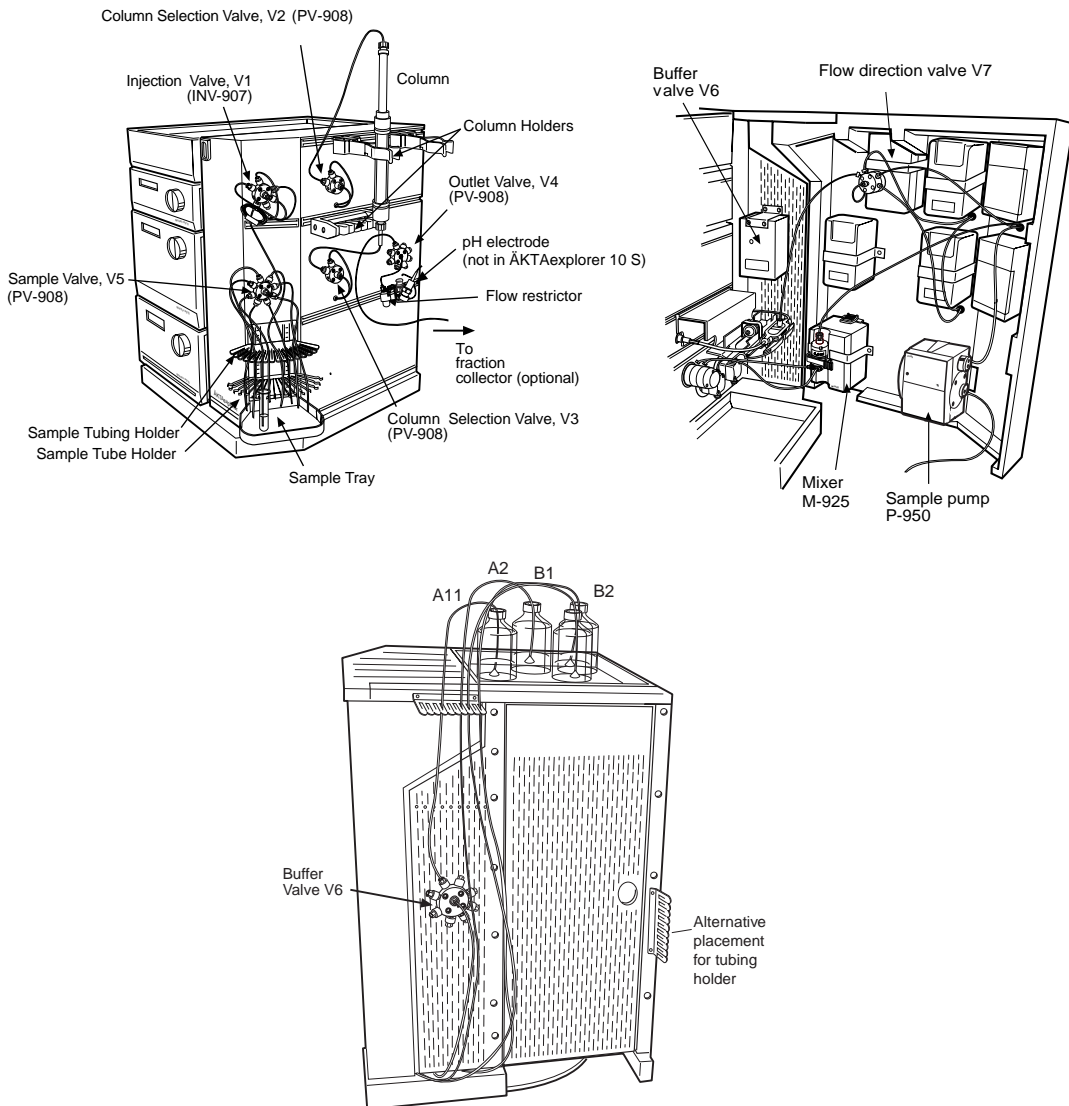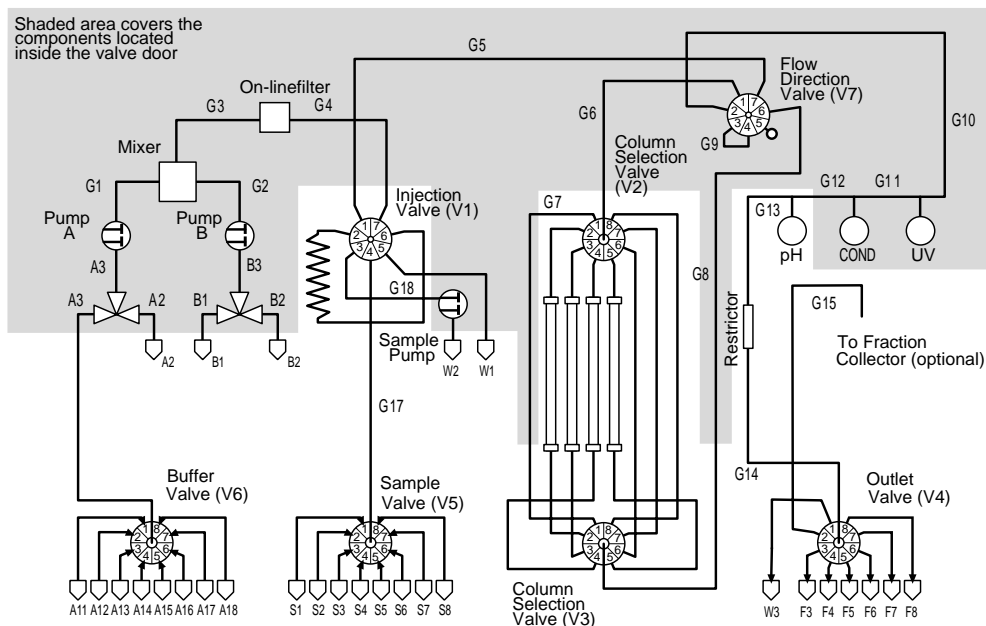
The table below shows the tubing available for ÄKTAexplorer 100, and the location in the system. The tubing used depends on which tubing kit is installed. At delivery 0.75 mm i.d. tubing is installed.

Tubing i.d.	Tubing o.d.	Material	Colour	Max. pressure	Volume of 10 cm	Connected
0.5 mm	1/16"	PEEK	Orange	25 MPa	19.6 µl	From Injection Valve to UV Flow Cell (Tubing kit 0.5)
0.5 mm	1/16"	Tefzel	Clear	7 MPa	19.6 µl	From UV Flow cell to fraction collector (Tubing kit 0.5)
0.75 mm	1/16"	PEEK	Green	10 MPa	44.2 µl	From Pump P-900 to UV Flow cell. G17, G18 and W1 (Installed at delivery)
0.75 mm	1/16"	Tefzel	Clear	7 MPa	44.2 µl	From UV Flow cell to fraction collector (Installed at delivery)
1.0 mm	1/16"	PEEK	Grey	3.4 MPa	78.5 µl	From Pump P-900 to fraction collector (Tubing kit 1.0)
1.0 mm	1/16"	Tefzel	Clear	5 MPa	78.5 µl	From all inlets to Pump P-900 (Sample Tubing kit 1.0))
1.6 mm	1/8"	Teflon	Clear	3.4 MPa	201.1 µl	W2 (Installed at delivery)
2.9 mm	3/16"	Teflon	Clear	3.4 MPa	660 µl	From all inlets to Pump P-900 (Installed at delivery)

A.6 Changing tubing kits –ÄKTAexplorer 10

Two different tubing kits, with different internal diameters, are available for use from the Injection Valve to the Outlet valve (or the fraction collector if applicable) in ÄKTAexplorer 10:

- Tubing kit 0.25 (G5, G7–G11). PEEK tubing, blue, marked G. Installed from factory at delivery. Used for most columns.

- Tubing kit 0.50 (H5, H7–H12). PEEK tubing, orange, marked H. For low-pressure columns, at high flow rates, and/or when the pH electrode is used.

The system is delivered with Tubing kit 0.25 installed. Tubing kit 0.50 should be fitted when columns with a low max pressure are used at high flow rates, or when the pH flow cell is installed to house the Ph electrode.

WARNING! Incorrectly fitted tubing may loosen, causing a jet of liquid to spray out. This is especially dangerous if hazardous chemicals are in use. Connect the tubing by first inserting the tubing fully, then tightening the connector fingertight. 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! The bend radius of PEEK tubing must never be less than 10 cm (with the exception of heat treated, preformed tubing). A smaller radius decreases the allowed maximum pressure and the tubing may break.

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

When changing from/to Tubing kit 0.25 to/from Tubing kit 0.50, change the tubings designated G5, G7–G11 with H5, H7–H12. Refer to the flow diagram in section A.4 for their location in the fluid handling path.

Note: Tubing kit 0.50 contains one more capillary (H12) than Tubing kit 0.25. This is because the pH flow cell is not mounted from the factory.

Tubing[1]	Length (mm)	i.d. (mm)	Location
G1	300	0.50	Pump P-900A (inner) to mixer (left)
G2	300	0.50	Pump P-900B (outer) to mixer (right)
G3	150	0.50	Mixer to on-line filter
G4	460	0.50	On-line filter to Injection valve pos. 7
G6	620	0.50	Column valve V2 (port 1) to Column valve V3 (port 1)
A3	150	1.6	SV-903A (IN) to pump P-900A
B3	150	1.6	SV-903B (IN) to pump P-900B
A11–A18	1250	1.6	Buffer vessels A11–A18 to Buffer valve V6 (port 1–8)
A1	750	1.6	Buffer valve V6 (Centre port) to SV-903A (NO)
A2	2000	1,6	Buffer vessels A2 to SV-903A (NC)
B1	1800	1,6	Buffer vessels B1 to SV-903B (NO)
B2	1800	1,6	Buffer vessels B2 to SV-903B (NC)
W1	1300	0.75	Injection valve (port 4) to waste
W2	1300	0.75	Injection valve (port 5) to waste
W3	1000	0.75	Outlet valve (port 1) to waste
F3	1000	0.50	Outlet valve (port 3, flowthrough)
Tubing kit 0.25 mm			
G5	270	0.25	Injection valve pos. 1 to Column valve V2 (centre port)
G7	550	0.25	Column valve V3 (centre port) to top of UV cell
G8	160	0.25	UV cell to Conductivity cell
G9	450	0.25	Conductivity cell to Flow restrictor
G10	120	0.25	Flow restrictor to Outlet valve (centre port)
G11	500	0.25	Outlet valve (port 2) to fraction collector
Tubing kit 0.50 mm			
H5	270	0.50	Injection valve pos. 1 to Column valve V2 (centre port)
H7	550	0.50	Column valve V3 (centre port) to top of UV cell
H8	160	0.50	UV cell to Conductivity cell
H9	450	0.50	Conductivity cell to Flow restrictor
H10	120	0.50	Flow restrictor to Outlet valve (centre port)
H11	500	0.50	Outlet valve (port 2) to fraction collector
H12	110	0.50	pH flow cell to Flow restrictor

_____

[1] G = General tubing, H = High flow tubing, W = Waste tubing, A = Inlet tubing A, B = Inlet tubing B, F = Fraction tubing

A.7 Changing tubing kits – ÄKTAexplorer 100

There are three different tubing kits available in ÄKTAexplorer 100:

- Tubing kit 0.75 (G1-G15, W3). Installed from factory at delivery.

- Tubing kit 1.0 (H1-H15, W4). Should be fitted when using low pressure columns at high flow rates.

- Tubing kit 0.5 (L1-L6). Should be fitted when using columns that give peak volumes less than 1 ml. When Tubing kit 0.5 is used, the Flow direction valve V7 is bypassed, i.e. reversed flow is not possible, and also the pH flow cell is bypassed.

WARNING! Incorrectly fitted tubing may loosen, causing a jet of liquid to spray out. This is especially dangerous if hazardous chemicals are in use. Connect the tubing by first inserting the tubing fully, then tightening the connector fingertight. 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! The bend radius of PEEK tubing must never be less than 10 cm (with the exception of heat treated, preformed tubing). A smaller radius decreases the allowed maximum pressure and the tubing may break.

WARNING! The maximum allowed pressure for the tubing in the Tubing kit 1.0 is 3.4 MPa (34 bar, 493 psi). Set a pressure limit in UNICORN that is less than this value. If higher pressures are used, the tubing may break, releasing a jet of liquid.

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

When changing from Tubing kit 0.75 to Tubing kit 1.0 or vice versa, change the following tubing (the Tubing kit 1.0 references are shown in parentheses, H1-H15, W4). Refer to the flow diagram in section A.4 for their locations in the fluid handling path:

Tubing	Length (mm)	Location
G1 (H1)	330	Pump P-900A (inner) to mixer (left)
G2 (H2)	330	Pump P-900B (outer) to mixer (right)
G3 (H3)	150	Mixer to on-line filter
G4 (H4)	460	On-line filter to Valve 1 pos. 7
G5 (H5)	470	Valve 1 pos. 1 to valve 7 pos. 7
G6 (H6)	410	Valve 7 pos. 1 to valve 2 centre
G7 (H7)	620	Bypass, valve 2 pos. 1 to valve 3 pos. 1
G8 (H8)	470	Valve 3 centre to valve 7 pos. 6
G9 (H9)	180	Valve 7 pos. 3 to valve 7 pos. 4
G10 (H10)	370	Valve 7 pos. 2 to UV cell
G11 (H11)	160	UV cell to Conductivity cell
G12 (H12)	450	Conductivity cell to pHcell
G13 (H13)	110	pH cell to restrictor
G14 (H14)	120	Restrictor to valve 4 centre port
G15 (H15)	500	Valve 4 pos. 2 to fraction collector
W3 (W4)	-	Valve 4 pos. 1 to waste

Note: Capillaries with other designations than shown in the table above may be included in the tubing kits (e.g. G16/H16/L7). Simply ignore these capillaries and throw away if you like.

Note: In applications where pH measurement is not relevant, for example RPC, the pH Flow Cell can be replaced with the Union 1/16" female /1/16" female supplied.

The following table lists the tubing to connect when changing from Tubing kit 0.75 to Tubing kit 0.5. Refer to the flow diagram below for their location in the fluid handling path:

Tubing	Length (mm)	Location
L1	270	Valve V1 pos. 1 to Valve V2 centre
L2	550	Valve V3 centre to UV cell
L3	160	UV cell to Conductivity cell
L4	450	Conductivity cell to Restrictor
L5	120	Restrictor to Valve 4 centre
L6	500	Valve 4 pos.2 to fraction collector

Note: *Capillaries with other designations than shown in the table above may be included in the tubing kits (e.g. G16/H16/L7). Simply ignore these capillaries and throw away if you like.*

Note: *Both the pH flow cell and the flow direction valve are no longer in the fluid handling path.*
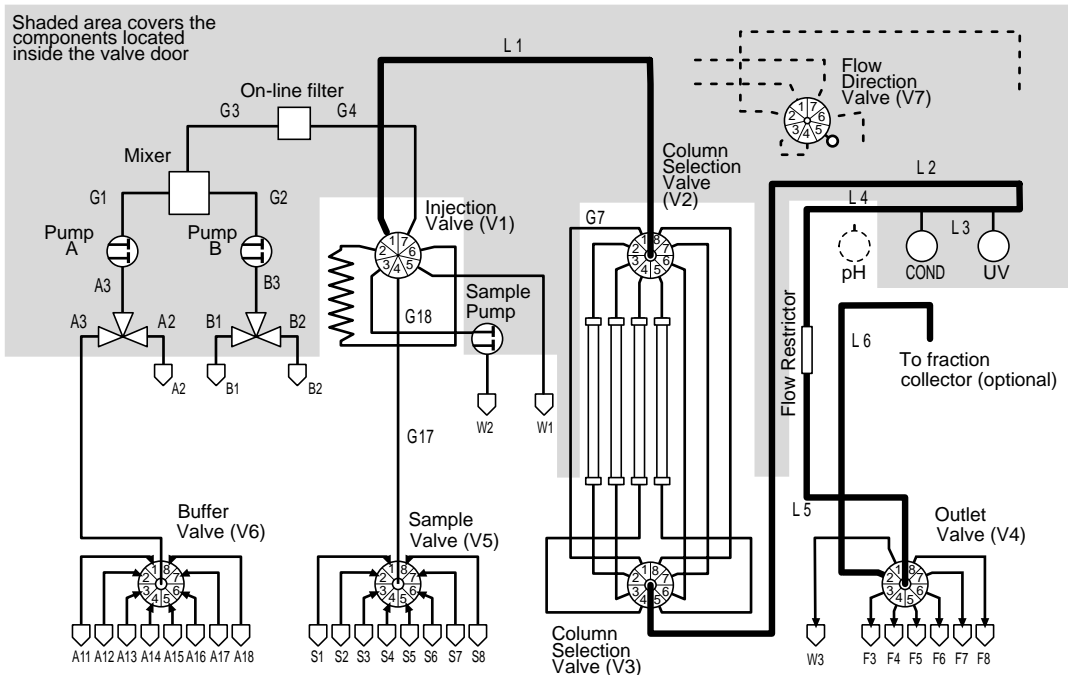
When changing back to Tubing kit 0.75 from Tubing kit 0.5, connect the following tubing. Refer to the flow diagram in section A.4 for the location in the fluid handling path:

Tubing	Length (mm)	Location
G5	470	Valve 1 pos. 1 to valve 7 pos. 7
G6	410	Valve 7 pos. 1 to valve 2 centre
G8	470	Valve 3 centre to valve 7 pos. 6
G9	180	Valve 7 pos. 3 to valve 7 pos. 4
G10	370	Valve 7 pos. 2 to UV cell
G11	160	UV cell to Conductivity cell
G12	450	Conductivity cell to pH cell
G13	110	pH cell to Restrictor
G14	120	Restrictor to valve 4 centre port
G15	500	Valve 4 pos. 2 to fraction collector

B   Components description

A complete description of each component can be found in their respective manuals and instructions.

B.1   Pump P-900

Pump P-900 is the collective name for a pump family. It is a high performance laboratory pump for use where accurately controlled liquid flow is required. It is a low pulsation pump equipped with 2 pump modules; A and B. This allows for binary gradients with high pressure mixing. A pressure sensor is connected to pump module A (left hand pair of pump heads).
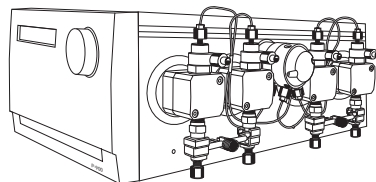
The model installed in ÄKTAexplorer 10 has 10 ml pump heads and is referred to as Pump P-903. P-903 has an operating flow rate range of 0.001–10 ml/min in isocratic mode and in gradient mode, and a pressure range of 0–25 MPa (250 bar, 3625 psi).

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

B.2   Valve SV-903

Valve SV-903 (Pump switching valve) is a 2-way 3-port valve. It is used with Pump P-900 and is powered and controlled from the pump. The valve may be used as a switching valve for gradient formation and BufferPrep or as a sample application valve for switching between sample and buffer solutions.

B.3   Monitor pH/C-900

Monitor pH/C-900 is a combined monitor for accurate, on-line monitoring of pH, conductivity and temperature in a wide range of liquid chromatography applications. Its accurate response coupled with high precision over a wide measuring range makes it ideal for use in all chromatography techniques, from reversed phase with very low conductivity eluents to hydrophobic interaction chromatography in high salt solutions.
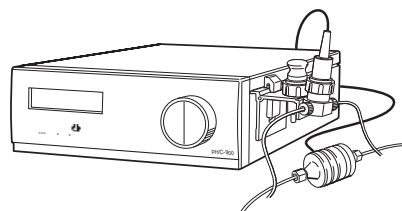
Monitor pH/C-900 consists of a control unit, a flow cell for conductivity and temperature, a flow cell with a holder for the pH electrode and the pH electrode.

B.4 Monitor UV-900

Monitor UV-900 is a multi-wavelength UV-Vis monitor that uses advanced fibre optic technology to monitor with high sensitivity at up to three wavelengths simultaneously in the wavelength range 190-700 nm. The use of fibre optics together with a unique flow cell design ensures a high signal-to-noise ratio with a minimal drift and refractive index effects.
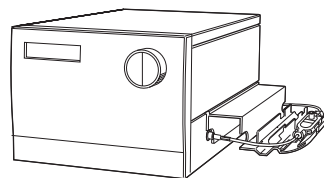
Monitor UV-900 consists of a main unit, optical fibres and a choice of two flow cells (optical pathlength 2 mm, internal volume 2 µl, or pathlength 10 mm, internal volume 8 µl) The 2 mm flow cell is delivered with ÄKTAexplorer.

B.5 UV flow cells

The type of flow cell used depends on the sample amount applied and the size of the column.
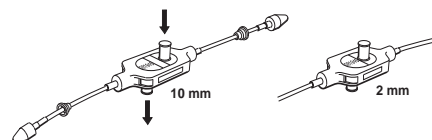
ÄKTAexplorer 10 is delivered with the 10 mm cell fitted. A 2 mm cell is available as an accessory. If a lower detection sensitivity is desired, due to output signal limitation, the 2 mm flow cell should be used.

ÄKTAexplorer 100 is delivered with the 2 mm cell fitted. A 10 mm cell is available as an accessory. For higher detection sensitivity, the 10 mm flow cell should be used.

B.6 Pump P-950

Pump P-950 is a single-channel laboratory pump for use as a laboratory pump to fill sample loops and to inject the sample directly onto the column. The pump is a standard component in all ÄKTAexplorer systems except in ÄKTAexplorer 10 and 10 XT.
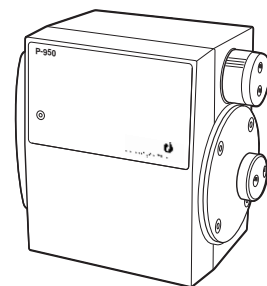
The sample is drawn into three liquid chambers in the pump. A stepper motor assembly performs the pumping action of the chambers. This assembly acts on the chambers in a sequential order, which gives a smooth flow from the pump. The pump produces flow rates up to 50 ml/min.

B.7 Mixer M-925

Mixer M-925 is a dynamic, dual chamber mixer powered and controlled from Pump P-900. All eluents commonly used in ion exchange, hydrophobic interaction, affinity and reversed phase chromatography can be mixed with a high degree of accuracy and reproducibility. The mixer is positioned directly after Pump P-900 in ÄKTAexplorer.
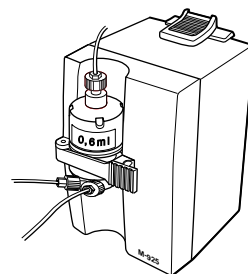
Mixer M-925 has three interchangeable mixing chambers (2, 5, and 12 ml) for optimal mixing in the entire flow rate range of ÄKTAexplorer 10 and ÄKTAexplorer 100.

B.8 Valve INV-907

Valve INV-907 is seven port motorised valve. In ÄKTAexplorer 100, there are two of them; one used as a sample injection valve and the other for reversed flow through the column. In ÄKTAexplorer 10, the valve is only used for sample injection.
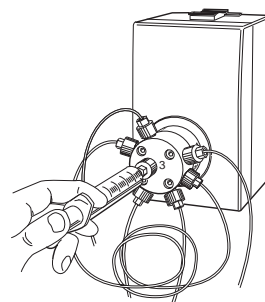
When used as a sample injection valve, three operating positions make it possible to:

- Load a sample loop without disturbing column equilibration.

- Inject the sample onto the column.

- 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 sample injection valve.

- Using a range of fixed volume loops for applying samples from 100 µl to 2 ml with accuracy and precision. Partially filling a loop allows sample application of volumes smaller than 100 µl.

- Using Superloop 10 ml, Superloop 50 ml, and 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.

Larger volumes are applied via the sample pump, Pump P-950, which allows the application of several litres of sample.

The second seven port valve in ÄKTAexplorer 100 is for reversed flow through the column, and is used for column cleaning as well as when reversed elution is preferred.
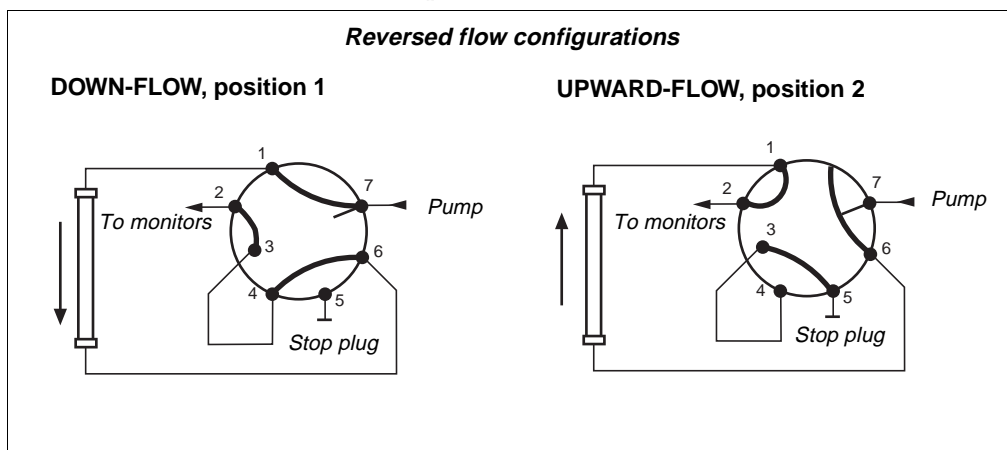
B.9 Valves IV-908 and PV-908

For eluent switching, one IV-908 is used in ÄKTAexplorer. It is connected before Pump P-900 and allows switching between 8 different buffers or samples.

ÄKTAexplorer 10 contains three PV-908 valves. Two of them are used for automatic column switching, which facilitates media scouting. The third is utilized as an Outlet valve, connecting the outlet to waste, a fraction collector (optional) or flowthrough.
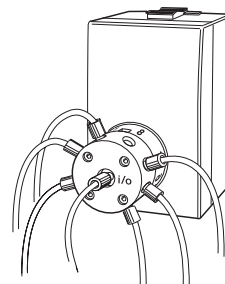
ÄKTAexplorer 100 contains four PV-908 valves, of which two are used for automatic column switching. One PV-908 can be used for automatic sample application of up to 8 samples. The last one is utilized for collecting up to seven large fractions.

Valve PV-908, with a pressure limit of 25 MPa, and IV-908, with a pressure limit of 2 MPa, are motorised 8-way valves. Compared with PV-908, IV-908 allows higher flow rates at lower back-pressures since it has larger diameter channels and is hence used on the inlet side of the system.

B.10 Flow restrictors FR-904 and FR-902

The flow restrictor generates a steady back-pressure to prevent air bubbles being formed after the column in the flow cells. There are two flow restrictors delivered with the system.

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

FR-902 is set from factory to 0.2 MPa and is used when using low pressure columns and/or when the pH electrode is mounted in the pH flow cell.

B.11 On–line filter
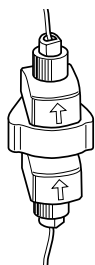
The On-line filter is fitted between the output of Mixer M-925 and position 7 of the Injection Valve. Arrows on the On-line filter indicates the flow direction. It generates a back-pressure of 0.5 MPa maximum.

The filter is a depth type filter and has a pore size of 2 μm. The filter used in ÄKTAexplorer 10 systems is made of titanium. In ÄKTAexplorer 100 systems, it is made of polypropene.

The filter should be replaced every week. When changing the filter, use a tool if necessary, to unscrew the filter body. When assembling the on-line filter, tighten the filter body by hand only. Never use a tool.

B.12 Fraction collector (optional)

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

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

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

Fast tube change minimises spills between tubes, eliminating it entirely below flow rates of 5 ml/min. Drop synchronisation eliminates sample loss during tube change.

C   BufferPrep details

C.1 Creating your own recipes

1   Select **File:BufferPrep Recipe** in the **Method Editor**.

2   Click on **New** to open the **NewRecipe** box.

3   Select a buffer from the list. If the required buffer is not listed, a new buffer can be defined, see section *Defining a new buffer substance* below. Up to 5 buffering components can be added.

4   Set the stock concentration of the buffer.

Buffer concentrations of 2–4 times higher compared to normal preparation should be used. When BufferPrep is used the buffer will be diluted between 2–10 times, dependent on the amount of acid/base that has to be used to reach the desired pH.

It is recommended that the total concentration for all buffer substances selected for the recipe should be between 0.03 M and 0.2 M (typically 0.1 M for ion exchange chromatography).

5   Select either **Acid** or **Base** (HCl or NaOH) from the pull-down list and set the inlet concentration (typically 0.1 M).

6   Select a salt from the pull-down list. If the required salt is not listed, a new salt can be defined, see section *Defining a new salt* below.

7   Set the maximum outlet concentration of the salt to 100% B (usually 1.0 M). The maximum outlet salt concentration is half the concentration of the inlet salt stock solution.

8   Set the pH range required for the buffer.

Note: To set a useful pH range the pKa must be known. Click on the Buffer Substance button and select the buffer component. The pKa values are shown in the list. Typically, a pH range ± 0.5 units around the pKa is useful. For exact ranges, check the buffer tables.

9   To add notes about the recipe, click on **Notes** and enter the required text in the box displayed. Click on **OK** to close the Notes box.

10   When you have created your recipe, click on **Save**. The recipe is checked and if the pH range selected is not feasible, a warning is displayed giving the error condition and making suggestions for its correction. Correct the recipe and click on **Save** again.

Note: The check does not include whether the buffering capacity is large enough over the entire pHrange.

11   Give the recipe a name and click on **Save as.**

C.2 Defining a new buffer substance

Before defining a new buffer substance ensure that all pKa values for the substance are available. The pKa values entered should be true pKa (i.e. the pKa value at indefinite dilution) as opposed to apparent pKa (i.e. measured at a non-zero concentration). Refer to section C.5 for information on true and apparent pKa. The pKa values should be given at 25 °C.

1   In the **NewRecipe** dialogue box, click on the **Buffer Substance** button and select **New** in the buffer definition dialogue box.

2   Enter the name of the new buffer substance.

3   Enter the pKa values. Up to 3 values can be entered for each buffering component. The pKa values must be entered in order of increasing value, starting with the lowest pKa value. When the buffering component has less than three pKa, the other pKa values should be set to zero.

4   Enter the dpKa/dT values (the change of pKa dependent on temperature) for each pKa. Zero means that the pKa does not change with temperature.

5   Enter the number of acidic protons for the buffer substance in the form that is actually weighed in, and click on **Replace** (Example: For NaH_2PO_4 enter 2, for Na_2HPO_4 enter 1, for Tris enter 0).

6   Enter the charge of the completely deprotonated ion. This will be a negative value for an acid, 0 for a base. Click on **Replace** (Example: For NaH_2PO_4 enter –3, for Tris enter 0).

7   Click on **OK** to add the new buffer substance to the list of available buffers.

C.3 Defining a new salt

When defining a new salt ensure that the new salt is inert i.e. a salt with no buffering properties.

1   In the **NewRecipe** dialogue box, click on the **Salt** button and select **New** in the salt definition dialogue box.

2   Enter the name of the new salt.

3   Enter the charge of the anion (Example: for Cl^- enter –1, for SO_4^{2-} enter –2).

4   Enter the charge of the cation (Example: For Na^+ enter 1, for Mg^{2+} enter 2).

5   Click on **OK** to add the new salt to the list of available salts.

C.4 Correction factors

To obtain higher pH accuracy, the recipe can be fine tuned around a specific pH at the flow rate to be used. When scouting over a broad pH range, fine tuning is less beneficial. Run BufferPrep manually at 0 and 100% B as follows:

1   From the **System Control:Manual** menu select the recipe under **Other, BufferPrep_pH** and **Flow** under **Pump**. Start a run manually at 0% B. Ensure the buffer valve is set correctly to the required inlet (A11–A18).

2   Check the pH reading when stable.

3   Change to 100% B by setting the **Gradient** instruction in **Manual:Pump** to 100% B for **Target** and 0 for **Length**. Click on **Execute**.

4   Check the pH reading when stable at 100%.

5   If the reading is acceptable at both 0 and 100% B, the correction factors do not need to be changed.

6   If the pH reading is not acceptable, select **Correction Factors** in the **New/Edit Recipe** box for the recipe in **File:BufferPrep Recipes** in the Method Editor. Enter the pH deviation at 0 and 100% B. (For example, if the pH set is 7.0 and the actual pH is 7.1 enter 0.1, or if the actual pH is 6.9, enter–0.1).

   Note: Some of the pre-programmed recipes have default correction factors. Add your deviation to these to obtain the correct value. Example: If your pre-set correction factor is –0.2 and your reading at pH 7 is 7.1, enter -0.2+0.1 = -0.1.

7   Save the recipe.

C.5 Examples and tips

Buffering capacity

If the buffer capacity of the broad range CIEX and AIEX recipes are too low, there are two alternatives.

*   Switch to a recipe with one buffer component with a pKa close to the required pH,

    or

*   increase the concentration of all buffers in the broad range recipe. Increase the acid concentration by the same factor as the buffer concentration. Note that a new recipe has to be created. The ionic strength of the start eluent will also be increased.

Ionic strength

Buffer components with several pKa values will give a higher ionic strength at the start when the pH is set above the second pKa and even higher above the third pKa for acidic buffer components. This may cause problems if you have peaks that elute early in the gradient. To reduce this, use low concentrations or if possible change to buffer components that only have one pKa.

Example
A recipe with 0.1 M Citrate (pKa 3.13, 4.76, and 6.40) will have an ionic strength of approximately 0.22 at pH 6, but only 0.05 M at pH 4. Use instead a 0.03 M solution or use 0.1 M MES at pH 6.

pH range

Due to pKa change with increasing ionic strength, the useful pH-range may be limited below the pKa for basic buffer components and above the pKa for acidic buffer components. The pH range on the opposite side of the pKa can usually be extended beyond 0.5 pH units.

Example
A recipe with Na_2HPO_4 (pKa 2.15, 7.20 and 12.33) will not give accurate pH above approximately pH 7.6 (when titrated with HCl).

Converting apparent pKa values to true pKa

Only true pKa values must be entered into BufferPrep. When entering a new buffer substance with only apparent pKa known, a conversion to true pKa must be performed.

The apparent pKa is measured at a non-zero concentration. Find the true pKa (pKa at infinite dilution) by taking the apparent pKa and add the value found in the table below for the concentration at which the apparent pKa has been measured (usually at 0.1 M).

Conc [M]	Base pKa1	Base pKa2	Acid pKa1	Acid pKa2
0.02	-0.05	-0.26	+0.05	+0.26
0.05	-0.07	-0.37	+0.07	+0.37
0.1	-0.09	-0.47	+0.09	+0.47
0.2	-0.12	-0.60	+0.12	+0.60

Example
Bis-Tris has a listed pKa of 6.46 at a concentration of 0.1 M. This is a base with only one pKa. Using the table above we find that 6.37 should give more accurate results (6.46 - 0.09 = 6.37).

Zwitter ions

Using zwitter ions in a BufferPrep recipe can be difficult. All zwitter ions have at least two pKa values. Since BufferPrep has to know all pKa values for the buffer component, do not use a zwitter ionic buffer component if the pKa values are not known. The second important issue is to know in which form the component is, i.e. if the molecule contains acidic protons.

Example

These examples can be used as a template for zwitter ions:

Bicine	pKa1 = 1.84	dpKa1/dT = value unknown, insert 0
	pKa2 = 8.33	dpKa2/dT = -0.017
	number of acidic protons = 1	
	charge of deprotonated ion = –1	

HEPES	pKa1 ~ 3	dpKa1/dT = value unknown, insert 0
	pKa2 = 7.39	dpKa2/dT = -0.014
	number of acidic protons = 1	
	charge of deprotonated ion = –1	

Cold room

If the dpKa/dT values are correct, there is no problem using BufferPrep in a cold room. To fine tune the AIEX and CIEX recipes, use the following correction factors:

AIEX at 5 °C	0% B = +0.10	100% B = 0.00
CIEX at 5 °C	0% B = 0.00	100% B = –0.40

C.6   Receipe overview

Data for pre-programmed AIEX recipes

Gradient 0.0–1.0 M NaCl

Buffer	Titrate with	~ pH range	Default at 0%B	correction 100% B	Start conductivity low - high pH

5.0-9.5 pH AIEX mixture
  0.05 M 1-methyl-piperazine
  0.05 M Bis-Tris
  0.025 M Tris
	0.1 M HCl	5.0 - 9.5	0.0	0.0	3.2 - 0.8 mS/cm

6.0-9.0 pH AIEX mixture
  0.07 M Bis-Tris
  0.05 M Tris
	0.1 M HCl	6.0 - 9.0	0.0	0.0	2.5 - 0.4 mS/cm

0.1 M Bis-Tris	0.1 M HCl	5.8 - 7.7	+0.2	0.0	2.2 - 0.4 mS/cm
0.1 M 1-methyl-piperazine	0.1 M HCl	5.0 - 5.7 8.4 - 10.3	0.0 -0.1	-0.1 -0.2	2.4 - 0.6 mS/cm 3.3 - 2.8 mS/cm
0.1 M Piperazine	0.1 M HCl	6.0 - 6.7 9.2 - 10.5	-0.1 -0.2	-0.3 -0.4	2.4 - 1.1 mS/cm 3.4 - 2.9 mS/cm
0.1 M Tris	0.1 M HCl	7.5 - 8.5	0.0	-0.2	2.4 - 1.3 mS/cm

	2	3	4	5	6	7	8	9	10	11	12
5.0–9.5 pH AIEX				5 ——————————— 9.5							
6.0–9.0 pH CIEX					6 —————————9						
1-methyl-piperazine				5 — 5.7							
Bis-Tris				5.8 ——————7.7							
Piperazine				6 — 6.7							
Tris						7.5 —— 8.5					
1-methyl-piperazine							8.4 ———— 10.3				
Piperazine								9.2 —— 10.5			
	2	3	4	5	6	7	8	9	10	11	12
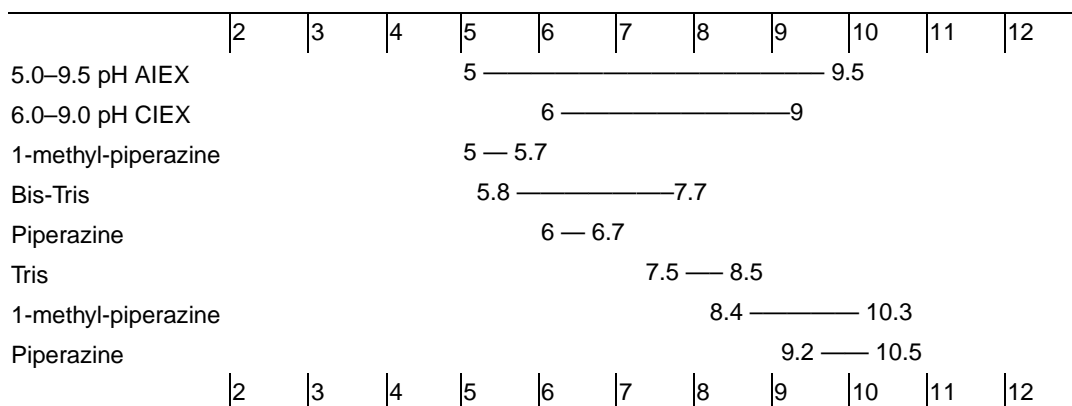
Data for pre-programmed CIEX recipes

Gradient 0.0 -1.0 M NaCl

Buffer	Titrate with	~ pH range	Default connection at 0%B	100% B	Start conductivity low - high pH
3.0-7.5 pH CIEX 0.03 M Phosphate Na_2HPO_4 0.03 M Formate Na 0.06 M Acetate Na	0.1 M HCl	3.0 - 7.5	0.0	-0.2	4.2 - 5.7 mS/cm
0.1 M Acetate Na	0.1 M HCl	3.8 - 5.7	0.0	-0.1	3.2 - 3.8mS/cm
0.1 M Bicine	0.1 M NaOH	7.0 - 9.0	+0.1	0.0	0.4 - 1.7 mS/cm
0.03 M Citrate Na3	0.1 M HCl	2.5 - 6.2	0.0	-0.2	4.2 - 3.0mS/cm
0.1 M Formate Na	0.1 M HCl	2.5 - 4.5	0.0	-0.2	4.4 - 3.5 mS/cm
0.1 M HEPES	0.1 M NaOH	6.6 - 8.2	+0.2	+0.3	0.6 - 1.6 mS/cm
0.1 M MES	0.1 M NaOH	5.5 - 6.7	+0.1	+0.1	0.7 - 1.6 mS/cm
0.03 M Phosphate Na_2HPO_4	0.1 M HCl	2.2 - 3.3 6.2 - 7.6	0.0 0.0	-0.3 -0.3	2.4 - 2.6 mS/cm 5.0 - 2.6 mS/cm

	2	3	4	5	6	7	8	9	10	11	12
3.0-7.5 pH CIEX		3.0 ——————————— 7.5									
Phosphate	2.2 —— 3.3										
Formate	2.5 ————— 4.5										
Citrate	2.5 ——————— 6.2										
Acetate	3.8 ————— 5.7										
MES	5.5 —— 6.7										
Phosphate	6.2 ———— 8.2										
HEPES	6.6 ———— 8.2										
Bicine	7.0 ———— 9.0										
	2	3	4	5	6	7	8	9	10	11	12
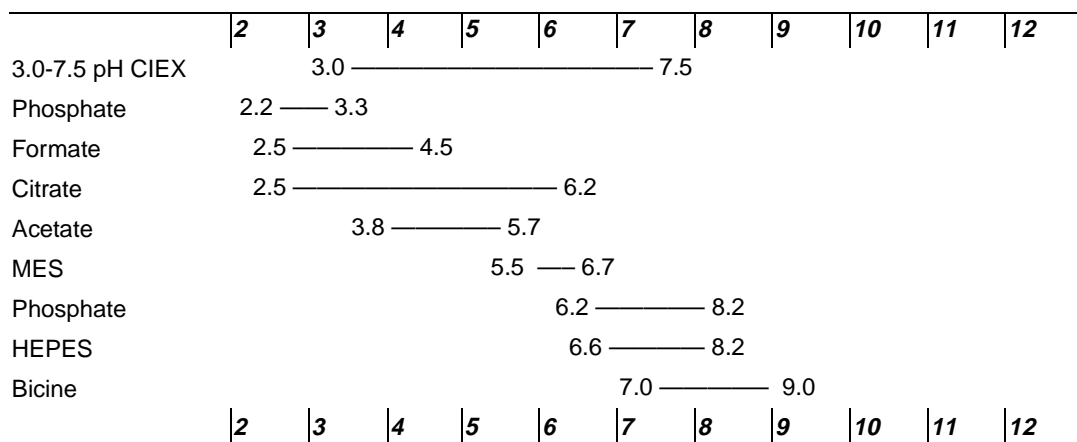
D   Technical specifications

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

Listed below are the relevant system specifications.

D.1   Operating data

Pump P-901

Flow rate range	
isocratic mode	0.01–100 ml/min in steps of 10 µl/min
gradient mode	0.01–100 ml/min in steps of 10 µl/min
Pressure range	0–10 MPa (100 bar, 1450 psi)
pH stability range	1–13, 1–14 (< 1 day exposure)
Viscosity	Max. 5 cP
Flow rate accuracy	
0.2–10.0 MPa	±2% or 20 µl/min whichever is greater
Flow rate reproducibility	
> 0.5 ml/min	rsd < 0.5%
Gradient composition	
accuracy	< ±1% at 0.5–100 ml/min
reproducibility	rsd < 0.25% at 0.5–100 ml/min
Internal volume	< 1800 µl/pump module

Pump P-903

Flow rate range	
isocratic mode	0.001–10 ml/min in steps of 10 µl/min
gradient mode	0.001–10 ml/min in steps of 10 µl/min
double mode	0.001–20 ml/min in steps of 10 µl/min
Pressure range	0–25 MPa (250 bar, 3625 psi)
pH stability range	1–13, 1–14 (< 1 day exposure)
Viscosity	Max. 5 cP
Flow rate accuracy	
0.2–25.0 MPa	±2% or 20 µl/min whichever is greater, with compression compensation activated
Flow rate reproducibility	
> 0.05 ml/min	rsd < 0.5%
Gradient composition	
accuracy	< ±1% at 0.05–10 ml/min
reproducibility	rsd < 0.25% at 0.05–10 ml/min
Internal volume	< 600 µl/pump module

Monitor UV-900

Wavelength range	190-700 nm in step of 1 nm, 3 wavelengths simultaneously
Bandwidth	4 nm
Wavelength accuracy	± 2 nm
Wavelength reproducibility	± 0.01 nm
Wavelength switch time	< 500 ms (one cycle from 214 nm to 254 nm and back to 214)
Linearity	< 2% deviation up to 2 AU at 260 nm with Uracil at pH 2
Noise[1] (at 230 nm)	< 6×10^{-5} AU, with 10 mm cell, H_2O at 1 ml/min
Drift[1] (at 254 nm)	< 2×10^{-4} AU/h
Flow cell	
Max. flow rate	100 ml/min
Max. pressure	2 MPa (20 bar, 290 psi)

[1] *Typical values at room temperature after warm/up*

Conductivity unit

Conductivity range	1 µS/cm–999.9 mS/cm
Deviation from theoretical conductivity	Max. ± 2% of full scale calibration range or ± 10 µS/cm whichever is greater in the range 1 µS/cm–300 mS/cm
Reproducibility	Max. ± 1% maximum or ± 5 µS/cm whichever is greater in the range 1 µS/cm–300 mS/cm
Noise	Max. ± 0.5% of full scale calibrated range
Flow cell	
Max. flow rate	100 ml/min
Max. pressure	5 MPa (50 bar, 725 psi)

pH unit (if applicable)

pH range	0 to 14 (spec. valid between 2 and 12)
Accuracy	± 0.1 pH unit, temperature compensated within +4 to +40 °C
Long term drift	Max 0.1 pH units deviation/10 h
Flow cell	
Max. flow rate	100 ml/min
Max. pressure	0.5 MPa (5 bar, 72 psi)

Sample Pump P-950

Flow rate range	0.1–50 ml/min in steps of 0.1 ml/min
Pressure range	1.0 MPa (10 bar, 145 psi)

D.2 Physical data

Degree of protection	IP 20
Power requirement	100–120/220–240 V ~, 50–60 Hz
Power consumption	370 VA
Fuse specification	T 6.3 AL
Dimensions, H x W x D	450 x 480 x 610 mm
Weight	66.8 kg
EMC standards	This product meets the requirements of the EMC Directive 89/336/EEC through the harmonized standard EN 61326-1 (emission and immunity). Note: The declaration of conformity is valid for the instrument if it is: • used in laboratory locations • used in the same state as it was delivered from Amersham Pharmacia Biotech except for alterations described in the User Manual • used as a "stand alone" unit or connected to other CE labelled Amersham Pharmacia Biotech modules or other products as recommended.
Safety standards	This product meets the requirement of the Low Voltage Directive (LVD) 73/23/EEC through the harmonized standard EN 61010-1.
Environment	+4 to +40 °C, 10–95% relative humidity (non-condensing), 84–106 kPa (840–1060 mbar atmospheric pressure).

D.3 Hardware requirements

- Compaq™ PC, Pentium II/333 MHz or later
  (minimum Pentium/90 MHz

- 64 Mb RAM (minimum 32 Mb) for one system
  128 Mb RAM (minimum 64 Mb) for two or more systems

- 1 Gb of available hard disk space, NTFS file system (minimum
  150 Mb)

- Colour monitor, 1024 x 768 pixels (minimum 800 x 600), small
  fonts, 64k colours

- 1 ISA slot per connected system

- CD-ROM drive

- 1.44 Mb (3.5") diskette drive

- Mouse

- Supported printers:
  HP DeskJet 660C
  HP DeskJet 690C
  HP DeskJet 870Cxi
  HP DeskJet 895 C
  HP DeskJet 2500 C
  HP LaserJet 4M
  HP LaserJet 5MP
  HP LaserJet 4000 N

D.4 Software requirements

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

D.5 Network requirements

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

- Supported network cards:
  3COM Etherlink III
  Compaq Netelligent 10/100 TX Embedded UTP Controller
  Compaq Integrated NetFlex-3 Controller
  AMD PCNET PCI Ethernet Adapter (Integrated)

- Novell® NetWare® version 4.50.189 or later, or
  Microsoft Windows NT Server 4.0.
  The UNICORN software works on earlier versions as well even
  though some versions of the Novell NetWare driver have known
  problems.

- A valid network connection

D.6 ÄKTAexplorer component materials

The wetted materials of ÄKTAexplorer are listed below:

	FFKM	PEEK	PTFE	FEP	ETFE	CTFE	ECTFE	PP	PVDF	PE	Titanium alloy	Quartz	Glass	Gold	Alum. oxide	Stainl. st. (Elgiloy)	Ruby/ sapphire	Kalrez	Simriz	Ceramic
Pump P-900	X	X							X	X	X				X	X	X			X
Monitor UV-900		X	X	X	X						X	X								
Monitor pH/C-900	X	X	X	X		X					X		X							
Mixer M-925	X	X	X	X	X						X									
INV-907	X	X	X																	
IV/PV-908	X	X	X																	
SV/FV-903	X	X	X																	
Pump P-950	X	X	X						X	X	X							X		
On-line filter	X	X	X					X												
Flow restrictor	X	X	X	X			X							X						
Tubing	X	X	X		X															
Inlet filters		X	X					X	X		X									
Unions/ Connectors	X	X	X		X															

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

E   Chemical resistance guide and chemical compatibility

The chemical resistance of ÄKTAexplorer 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 < 1 day	Exposure up to 2 months	Comments
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, CTFE, PP and PE are affected by long term use
Cyclohexane	OK	OK	
Detergents	OK	OK	
Dimethyl sulphoxide	Avoid	Avoid	PVDF is affected by long term use
1, 4-Dioxane	Avoid	Avoid	ETFE, PP, PE and PVDF are affected by long term use
Ethanol	OK	OK	
Ethyl acetate	OK	Avoid	Silicone not resistant. Pressure limit for PEEK decreases.
Ethylene glycol	OK	OK	
Formic acid	OK	OK	Silicone not resistant
Glycerol	OK	OK	
Guanidinium hydrochloride	OK	OK	
Hexane	OK	Avoid	Silicone not resistant. Pressure limit for PEEK decreases.
Hydrochloric acid, 0.1 M	OK	OK	Silicone not resistant
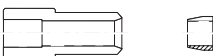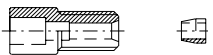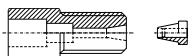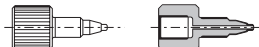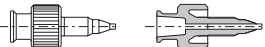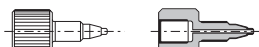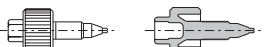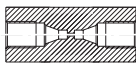
Chemical	Exposure < 1 day	Exposure up to 2 months	Comments
Hydrochloric acid, > 0.1 M	OK	Avoid	Silicone not resistant. Titanium is affected by long term use
Isopropanol	OK	OK	
Methanol	OK	OK	
Nitric acid, diluted	OK	Avoid	Silicone not resistant
Nitric acid, 30%	Avoid	Avoid	Elgiloy is affected by long term use
Phosphoric acid, 10%	OK	Avoid	Titanium, aluminium oxide and glass 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	ETFE, CTFE, PP and PE are not resistant
Toluene	OK	Avoid	Pressure limit for PEEK decreases
Trichloroacetic acid, 1%	OK	OK	
Trifluoroacetic acid, 1%	OK	OK	
Urea	OK	OK	
o-Xylene p-Xylene	OK	Avoid	PP and PE are affected by long term use

F Accessories, spare parts and consumables

Item	Quantity per pack	Code no.
Pump P-901		
Pump P-901	1	18-1108-56
Seal kit, 100 ml, including 2 piston seals 2 rinse membranes	1	18-1113-12
Piston kit, 100 ml	1	18-1112-13
Tubing kit for rinsing system	1	18-1113-32
Purge kit	2	18-1124-53
Pump head capillaries, 100 ml (capillaries (2) for one pump module)	1	18-1117-52
Rinsing/draining housing	1	18-1112-03
Pump head, 100 ml complete	1	18-1128-48
Purge valve	1	18-1128-87
O–ring for purge valve	10	19-0036-01
Check valve kit, including 1 inlet check valve 1 outlet check valve	1	18-1128-66
Pump P-903		
Pump P-903	1	18-3100-00
Seal kit, 10 ml, including 4 piston seals 4 rinse membranes	1	18-1120-77
Piston kit, 10 ml	1	18-1120-75
Tubing kit for rinsing system	1	18-1113-32
Purge kit	2	18-1124-53
Pump head capillaries, 10 ml	1	18-1120-81
Rinsing/draining housing, 10 ml	1	18-1120-76
Pump head, 10 ml complete	1	18-1128-47
Purge valve	1	18-1128-87
O–ring for purge valve	10	19-0036-01
Check valve kit, including 1 inlet check valve 1 outlet check valve	1	18-1128-66

Item	Quantity per pack	Code no.
Monitor pH/C-900		
Monitor pH/C-900 complete but without pH electrode	1	18-1107-76
pH electrode, round tip, including flow cell and holder	1	18-1134-84
pH electrode, round tip	1	18-1111-26
pH flow cell, round tip, including dummy electrode	1	18-1112-92
Dummy electrode, round tip	1	18-1111-03
Conductivity flow cell	1	18-1111-05
Monitor UV-900		
Monitor UV-900 complete but without flow cells	1	18-1108-35
Flow cell UV-900, 2 mm	1	18-1111-10
Flow cell UV-900, 10 mm	1	18-1111-11
Fibre detachment tool	1	18-1111-16
Mixer M-925		
Mixer M-925 including one UniNet cable	1	18-1118-89
Mixing chambers:		
0.6 ml	1	18-1118-90
2 ml	1	18-1118-91
5 ml	1	18-1118-92
12 ml	1	18-1118-93
Pump P-950		
Pump P-950, complete, including one UniNet cable	1	18-6083-01
Mounting kit P-950	1	18-1142-24
Valves IV-908, PV-908		
Valve IV-908 including one UniNet cable	1	18-1108-42
Valve PV-908 including one UniNet cable	1	18-1108-41
Valve kit, including channel plate and distribution plate		
IV-908	1	18-1109-07
PV-908	1	18-1109-06
Number Plates 0–9	1	18-1109-09
Mounting bracket	1	18-1109-11

Item	Quantity per pack	Code no.
Valve INV-907		
Valve INV-907 including one UniNet cable (fill port, needle and syringe holder are not included)	1	18-1108-40
Injection kit INV-907 including fill port, needle and syringe holder	1	18-1110-89
Valve kit INV-907 including channel plate and distribution plate	1	18-1109-05
Sample loops 100 µl 500 µl 1 ml 2 ml	 1 1 1 1	 18-1113-98 18-1113-99 18-1114-01 18-1114-02
Mounting bracket	1	18-1109-11
Valve SV-903		
Valve SV-903, including mounting bracket	1	18-1114-49
Cables		
UniNet, 0.18 m	1	18-1109-72
UniNet, 0.3 m	1	18-1109-73
UniNet, 0.7 m	1	18-1109-74
UniNet, 1.5 m	1	18-1117-75
UniNet, 3.0 m	1	18-1109-75
UniNet, 15.0 m	1	18-1117-74
Mains cable, 120 V	1	19-2447-01
Mains cable, 240 V	1	19-2448-01
Signal Cable, 6 pin mini DIN-open	1	18-1110-64

Item	Quantity per pack	Code no.
Connectors and unions		
Fingertight Connector, 1/16" for 1/16" o.d. tubing, PEEK	10	18-1112-55
Tubing connector for 3/16" o.d. tubing, PEEK	10	18-1112-49
Ferrules for 3/16" o.d.tubing, PEEK	10	18-1112-48
Tubing connector for 1/8" o.d. tubing, PEEK	10	18-1121-17
Ferrules for 1/8" o.d.tubing, PEEK	10	18-1121-18
Tubing connector for 1/16" o.d. tubing, PEEK	10	18-1127-07
Ferrules for 1/16" o.d.tubing, PEEK	10	18-1127-06
Union 1/16" female/M6 male, PEEK	6	18-1112-57
Union M6 female/1/16" male, PEEK	8	18-1112-58
Union Luer female/1/16" male, PEEK	2	18-1112-51
Union 5/16"–32 female/HPLC male, PEEK	8	16-1142-08
Stop plug, 1/16", PEEK	5	18-1112-52
Stop plug, 5/16", PEEK	5	18-1112-50
Union, 1/16" female/1/16" female, titanium	1	18-3855-01
Union Luer male/M6 female	1	18-1027-12
Tubing connector/M6 male	1	18-1017-98

Item	Quantity per pack	Code no.
Tubing		
Inlet Filter assembly, 1 net, 1 filter	1	18-1113-15
Inlet filter set, 10 nets and 10 filters	10	18-1114-42
PEEK tubing, i.d. 0.25 mm, o.d. 1/16"	2 m	18-1120-95
PEEK tubing, i.d. 0.50 mm, o.d. 1/16"	2 m	18-1113-68
PEEK tubing, i.d. 0.75 mm, o.d. 1/16"	2 m	18-1112-53
PEEK tubing, i.d. 1.0 mm, o.d. 1/16"	2 m	18-1115-83
Tefzel tubing, i.d. 0.75 mm, o.d. 1/16"	2 m	18-1119-74
Teflon tubing, i.d. 1.6 mm, o.d. 1/8"	3 m	18-1121-16
Teflon tubing, i.d. 2.9 mm, o.d. 3/16"	3 m	18-1112-47
Tubing Kit[1], i.d. 0.25 mm, PEEK	1	18-1122-12
Tubing Kit[1], i.d. 0.50 mm, PEEK	1	18-1123-21
Tubing Kit[2], i.d. 0.5 mm, o.d. 1/16"	1	18-1121-64
Tubing Kit[2], i.d. 0.75 mm, o.d. 1/16"	1	18-1122-14
Tubing Kit[2], i.d. 1.0 mm, o.d. 1/16"	1	18-1121-65
Sample Tubing Kit, 1.0 mm, including tubing (2), i.d. 1.0 mm length 1250 mm and two connectors and 2 ferrules	1	18-1115-77

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[1] Contains tubing as listed in table in section *A.6 Changing tubing kits – ÄKTAexplorer 10*.

[2] Contains tubing as listed in table in section *A.7 Changing tubing kits – ÄKTAexplorer 100*.

Item	Quantity per pack	Code no.
Accessories		
On-Line filter, (10 ml systems)	1	18-1118-01
On-Line filter kit, (10 ml systems) 2 filters	2	18-1120-94
On-Line filter, (100 ml systems)	1	18-1112-44
On-Line filter kit, (100 ml systems) 10 filters and 2 nets	10	18-1027-11
On-Line filters, (100 ml systems) 25 filters	25	18-1130-23
Short column holder	1	18-1113-17
Column Holder, for up to six small columns	1	18-1113-18
Tubing Cutter	1	18-1112-46
Flow Restrictor, FR-904, 0.4 MPa	1	18-1119-63
Flow Restrictor, FR-902, 0.2 MPa	1	18-1121-35
Lab rod holder	1	18-1113-19
Sample Holder, SH-900, including 1 Sample Tray, 2 Sample Tube Holders, 1 Sample Tubing Holder, and 1 Mounting plate	1	18-1110-78

Item	Quantity per pack	Code no.
User Manuals		
ÄKTAexplorer User Manuals and Instructions (Box containing User Manuals/Instructions for all components in ÄKTAexplorer)		18-1141-01
ÄKTAexplorer Installation Guide		18-1139-59
Making your first runs		18-1140-78
UNICORN version 3.10 User Manual		18-1134-58
UNICORN version 3.20 Frac-950 module for UNICORN applications		18-1138-53
ÄKTAexplorer System Manual		18-1140-45
ÄKTAexplorer Method Handbook		18-1124-23
Pump P-900 User Manual		18-1120-04
Monitor UV-900 User Manual		18-1120-05
Monitor pH/C-900 User Manual		18-1120-06
Short Instruction Pump P-900		18-1120-08
Short Instruction Monitor UV-900		18-1120-09
Short Instruction Monitor pH/C-900		18-1120-10

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