ÄKTA design

ÄKTAxpress

User Manual

18-1178-00

Amersham Biosciences

Table of Contents

1 Preface

About this chapter

This chapter contains regulatory, contact and safety information.

In this chapter

This chapter contains the following sections

1.1 Important user information

Important user information

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

Warning symbols

The following warning symbol is used in the documentation.

> **WARNING!**
> The Warning symbol highlights instructions that must be followed to avoid personal injury. Do not proceed until all stated conditions are clearly understood and met.

Caution notices

The following caution notice is used in the documentation.

> **CAUTION!**
> The Caution sign highlights instructions that must be followed to avoid damage to the product or other equipment. Do not proceed until all stated conditions are clearly understood and met.

Note signs

The following note sign is used in the documentation.

Note: *The Note sign indicates information important for trouble free and optimal use of the product.*

Comments on manual

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

Amersham Biosciences AB, SE-751 84 Uppsala, Sweden

Copyright

1.2 Product information

CE certification

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

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

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

Class A product

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

Terms and conditions of sale

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HiTrap Chelating HP Purification and preparation of fusion proteins and affinity peptides comprising at least two adjacent histidine residues may require a license under US pat 6,284,933 and US pat 5,310,663, including corresponding foreign patents (assignee: Hoffman La Roche, Inc).

Office addresses

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1.3 Safety

Read the Safety Handbook

Read the safety warnings and cautions before starting the installation.

The warnings are available in English, German, Spanish, French, Italian and Swedish in the *ÄKTAxpress Safety Handbook*.

2 Introduction

About this chapter

This chapter contains an introduction to ÄKTAxpress™ and the basic operating principles. It also gives a background to multi step purification of recombinant proteins, and general information about the user documentation.

In this chapter

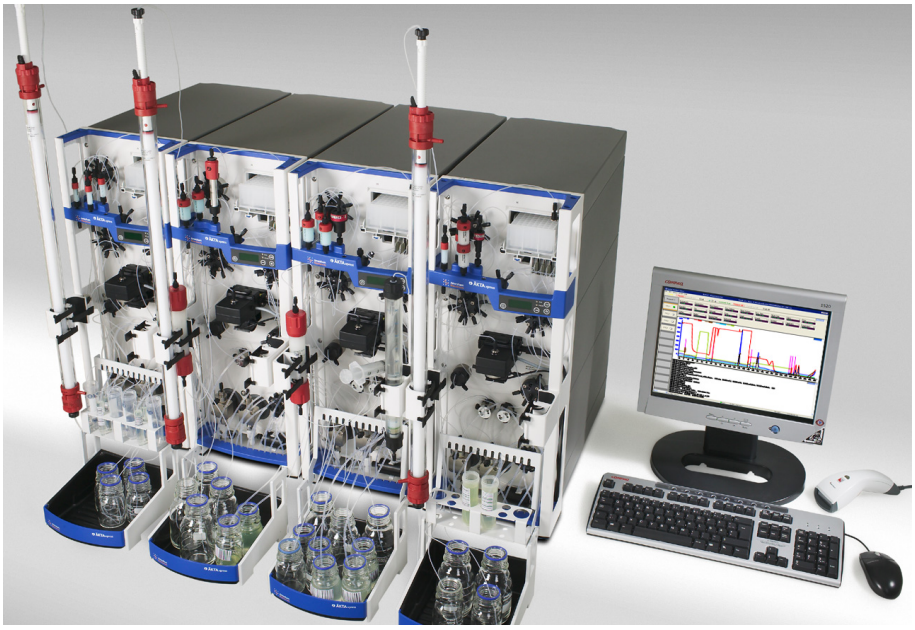
This chapter contains the following sections

2.1 Introducing ÄKTAxpress

Introduction

This section gives a general overview of ÄKTAxpress.

ÄKTAxpress



ÄKTAxpress is a liquid chromatography system intended for automated, multi step purification processes. The system has been developed and optimized for purification of His-tagged and Glutathione S-Transferase (GST)-tagged recombinant proteins from clarified cell lysates. It supports a number of purification protocols containing up to four chromatography steps, for example, affinity chromatography followed by desalting, ion exchange, and gel filtration. All supported multi step protocols include tag cleavage as an option.

The standard delivery of ÄKTAxpress includes four separation systems as shown in the illustration above. The flask holder is optional.

ÄKTAxpress features

ÄKTAxpress provides the following features:

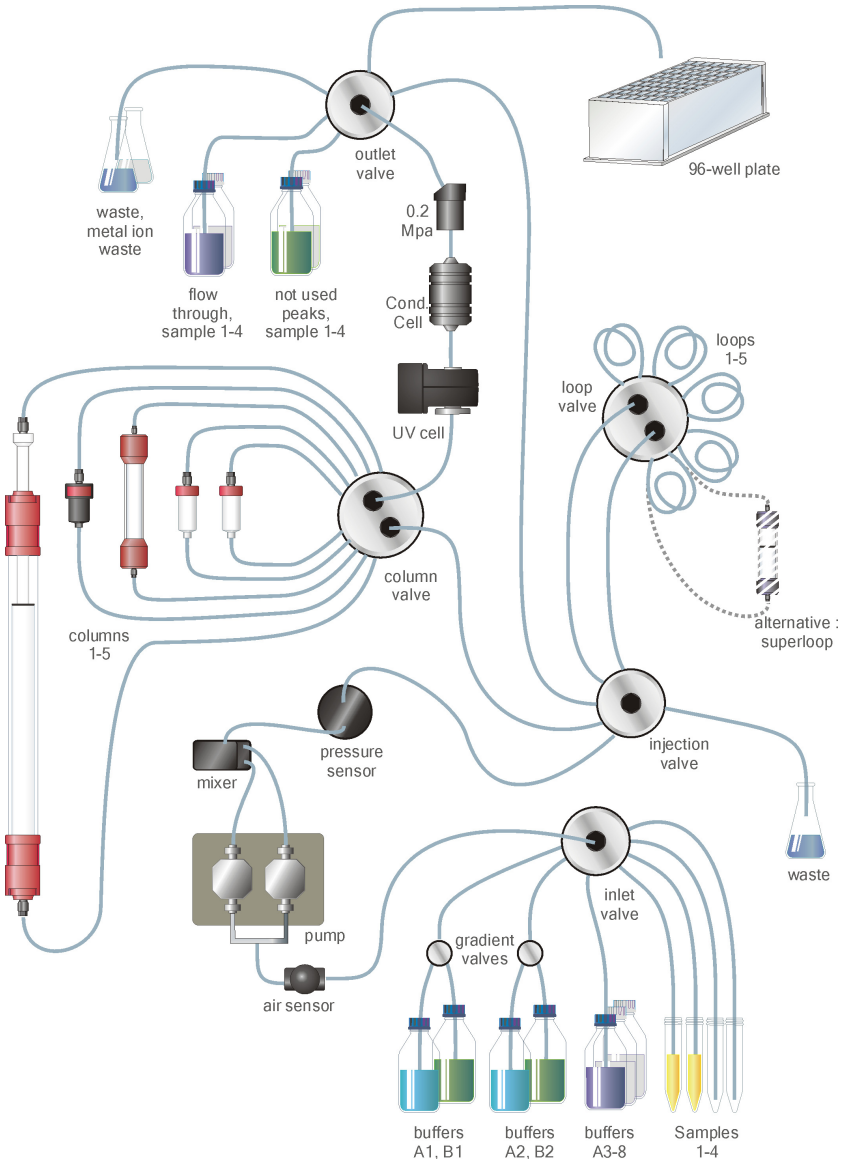
- A Method Wizard to make it easy to create method plans.
- Automatic purification of up to four samples in a single run.
- Purification protocols containing up to four chromatography steps.
- Automated affinity tag removal in combination with any multi step purification protocol supported by ÄKTAxpress.
- Up to 50 mg of protein can be purified per sample.
- Up to twelve ÄKTAxpress system can be run in parallel, controlled from one computer. Different purification protocols can be run simultaneously.
- The systems can be placed next to each other or they can be separated if preferred.

The separation system

ÄKTAxpress comprises a number of separation systems and a computer with monitor running the control and analysis software.

The separation system is a compact chromatography unit containing the components required for automated, multi step purification processes.

The illustration below is a schematic drawing showing the components in the flow path.



The main components are as follows:

- Combined sample and buffer pump.
- Switch valves for buffer selection and gradient formation.
- Separate rotary valves for sample- and buffer selection, manual injection, column selection, loop selection, and outlet flow direction.
- UV and conductivity cells.

- Loops for intermediate fraction collection.
- Fraction collector with deep-well microplate.

The columns required for the chosen purification can be attached to the system either by threading them into the column block (small columns) or by securing them with snap locks in the column holder (large columns).

The separation system is equipped with a control panel with push-buttons and display. The push buttons allow the operator to start and pause a run. The display indicates running status, identity of the system, error codes, etc.

The UNICORN control system

UNICORN™ is a complete package for control and supervision of ÄKTAxpress. It consists of software for interfacing the controlling computer to the separation system via the USB/CAN converter box supplied. The software runs under Microsoft® Windows®.

The Method Wizard in UNICORN provides easy creation of method plans for purification, and preparation and maintenance of the separation system and columns.

Before a run, the operator chooses purification method plan and the number of samples for each separation system. The systems can then be started separately or simultaneously from UNICORN, or from the control panel on each system.

The illustration below shows the System Control module in UNICORN when using four separation systems.

Several purification protocols in parallel

A single separation system can handle one purification protocol at a time. The standard ÄKTAxpress delivery package includes four systems, thus allowing up to four different purification protocols to be run simultaneously. Totally, UNICORN provides individual and simultaneous control of up to twelve separation systems by one single computer.

The individual systems can also be separated from each other, thus allowing operation in cold room and room temperature in parallel.

Required installations

ÄKTAxpress separation system and the UNICORN computer should be installed and tested by Amersham Biosciences personnel.

2.2 Purification of recombinant proteins

Introduction

This section introduces the reader to purification of recombinant proteins, and thus gives a background to the development of ÄKTAxpress.

Protein purification

Success in isolation and purification of a target protein is highly based on the knowledge of the protein characteristics. Protein purification serves to deliver a suitable amount of pure target protein in its native biological state for use in further studies. The requirements on the end product may vary considerably. For instance, a certain enzyme may be considered pure enough for activity studies at a relatively moderate mass purity, provided that the end product is free of substances interfering with the activity of the enzyme. Pure enough for structure analysis of a protein, on the other hand, normally means a rather high mass purity, since interfering substances may affect the techniques used in structure determinations. The purification itself is nearly never a final goal, but rather a necessary step on the way to perform protein research in many different scientific areas.

Multi step purification of recombinant proteins

The need to produce pure target proteins quickly and easily has increased the usage of recombinant proteins greatly in recent years. So has also the wealth of techniques and products used for their amplification and purification.

The advantage of using a fusion protein to facilitate purification of a recombinant protein is widely recognized. Fusion proteins are simple and convenient to work with and, for many applications, a single step purification using affinity chromatography is sufficient to achieve the desired level of purity. However, if there is no suitable fusion system, or when higher degree of purity is required, a multi step purification process will be necessary.

ÄKTAxpress for multi step purification

ÄKTAxpress has been developed to meet the growing demand for quick and easy purification of recombinant proteins with high purity. The system is based on purification strategies that involve combinations of protein capture on affinity columns, buffer exchange in desalting columns, intermediate purification in ion exchange columns, and polishing in gel filtration or ion exchange columns. Consequently, ÄKTAxpress supports a number of purification protocols containing up to four steps. The intermediate protein peaks between the steps are collected in loops.

The purification protocols in ÄKTAxpress are primarily designed for purification of the most common fusion proteins—$(His)_6$-tagged and Glutathione S-Transferase (GST)-tagged proteins. Tag removal is optional in all protocols.

Up to twelve ÄKTAxpress systems can work in parallel, where each system can handle up to four different samples through the whole purification procedure independently of the other systems. This increases the protein production throughput which is a key issue, for example, in the field of structural and functional genomics.

2.3 Basic operating principles

Introduction

This section gives a brief introduction to the function of ÄKTAxpress. It describes the main steps during a purification run as shown in the table below.

Step	Action
1	Sample application and wash
2	Elution
3	Intermediate fraction collection
4	Final fraction collection

A functional description of ÄKTAxpress and a detailed description of the steps during the purification process can be found in section **3.3 Purification process overview** on page 47.

Sample application and wash

The inlet valve selects the appropriate sample inlet. The pump then loads the sample onto the affinity column which captures the protein.

If using several samples during the run, the sample loading and the subsequent wash steps will be executed serially for all samples before elution of the first protein starts. Each sample is therefore loaded onto its own affinity column and the captured proteins will be stored on the columns while waiting for the elution step.

Elution

The inlet valve selects the appropriate buffer inlet. If a gradient is required, two buffer inlets can be selected simultaneously using a switch valve. The pump transports the buffer through the column to elute the captured protein.

Intermediate fraction collection

The eluted protein peak is detected by the UV cell. If the protein is to be further purifed in a subsequent step, the peak volume will be directed to one of the loops. The loops are used to store the intermediate fractions. The largest peak is then loaded directly from the loop onto the next column for the next purification step.

Final fraction collection

When the last purification step is finished and the eluted protein peak has been detected by the UV cell, the outlet valve directs the peak volume to the deep-well microplate in the fraction collector.

2.4 ÄKTAxpress user documentation

Introduction

This section gives an overview of the user documentation for ÄKTAxpress.

ÄKTAxpress user documentation package

The table below describes all user manuals included in ÄKTAxpress user documentation package:

User manual	Content
ÄKTAxpress User Manual	How to use the system, including concepts, methodology, operation, evaluation, troubleshooting, and maintenance. This document is also available as an on-line manual.
ÄKTAxpress Cue Cards	Short step-by-step instructions and tables for the daily work, such as creating and running a method plan, evaluating the results, preparation and maintenance of the system and columns. Video sequences are supplied showing the basics of operation.
ÄKTAxpress Installation Guide	How to prepare for the initial installation, to perform the installation, to run the installation test, and to expand an existing installation with additional systems.
ÄKTAxpress Safety Handbook	Safety instructions that must be followed for the use of ÄKTAxpress, in 6 languages: English, German, Spanish, French, Italian and Swedish.
UNICORN ÄKTAxpress User Reference Manual	Detailed instructions on the use of UNICORN. This document is also available as an on-line manual.

User manual	Content
UNICORN Administration and Technical Manual	Information for the system administrator about for example user administration, network installation and troubleshooting. This document is also available as an on-line manual.

Document structure

This manual is divided into chapters. Each chapter starts with a brief overview that presents the contents and the headings for the sections that the chapter contains. The section begins with an introduction that summarizes the content.

Each section is divided into blocks of information with separating lines. The blocks are identified by labels in the margins. This makes it easier for you to quickly scan a page to find the exact topic you are looking for.

ÄKTAxpress User Manual

The table below describes the content in each chapter in this manual.

Chapter	Contents
1. Preface	Regulatory, contact and safety information.
2. Introduction	General system overview of ÄKTAxpress and basic operating principles, background to purification of recombinant proteins, and information about the user documentation.
3. ÄKTAxpress overview	Description of ÄKTAxpress and a purification process overview.
4. Methodology	A guide to selecting purification protocol, buffers, and columns.
5. Method Wizard	Description of the Method Wizard (Prepare, Purify, and Maintenance).
6. Operation	Instructions for preparing the system for a run and performing a run on one or several systems.
7. Evaluation	Viewing and evaluating the results, printing chromatograms, and generating reports.

Chapter	Contents
8. Maintenance	Maintenance schedules and instructions for preventive maintenance and replacing spare parts.
9. Troubleshooting and corrective actions	Overview of error symptoms, possible causes, and corrective actions. Error codes.
10. Reference information	Detailed hardware description, technical and chemical specifications, ordering information.

Typographical conventions

Menu commands, field names and other text items from the software or on control buttons are quoted exactly as they appear, in a bold typeface:

- *Example*: **Run Setup**

Search paths are shown in a bold typeface with a separating colon between each level:

- *Example*: **View:Windows:Customise** (i.e. the menu command **Customise** in the sub-menu **Windows** from the **View** menu).

Text entries that UNICORN generates or that the user must type are represented by a monotype typeface:

- *Example*: `Connection change`

Prerequisites

The following prerequisites must be fulfilled before you can use this manual in the way it is intended.

- You need to have a general understanding of how the PC and Windows® work. In most cases universal computer functions will not be explained.
- UNICORN must be installed and configured correctly on the computer.
- You need to understand the basic concepts of liquid chromatography. Terminology and functionalities will be explained only when they differ from normal practice.
- Before you try to operate ÄKTAxpress you must study and understand *ÄKTAxpress Safety Handbook*.

3 ÄKTAxpress overview

About this chapter

This chapter contains an overview of the UNICORN control system and a brief physical and functional description of the components in ÄKTAxpress. It also contains an overview of the purification process steps.

In this chapter

This chapter contains the following sections

3.1 UNICORN overview

Introduction

This section is an overview of the UNICORN control system.

The UNICORN control system

UNICORN™ is a complete package for control and supervision of ÄKTAxpress. It consists of software for interfacing the controlling computer to the separation systems via the USB/CAN converter box supplied.

The UNICORN software runs under Microsoft Windows.

Software modules

UNICORN consists of four integrated modules which are described in the table below.

Module	Function
UNICORN Manager	File handling and administration.
Method Editor	To create and edit methods and method plans.
System Control	To start method plans and to control and monitor the separations processes on-line.
Evaluation	To evaluate and present stored results from the separation processes.

Method Wizard

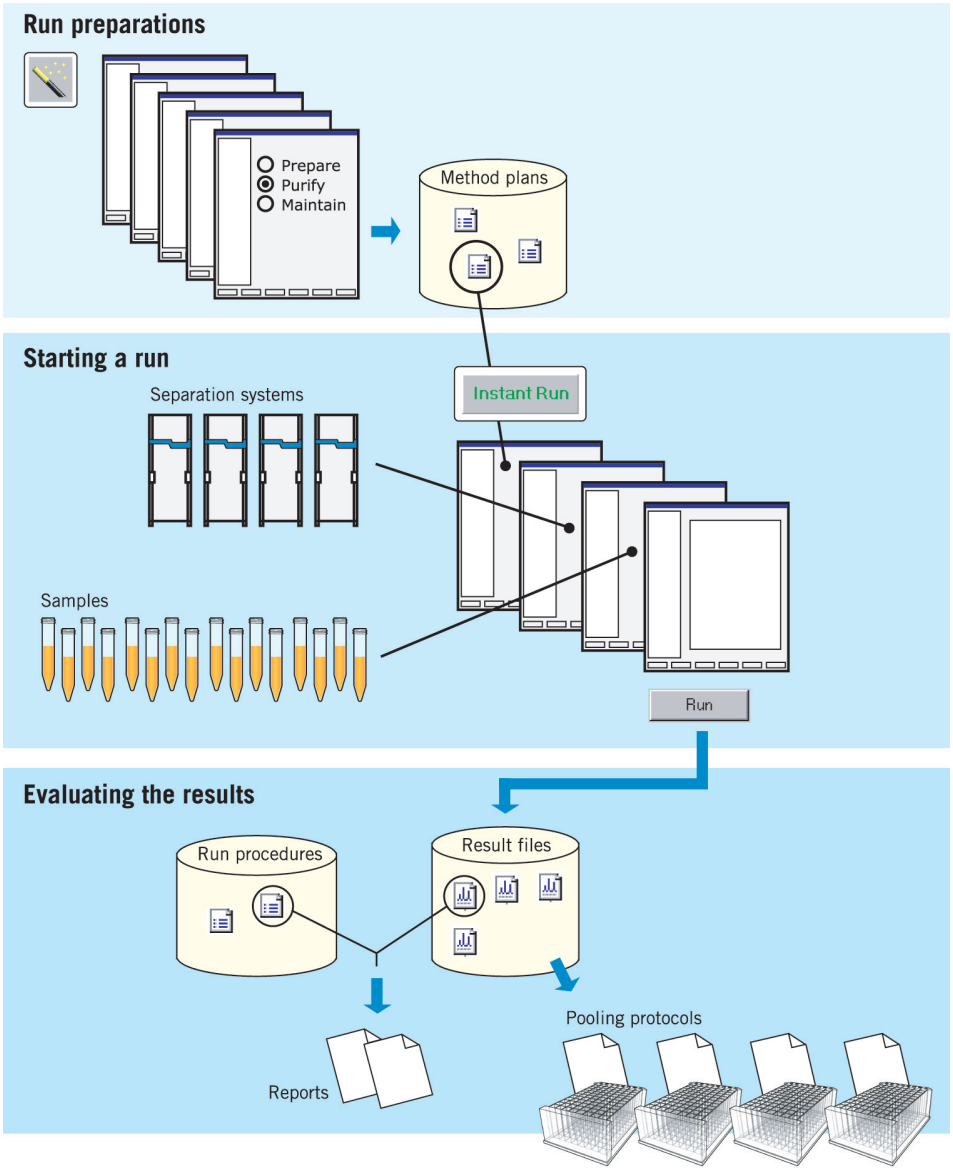
The Method Wizard included in UNICORN provides easy creation of method plans for purification, preparation and maintenance of the separation systems and columns. The Method Wizard also allows editing of stored method plans.

Before a run, the operator chooses method plan and the number of samples for each separation system. The systems can then be started separately or simultaneously from UNICORN.

For more information about the Method Wizard, see **5 Method Wizard** on page 84.

Operation overview

The step-by-step operation of the separation system is described below:



Create

A new method plan is created using the Method wizard in the Method Editor module. If required, a traditional method can also be created in the Method Editor.

Run

Method plans are started on single or multiple systems using a wizard in the System Control module. Methods can only be started on a single system. A method cannot be started using the wizard.

Evaluate

Result files are created continuously and when the run has finished, the result files can be processed using standard run procedures or own developed run procedures. Automatic pooling of fractions will be performed if single chromatograms are opened.

Help functions

The table below describes how to access the on-line help utility included in the UNICORN software:

If you want to access...	then...
the general help utility	open the **Help** menu in any of the software modules.
context specific help topics	• click the Help button in the dialog box, or • press the **F1** key on the keyboard.

3.2 Separation system overview

About this section

This section shows the location of the components in ÄKTAxpress, as well as the controls, indicators, and rating labels. It also describes the liquid flow path with a functional description of the components.

In this section

This section contains the following sub-sections

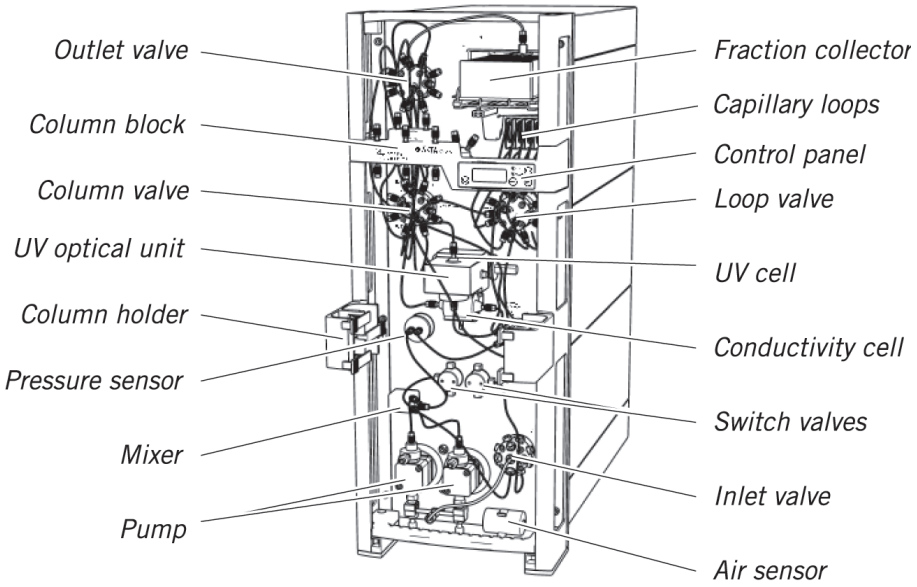
3.2.1 Location of the components

Introduction

This section contains an overview of the components in the separation system.

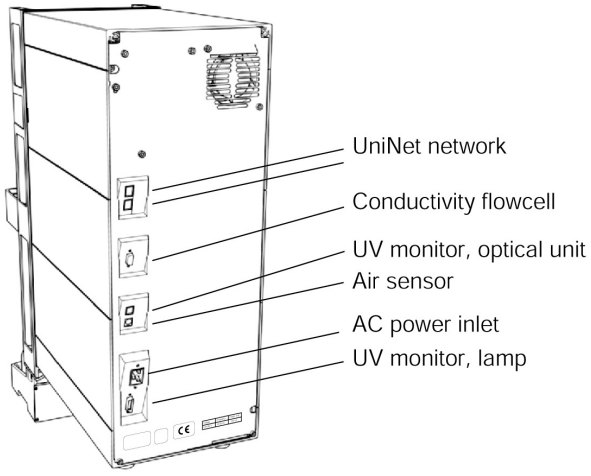
Fluid handling components

All fluid handling components are located on the front of the separation system.



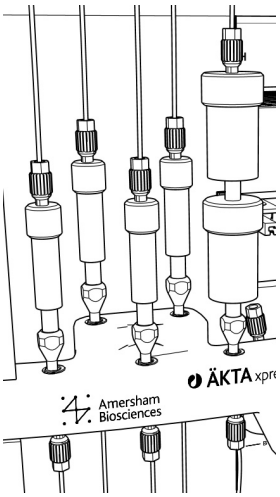
Electrical connectors

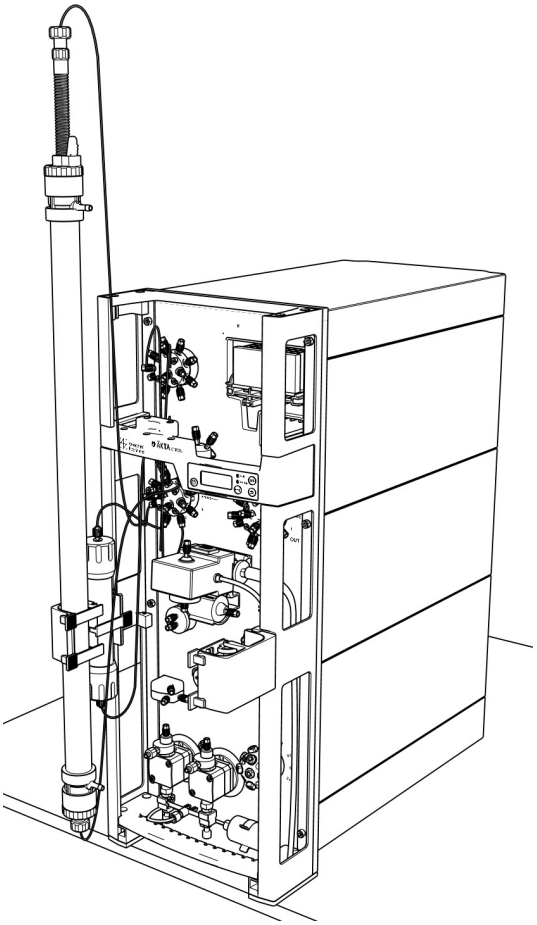
Connectors for power and communication are located on the rear of the system.

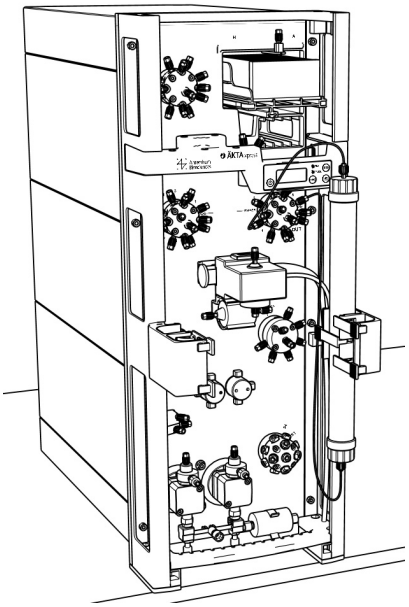


Location of columns

The columns can be attached to ÄKTAxpress in different places, depending on the size of the column:

Column type	Location
Small columns	Small columns are connected to the column block through different unions. See **6.5.4 Connecting columns and tubing** on page 163for information about the unions. The block has five ports. In some applications it is possible to use two columns connected in series. 

Column type	Location
Large columns (30 mm waist diameter)	Large columns are attached by using the *left hand* column holder with snap locks. The holder can hold two columns. 

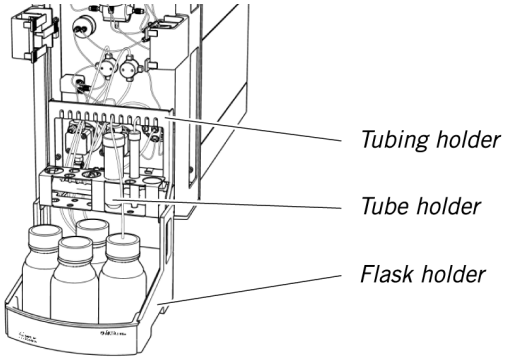
Column type	Location
Superloop	A Superloop™ can be attached in the *right hand* column holder.  ***Note:*** *Do not put gel filtration columns in the right hand side column holder. It will block the fraction collector and might get destroyed.*

Location of liquid tubes and containers

A tubing holder and tube holder are included in the system at delivery. The tubing holder is used for organizing the tubing. The tube holder can take tubes of two different sizes used for samples (S1–S4). The tubes can also be used for flow through fractionation (F3–F6) and nonselected peaks (F7–F10) if small volumes are expected.

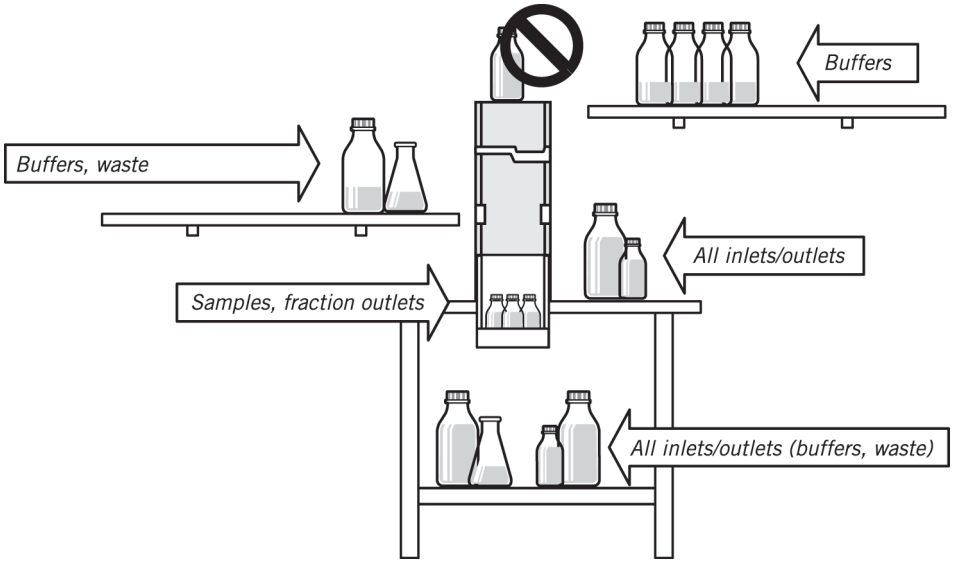
A flask holder is available as an option and can be mounted on the front of the system. The flask holder can be used for samples bottles (S1–S4), flow through fractionation bottles (F3–F6) and bottles for nonselected peaks (F7 to F10). The flask holder can take a maximum weight of 3.5 kg.

The holders can be used independently of each other.



Buffer and waste containers are usually large and may be stored directly on the floor or on suitable shelves.

Example of location of different liquid containers:

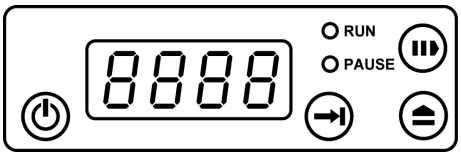


WARNING!

Never place liquid containers on the top of the separation system. If they become full and overflow, liquid may enter the system causing a short-circuit.

Control panel

The separation system is mainly controlled and monitored via UNICORN. The separation system is, though, also equipped with a control panel with controls and indicators on the horizontal front bar. The control panel allows for limited control of the system as well as information feedback.



For more information about the indicators and controls see **3.2.5 Indicators and controls** on page 43.

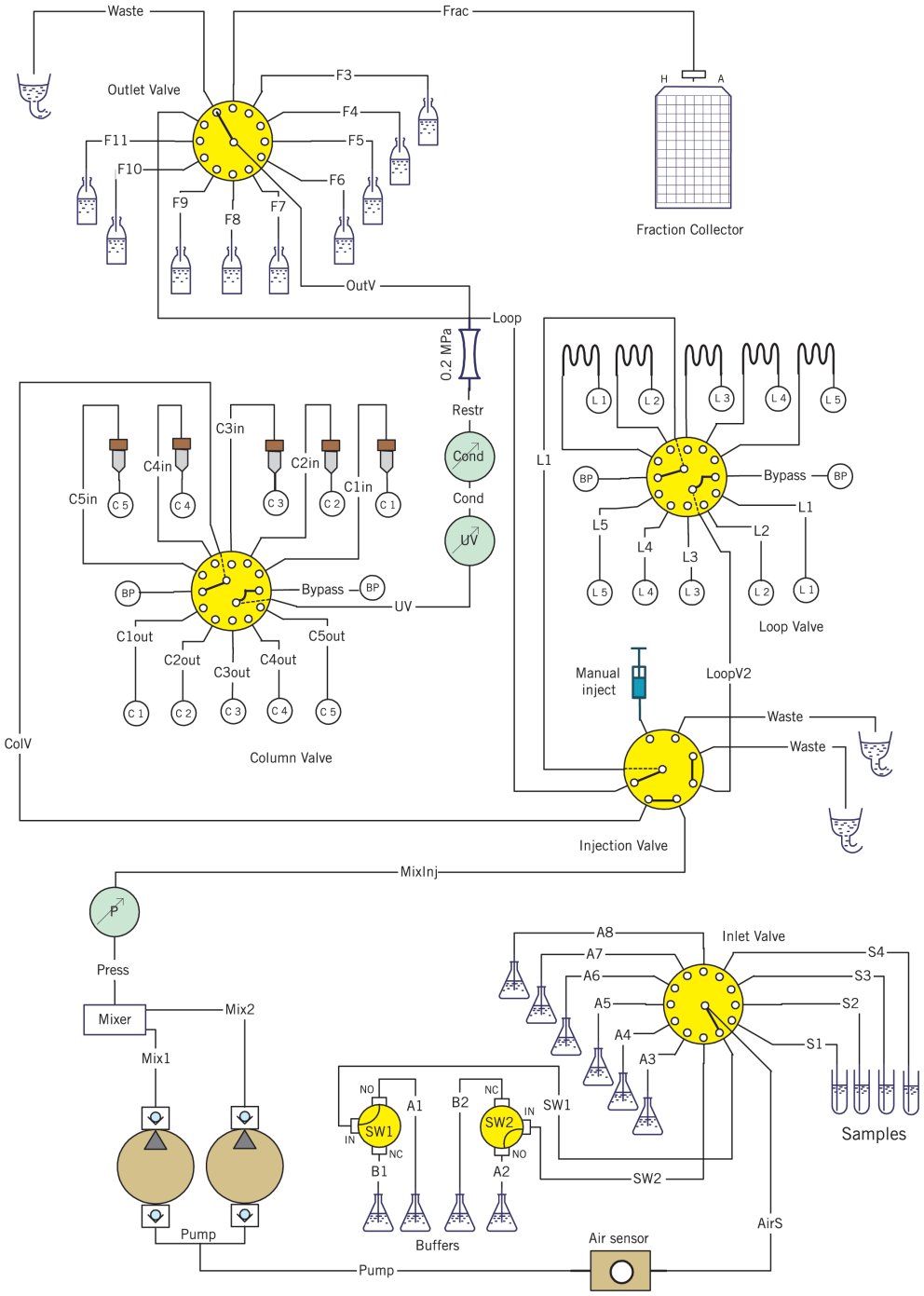
3.2.2 Liquid flow path

Introduction

This section contains an overview of the tubing and components configuration of the liquid flow path in ÄKTAxpress.

The liquid flow path

The illustration below shows the liquid flow path with the valves in the default positions.



3.2.3 Tubing and connectors

Introduction

This section describes the tubing and connectors included in the separation system.

Sample and buffer inlet tubing

All tubing located before the pump in the flow path, i.e. the *sample and buffer inlet tubing*, has inner and outer diameters (i.d. and o.d.) of 1.6 mm and 1/8" respectively. This tubing is connected with M6 fittings (not flanged, see illustration below) except for tubings connected to the pump, air sensor, and switch valves, where 5/16" fittings are used. The tubing material is Teflon®.

System and outlet tubing

All tubing located after the pump in the flow path, i.e. the *system and outlet tubing*, has i.d. and o.d. of 1.0 mm and 1/16" respectively. This tubing is connected with narrow-headed fingertight connectors. The tubing material is Tefzel®, except the outlet tubing to the fraction collector which is made of PEEK.

The system also includes stop plugs to be used in non-used ports in the valves and the column adapter.

Colored labels on the tubing

There are always a specific sample tubing (S1–S4), a flowthrough outlet tubing (F3–F6), and an outlet tubing for unselected peaks (F7–F10) dedicated for each one of the samples. These three tubings are marked with a label which has a unique color.

Sample no.	Tubing designation	Color
1	S1, F3, F7	Red
2	S2, F4, F8	Yellow
3	S3, F5, F9	Blue
4	S4, F6, F10	Green

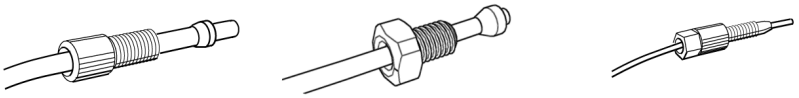
Length of inlet and outlet tubing

- The tubing for buffers (A1–A8, B1, B2), fraction outlet (F3–F11), and waste (Waste 1–3) are 1.5 m long.
- The tubing for samples (S1–S4) are 0.5 m long.

Note: *The tubing should never exceed these lengths.*

Tubing fitting types

The illustration below shows the three tubing fitting types used in ÄKTAxpress.



5/16" fitting *M6 fitting* *Narrow-headed fingertight connector*

Connecting the fittings

- When connecting an M6 or a 5/16" fitting, the orientation of the ferrule must be correct (see the illustration above).
- When connecting a fitting, the tubing must be inserted fully into the connection port before tightening the fitting.

3.2.4 Functional description

Introduction

This section briefly describes the function of the components in the liquid flow path of ÄKTAxpress.

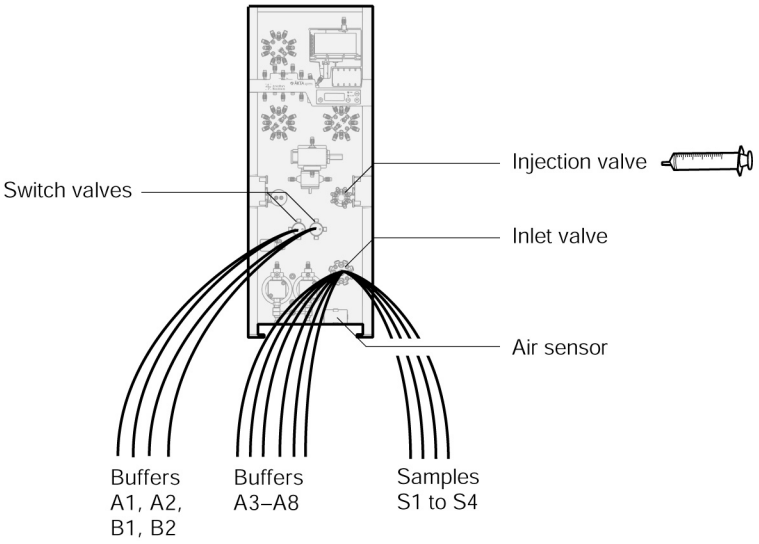
The functions of ÄKTAxpress can be divided into six categories:

- Sample and buffer handling
- Pump and related items
- Column handling
- UV and conductivity detection
- Intermediate fraction collection
- Final fraction collection

For a more detailed description of the components, see **10.1 System description** on page 308.

Sample and buffer handling

The parts for handling samples and buffers are located at the lower part of the separation system. They comprise two switch valves for buffer selection and gradient, a general inlet valve for samples and buffers, an injection valve and an air sensor.



Switch valves

The switch valves are mainly used for gradient formation. They have four buffer inlets, two on each valve, labelled A1, B1, A2, and B2.

Inlet valve

The flow from the switch valves continues to the inlet valve, which provides six additional buffer inlets, labelled A3–A8. The inlet valve also provides four sample inlet tubings, labelled S1–S4.

Injection valve

A sample volume can also be injected manually into the flow path through the injection valve using a syringe. The injection valve is located above the inlet valve.

Note: *Buffer containers, sample tubes, and other external hardware are not included at delivery.*

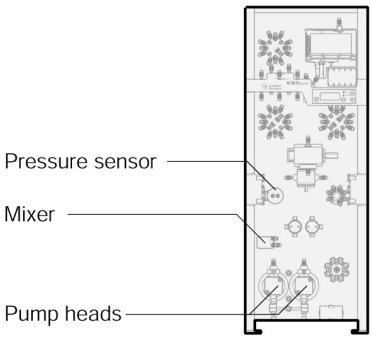
Air sensor

An air sensor is connected between the inlet valve and the pump. In method plans the air sensor is activated by default and used for:

- Detecting when the sample supply is empty during sample loading. The inlet valve then switches to a buffer inlet.
- Detecting when the buffer supply is empty during a run. The system is then set to PAUSE mode.

Pump and related items

The pump related items are located at the lower left hand side of the system.



Pump

The pump is located at the lower left-hand side of the separation system. It is a low-pulsation pump, equipped with two pump heads, which can deliver up to 65 ml/min at pressures up to 3 MPa.

The pump handles all liquid transport in the separation system.

Mixer

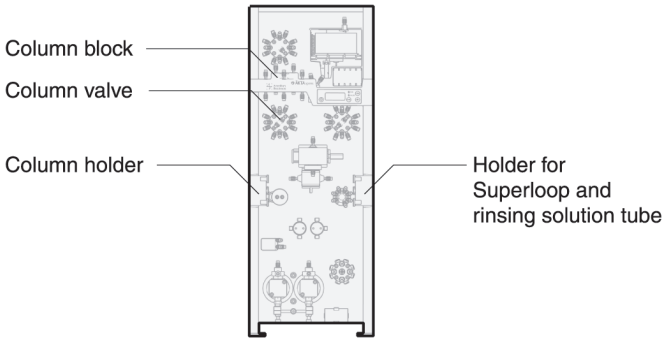
The buffers used for creating gradients are mixed in a static mixer which is located directly after the pump. The volume of the mixer chamber is 0.25 ml.

Pressure sensor

A pressure sensor is connected directly after the mixer for continuous detection of the system pressure.

Column handling

The column handling items are the column valve, and two types of column supports: a column block for small columns and column holder for larger columns.



Column valve

The column valve is located at the upper left hand side of the front panel. The valve makes it possible to select between up to five columns and a by-pass tubing.

Column block

The column block located directly above the column valve can take up to five small columns with fingertight fittings.

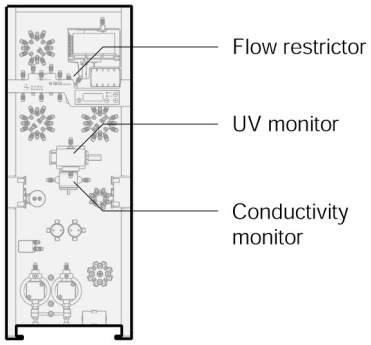
Column holder

The rail on the left hand side is equipped with snap locks for fastening up to 2 large columns while the right hand side snap locks are used for a superloop and a tube for piston seal rinsing solution.

The online filter supplied can be connected to the top of the gel filtration columns using a 1/16" male/M6 female connector.

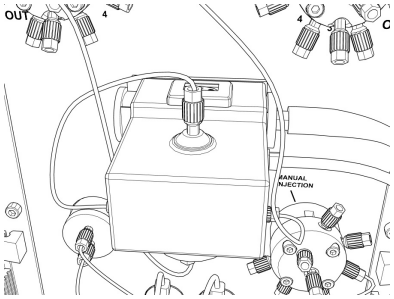
UV and conductivity detection

The UV cell and the conductivity cell are located at the middle part of the system.

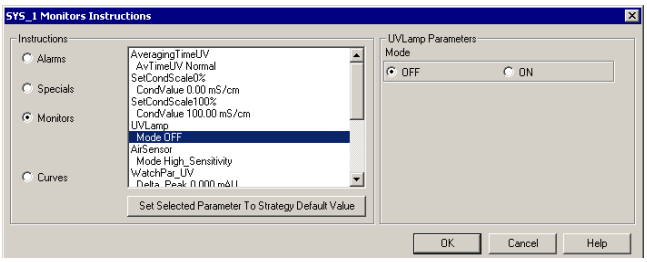


UV monitor

The UV cell is connected in the flow path directly after the column valve. It provides online detection of the absorbance of the eluted protein. The optical unit is equipped with a Hg lamp and a filter with a wavelength of 280 nm. If required, a wavelength of 254 nm can be used by turning the filter wheel and adjusting the housing.



The UV lamp is turned on by default. The lamp can be turned off in **System:Settings** in System Control by selecting **Monitors:UV lamp**. The warm up time is 60 minutes.

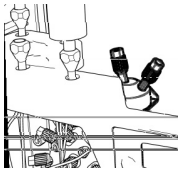


Conductivity monitor

The conductivity cell, connected directly after the UV cell, is primarily used for verifying gradients during the elution. The measurement range is 1 µS/cm to 999.9 mS/cm.

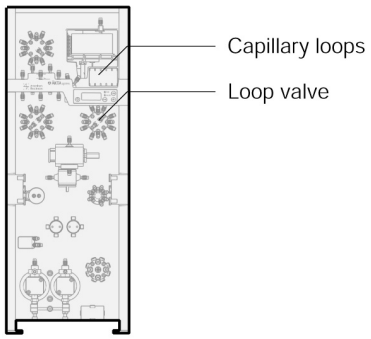
Flow restrictor

A flow restrictor is connected in the flow path directly after the conductivity cell to prevent air from precipitating or getting trapped in the UV cell. The flow restrictor creates a backpressure of 0.2 MPa.



Intermediate fraction collection

The intermediate fraction collection is handled by a loop valve and five capillary loops.

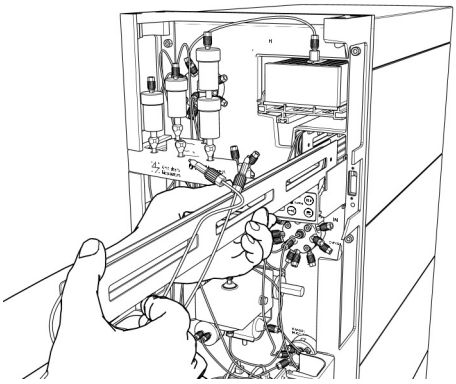


Loop valve

The loop valve is located at the upper right-hand side. It is used to direct an intermediate peak volume from the UV cell and the outlet valve into a suitable loop where it is stored for the next purification step.

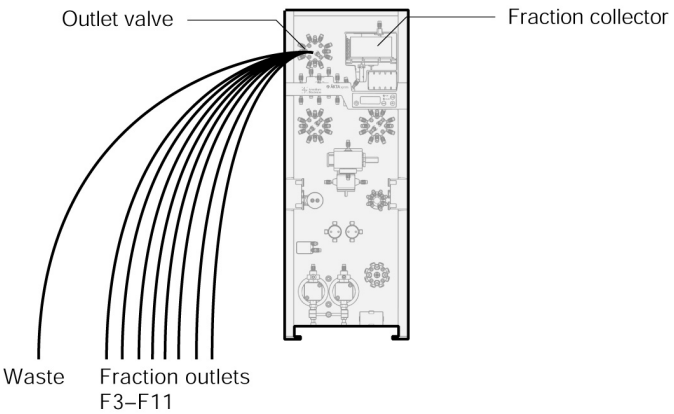
Capillary loops

Five loops, each one with a volume of 10 ml, are connected to the loop valve. The loops have i.d. 1.0 mm and are made of Tefzel tubing.



Final fraction collection

The final fractionation parts are the outlet valve and fraction collector located at the top of the system.



Outlet valve

During the last purification step, the outlet valve directs the peak volume to the fraction collector. The outlet valve also provides up to nine outlets for collecting flowthrough volumes and nonselected peaks, and a waste outlet.

The outlet valve is also a part of the intermediate fraction collection where it is used to direct the peak volume to the loop valve.

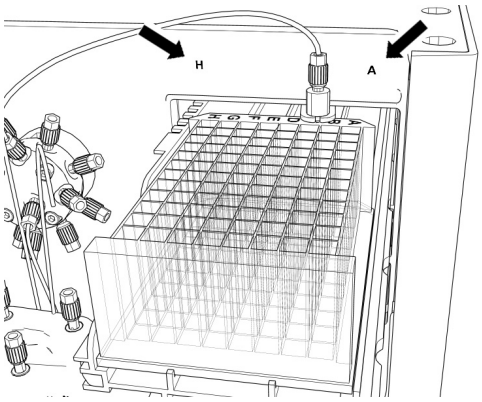
The flow-through and nonselected peaks containers should be placed on a shelf under the bench or in a flask holder (optional).

WARNING!

Never place flasks on the top of the separation system. If they become full and overflow, liquid might penetrate the system causing a short-circuit.

Fraction collector

The fraction collector is a X-Y collector made for 96 square well microplates (8×12 well positions). The volume of a well is typically 2.2 ml.



3.2.5 Indicators and controls

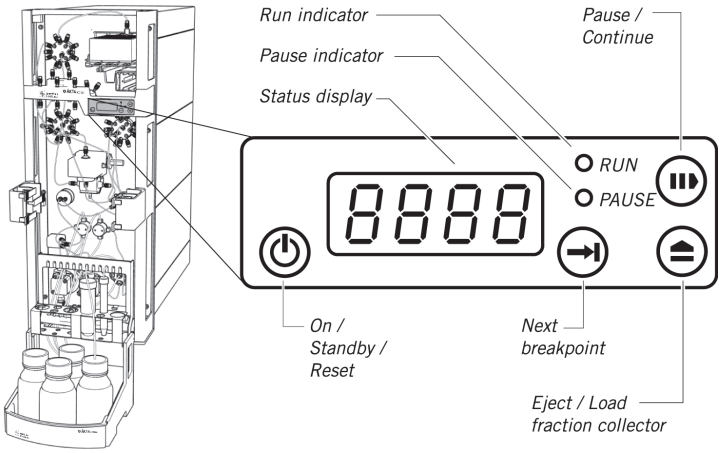
Introduction

This section describes the indicators and controls on ÄKTAxpress.

For information about the computer equipment, refer to the manufacturer's instructions.

Overview

The separation systems are equipped with the following indicators and controls.



Push buttons

The separation systems are equipped with the following push buttons.

Button	Function
On / Standby / Reset 	• In STANDBY mode: **Power on** (**Reset** of separation system incl. pump synchronization). • In END mode: **Standby**. No power to active components. • Pressing the button for 5 seconds in END/RUN/PAUSE mode: **Reset** of separation system incl. pump synchronization.
Pause / Continue 	• In RUN mode: **Pause**. • In PAUSE mode: **Continue**.
Next breakpoint 	• In RUN mode: **Go to next breakpoint** in the running method. Pressing the button in other modes will issue an error to UNICORN.

Button	Function
Eject / Load microplate 	• In END mode: **Eject** or **load** microplate. • In PAUSE mode: **Eject** or move the microplate to the position it had when the system was paused. • In RUN mode: No function.

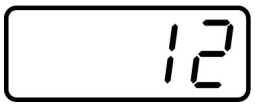
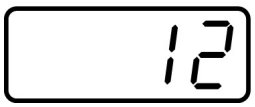
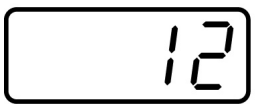
Indicators

The separation systems are equipped with the following indicators.

Indicator	Function
RUN	**Run** Green indicator: A run is active.
PAUSE	**Pause** Yellow indicator: The system is in PAUSE mode.
	**Status display** • Normal operation: System unit number (1–12), for example: **12**. • Error: 3-digit error code, for example: **E416** (UV-lamp broken)

Status indication

The indicators can show the following status.

Indicators	Status
 RUN PAUSE	**Standby**. The system is in standby. All active parts are turned off. The system can be turned on by pressing the **On** button.
 RUN PAUSE	**Idle**. No activity. The display shows the system unit number (e.g. 12).
 RUN PAUSE	**Run**. A run is performed.
 RUN PAUSE	**Pause**. The system is paused either manually or automatically by UNICORN.

Indicators	Status
 *Two segments flashing.*	**Communication error**. There is no connection with the ÄKTAxpress PC driver.
 *Display and PAUSE flashing. The display alternates between the error code and the system unit number.*	**Error**. The system is paused and an error code is displayed. For a description of the error codes, refer to **9.6 Error code list** on page 300.
	**Lamp test**. All indicators are lit for 3 seconds during the starting up sequence.
	**Program update**. A dialog is also shown on the UNICORN computer during the program update.

User control from the computer

The separation system is mainly controlled and monitored by UNICORN. A communication converter box is connected between the USB port in the computer and the UniNet port in the separation system as communication interface.

UNICORN can control up to twelve separation systems simultaneously.

More information about controlling the separation system from UNICORN is found in **6 Operation** on page 142.

3.2.6 Labels

Introduction

This section describes the labels on the separation systems.

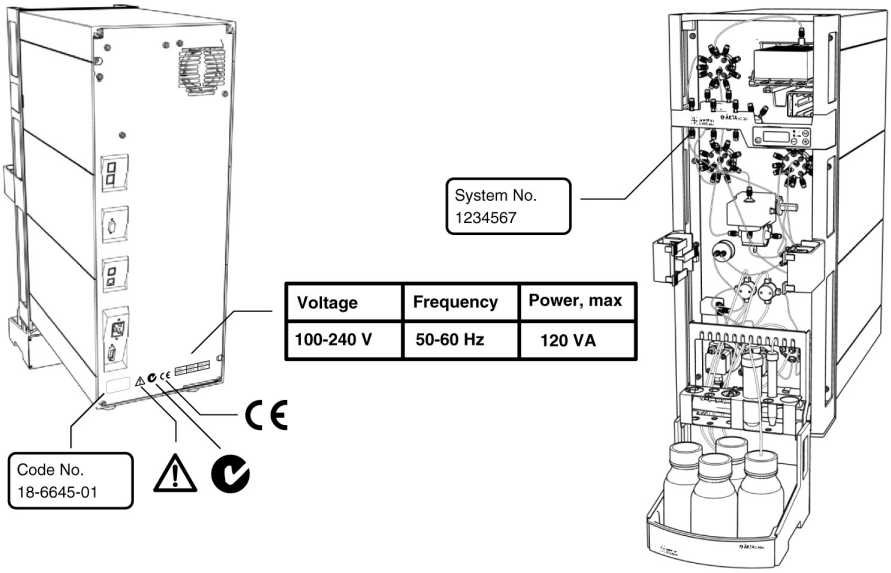
For information about the computer equipment, refer to the manufacturer's instructions.

Labels

Labels for safety, product no., CE, C-tick, and rating are located on the rear panel of the separation systems.

A system no. label is located on the left front rail.

The safety label warns the user of risk for personal injury. Do not proceed until the instructions are clearly understood and all stated conditions are met.



3.3 Purification process overview

About this section

This section gives an overiew of the steps during a typical purification process.

In this section

This section contains the following sub-sections

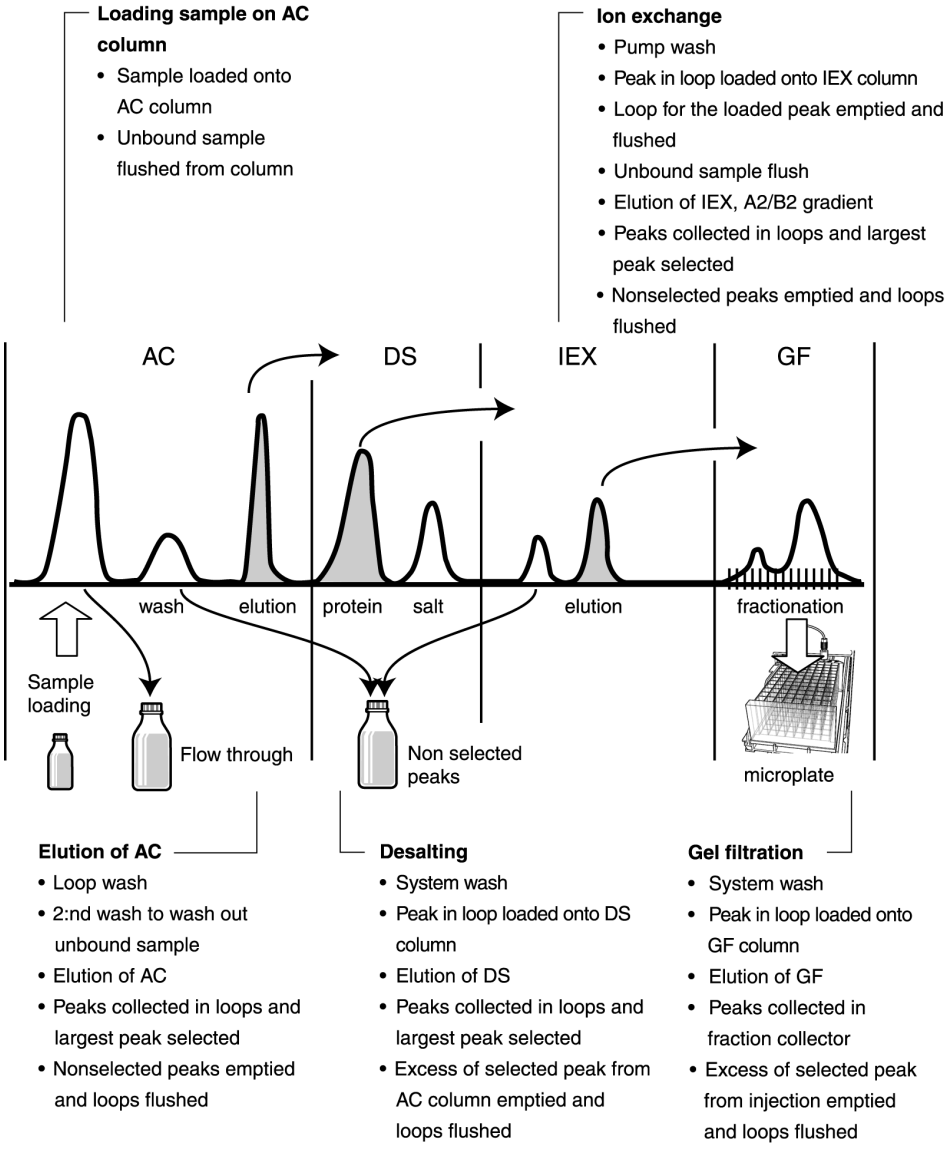
3.3.1 Process flow chart

Introduction

This section contains a flow chart of the process steps during a typical run with ÄKTAxpress.

Process flow chart

The example below shows the main steps during a four step purification of a single sample of a protein: Affinity (AC) – Desalting (DS) – Ion exchange (IEX) – Gel filtration (GF) which is equal to protocol E in the Method Wizard.



A detailed description of the process steps can be found in the section **3.3.2 Description of the process steps** on page 49.

3.3.2 Description of the process steps

Introduction

This section contains a detailed description of the process steps during the four step purification process described in the flow chart. The description is generalized to cover up to four samples.

A separate description of the tag cleavage procedure is also included.

Note: *The description covers the most important process steps only.*

Manual system setup before starting the run

The table below briefly describes how the operator prepares ÄKTAxpress for a run.

Step	Description
1	The buffer containers and the sample tube(s) are filled and sealed.
2	All inlet, outlet, and waste tubing is immersed in the correct flasks.
3	An empty microplate is placed in the fraction collector.
4	The correct columns are fitted and equilibrated (if not included in the method plan).
5	The inlet tubing is filled (if not included in the method plan) and the pump heads purged.

Starting the run

The table below briefly describes the procedure for starting a purification process.

Step	Description
1	A wizard dedicated for initializing the run is started in the System Control module.
2	The requested information in the wizard is filled in. See also **6.6.1 Starting a run using a method plan** on page 186.
3	The last page in the wizard, Summary, specifies the method requirements, totally and for each of the systems. It is recommended to print this list since it will not be saved. When clicking Run on the Summary page, all systems enter Pause mode. A final check of the setup can be made.
4	The run is started by clicking Continue or Continue all, or by pressing the Continue button on the front panel.

Loading the sample onto the AC column

The table below describes how the sample is loaded onto the AC column.

Step	Description
1	The sample S1 is loaded onto the first AC column. The inlet valve switches to A1 when the sample tube is empty (air sensor detection).
2	Air is removed through a Waste port in the injection valve.
3	Unbound sample is flushed from the column with buffer A1 and collected in a flowthrough collection flask.
4	When using several samples during the run, step 1–3 are repeated serially for all samples before proceeding to the next step.

Elution of the AC column

The table below describes the elution procedure of the AC column(s).

Step	Description
1	A LoopWash of all loops is performed with buffer A2.
2	A second wash is performed with buffer A1/B1 to wash out unbound sample. If peaks are detected, they will collected in F7–F10.
3	The AC column is eluted with a step gradient using buffer inlet A3 and the peaks collected in loops. The largest peak is selected.
4	The nonselected peaks are emptied from the loops to the collection tubes in outlet F7–F10, one for each sample. The loops are then flushed with buffer A2 to waste.

Desalting

The table below describes the desalting procedure of the collected peak volume.

Step	Description
1	A SystemWash is performed to fill the system with buffer A2
2	The AC peak collected in the loop is loaded onto the DS column (peak volume + flush volume).
3	The DS column is eluted with buffer A2 and the peak volume collected in a loop.
4	The excess of the selected peak from the AC column is emptied from the loop to the collection tubes F7–F10, one for each sample. The loop is then flushed with buffer A2 to waste.

Ion exchange

The table below describes the ion exchange procedure of the collected peak volume.

Step	Action
1	A PumpWash is performed to fill the pump with buffer A2
2	The DS peak collected in the loop is loaded onto the IEX column (peak volume + flush volume).
3	The loop with the loaded peak is flushed with buffer A2 to waste.
4	Unbound sample is flushed from the IEX column to waste with buffer A2. Peaks are collected in outlet F7–F10.
5	The IEX column is eluted with a buffer A2/B2 gradient and the peak volumes are collected in loops.
6	The nonselected peaks are emptied from the loops to the collection tubes F7–F10, one for each sample. The loops are then flushed with buffer A2 to waste.

Gel filtration

The table below describes the gel filtration procedure of the collected peak volumes.

Step	Description
1	SystemWash is performed to fill the system with buffer A4.
2	The largest IEX peak collected in the loops is loaded onto the GF column. The maximum volume is 5 ml.
3	The GF column is eluted with buffer A4.
4	The eluted peaks are collected in the fraction collector.
5	The excess of the selected peak from the injection onto the GF column is emptied from the loops to the collection tubes F7–F10, one for each sample. The loops are then flushed with buffer A2 to waste.
6	Elution of the AC column, desalting, ion exchange, and gel filtration is repeated for each sample.

Tag cleavage

The table below describes the tag cleavage procedure and includes the elution of the AC column. Hence, when tag cleavage is used, this part replaces the "Elution of the AC column" part described previously in this section.

Step	Description
1	A second wash is performed with buffer A1/B1 to wash out unbound sample.
2	The column is filled with cleavage buffer A7 (optional).
3	The protease in the superloop is loaded onto the column(s).
4	The system is paused for the set incubation time. During the incubation the tags are cleaved from the protein.
5	LoopWash of all loops is performed with buffer A2.
6	The AC column is eluted with buffer A1 or the chosen concentration of B1, and the peak volume collected in a loop.
7	SystemWash is performed to fill the system with buffer A3.
8	Uncleaved protein and tags are flushed from the AC column with buffer A3 and collected in collection tubes F7–F10.

4 Methodology

About this chapter

This chapter gives a brief introduction to the methodology for ÄKTAxpress. It contains information and guidelines on how to choose protocols, perform affinity tag removal, choose columns and, finally, suggests buffers that can be used. Developing the perfect scheme for purifying a protein remains though an empirical process and therefore has to be optimized.

In this chapter

This chapter contains the following sections

4.1 Protocols and affinity tag removal

About this section

This section contains general descriptions of the current protocols that can be used with ÄKTAxpress and guidance for how to choose a protocol. It also describes the affinity tag removal process that can be automatically performed in ÄKTAxpress in combination with all supported multi step protocols.

In this section

This section contains the following sub-sections

4.1.1 Protocol descriptions and choice

Introduction

This section describes the different protocols that can be used with ÄKTAxpress and gives guidance on how to choose protocol for a purification.

Protocol definition

The name of a protocol is an abbreviation of the combination of chromatography techniques used in the purification.

Example: The protocol AC - DS - IEX includes purification with affinity chromatography, followed by desalting and finally ion exchange chromatography.

Abbreviations found in the protocol names:

Abbreviation	Chromatography technique
AC	Affinity chromatography
DS	Desalting
IEX	Ion exchange chromatography
GF	Gel filtration

Protocols for ÄKTAxpress

For ÄKTAxpress there are seven protocols that can be used. All of the automated protocols can be combined with automatic tag-removal. See sections **4.1.2 Description of affinity tags** on page 59 to **4.1.5 Conditions for cleavage** on page 64 for more information on affinity tags and removal of affinity tags.

Protocol	Type of protocol
AC - GF	Automated 2 step protocol [A]
AC - DS	Automated 2 step protocol [B]
AC - DS - IEX	Automated 3 step protocol [C]
AC - DS - IEX - DS	Automated 4 step protocol [D]
AC - DS - IEX - GF	Automated 4 step protocol [E]
DS	Manual 1 step protocol [F]
GF	Manual 1 step protocol [G]

Note: *In GF and DS (1 step protocols) the sample is loaded manually with a syringe before starting the run. In automated multi step protocols the sample is automatically loaded via the system pump.*

Consider when choosing protocol

Questions to be considered before choosing a purification protocol:

- What should the protein be used for after the purification?
- Should the protein be in any specific buffer to keep it stable and suitable for the next application?
- What purity is required?
- Is it important to have a charge and/or size homogenous sample after the purification?

The more purification steps that are performed, the higher the purity of the target protein will be. Protein losses increase with each successive purification step. ÄKTAxpress is optimized to perform the supported automated multi step protocols as efficiently as possible. It is, however, up to the user to combine purification techniques, columns and buffers to achieve the best purification for each specific target protein.

Guidance for choice of protocol

The table below gives guidance on some sample characteristics achieved after the last purification step for each protocol.

Protocol	Buffer exchange	Charge homogeneity	Size homogeneity	Effect on purity
AC-DS	×			+
AC-GF	×		×	++
AC-DS-IEX		×		++
AC-DS-IEX-DS	×	×		++
AC-DS-IEX-GF	×	×	×	+++
DS	×			–
GF	×		×	+

Run times and maximum number of samples

The run time mainly depends on the number of samples, the column properties, and the number of chromatography steps in the protocol. The run time is also affected by the temperature, the sample viscosity and amount (sample loading time) and the length of the wash steps after loading the sample onto the AC column. The run times in the tables below do not include the sample loading time.

Room temperature

Specifications for the seven protocols, when purifying a His-tagged protein in room temperature:

Protocol	Maximum number of samples	Estimated run times with max. number of samples	Estimated run times for one sample
(A) AC - GF	4	10.4 h	2.7 h
(B) AC - DS	4	4.3 h	1.2 h
(C) AC - DS - IEX	3	6.3 h	2.2 h
(D) AC - DS - IEX - DS	3	9.3 h	3.1 h
(E) AC - DS - IEX - GF	2	8.6 h	4.3 h
(F) DS	4	0.6 h	0.2 h
(G) GF	4	6.1 h	1.6 h

Cold room

Specifications for the seven protocols, when purifying a His-tagged protein at cold room temperature:

Protocol	Maximum number of samples	Estimated run times with max. number of samples	Estimated run times for one sample
(A) AC - GF	4	12.8 h	3.3 h
(B) AC - DS	4	5.2 h	1.4 h
(C) AC - DS - IEX	3	7.4 h	2.6 h
(D) AC - DS - IEX - DS	3	10.7 h	3.6 h

Protocol	Maximum number of samples	Estimated run times with max. number of samples	Estimated run times for one sample
(E) AC - DS - IEX - GF	2	10.1 h	5.1 h
(F) DS	4	0.7 h	0.2 h
(G) GF	4	7.6 h	2.0 h

4.1.2 Description of affinity tags

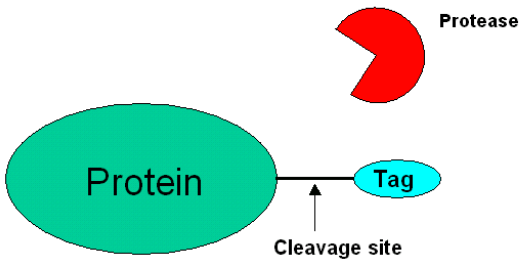
Introduction

This section gives a general description of affinity tags and their use, as well as a more detailed description of the two affinity tags that can be used with ÄKTAxpress.

About affinity tags

Affinity tags are often used to facilitate the purification of recombinant proteins. An affinity tag is included on DNA level for the recombinant protein, normally at the N- or C-terminus. The affinity tag can bind to an affinity chromatography medium with a suitable ligand allowing specific purification.

For several applications, the affinity tag has to be removed from the target protein. If a protease recognition sequence has been introduced between the affinity tag and the protein, then the affinity tag can be removed during the purification process of the protein. When the tagged protein is subjected to its specific protease, cleavage occurs at the recognition site. Cleavage can be performed in batch or on-column. All ÄKTAxpress multi step purification protocols can be combined with automatic on-column affinity tag cleavage.



Affinity tag types

Tags supported by ÄKTAxpress protocols:

Affinity tag type	Binds to	Size
His_n (polyhistidine-tag with n histidines)	Metal ions *Example*: Ni^{2+}, Zn^{2+}, Cu^{2+}, Co^{2+}	~1-3 kDa*
GST (glutathione S-transferase)	Glutathione	~26 kDa

** depending on the number of histidines and the used linker between the tag and the target protein.*

His-tags

His-tags are the most widely used affinity tags. They are normally composed of a sequence of 6 histidines that can bind with high affinity to metal ions. They are small and therefore often considered to have little effect on the structure of the proteins. The tag is also useful if purification under denatured conditions is required.

GST-tags

GST-tags often improve the expression and solubility of the target protein. The interaction with glutathione is highly specific and a one step purification therefore often results in a reasonably pure material.

Purification properties

Purification of His or GST fusion proteins is simple and uses mild elution conditions that minimize the risk of damage to the functionality of the target protein.

4.1.3 Description of proteases

Introduction

This section describes different types of proteases that can be used for tag cleavage.

About proteases

Proteases can be tagged or non-tagged. When using a His or GST-tagged protease, the cut-off target protein can be eluted while the protease is still bound to an immobilized metal affinity chromatography column (IMAC) or a Glutathione affinity chromatography column. When using a non-tagged protease, further purification is necessary to separate the target protein from the protease.

Protease types

The following list describes a few proteases commonly used for tag cleavage.

Protease	Common recognition sequence (* marks the cleavage site)	Optimal incubation temperature	Code number	Comment
Thrombin (non-tagged) 	LVPR*GS	22 °C	27-0846-01	–
Factor Xa (non-tagged) 	IEGR*-	12 °C	27-0849-01	Cuts with clean ends if an N-terminal affinity tag is used.
PreScission (GST-tagged) 	LEVLFQ*GP	4 °C	27-0843-01	Highly specific. Low optimal temperature.
TEV protease (His-tagged) 	ENLYFQ*G	30 °C	Invitrogen	Highly specific.

Note: *Low incubation temperature reduces risk of protein degradation.*

4.1.4 Description of the affinity tag removal process

Introduction

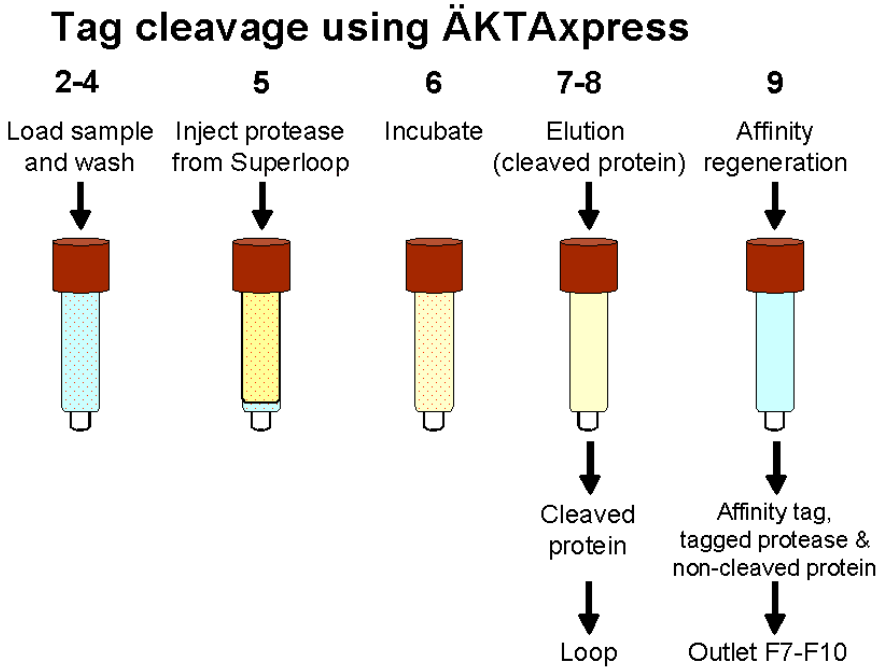
This section describes an on-column affinity tag removal process. The process is performed automatically in ÄKTAxpress.

Process overview

The table below describes the stages in an affinity tag removal method.

Stage	Description
1	The software assists the user to manually fill the superloop with the required protease solution at the beginning of the automated affinity tag removal method.
2	Affinity tagged target protein binds to an AC column.
3	Unbound protein and other material is washed off with buffer.
4	Optional: Cleavage buffer is added to the column.
5	Protease is loaded onto the column from the superloop.
6	The system is set to pause in order to allow on-column cleavage incubation. The protease cleaves the target protein from the tag.
7	The free target protein is washed out with AC binding buffer or Wash 2 buffer from the AC column and is collected in the capillary loop(s).Tagged protease, tags and non-cleaved protein stays on the column. If non-tagged protease has been used, the protease will also be released with the target protein.
8	The target protein can be further purified. Automatic affinity tag removal can be combined with any of the ÄKTAxpress multi step protocols.
9	Substances still bound to the column (i.e. the affinity tags, tagged protease and non-cleaved target protein) are eluted with elution buffer and collected in the appropriate outlet tubing (sample 1: F7, sample 2: F8, etc.).

The image below shows the different stages on the affinity column. The numbers indicate the steps described in the table above.



Performing automatic affinity tag removal with ÄKTAxpress

See **6.5.8 Performing automatical affinity tag removal** on page 175 for instructions on how to perform automatic affinity tag removal on ÄKTAxpress.

4.1.5 Conditions for affinity tag cleavage

Introduction

This section describes conditions important for affinity tag cleavage.

Conditions affecting cleavage

Protease:protein ratio, buffer composition, time and temperature are all important factors for efficient cleavage. All these conditions can be optimized to fit the specific protein. Different proteases need different amounts of time for cleavage. If cleavage is not performed at the protease's optimal temperature the cleavage time will be longer.

Note: *Try to keep the glycerol concentration low in the protease solution, since high glycerol concentration affects TEV activity negatively.*

Cleavage time

The table below provides guidance on appropriate conditions for on-column cleavage using ÄKTAxpress.

If...	at...	then...	with ... units[1] of protease per mg of protein
using PreScission™ Protease	+4 °C	cleave 8 h	20
using AcTEV™ (from Invitrogen)	room temperature	cleave 8 h	200

[1] Unit definitions:

- One unit AcTEV™ cleaves ≥ 85% of 3 µg control substrate in 1 h at 30 °C.
- One unit PreScission will cleave ≥ 90% of 100 µg of a test Glutathione S-transferase fusion protein in cleavage buffer (50 mM Tris-HCl, 150 mM NaCl, 1 mM EDTA, 1 mM DTT, pH 7.0 at 25°C) at 5 °C for 16 h.

Raising cleavage temperature

If ÄKTAxpress is used in a cold room, it is possible to raise the temperature in the column by using a column heater during the affinity and cleavage steps. The column heater is not controlled from UNICORN but can be placed on top of the system.

Note: *Raise the temperature carefully if the protein is temperature sensitive.*

Buffer suggestions

See **4.3.2 Buffer suggestions** on page 80 for guidelines on buffers for cleavage.

Further information

See Instructions and Application notes from Amersham Biosciences (**4.4 Documents for further information** on page 83) and other suppliers.

4.2 Column alternatives

About this section

This section contains general descriptions of the columns currently supported by ÄKTAxpress, guidelines for selection of appropriate column combinations for different applications and schedules for simplified column selections for the different protocols.

In this section

This section contains the following sub-sections

4.2.1 Descriptions of columns

Introduction

This section describes the different types of columns currently supported by ÄKTAxpress.

Column types

Four main column types are used for chromatography in ÄKTAxpress.

Column type (abbreviation)	Separation property
Affinity chromatography column (AC)	Function
Desalting column (DS)	Size
Ion exchange column (IEX)	Charge
Gel filtration column (GF)	Size

More information on the main column types is found below.

Affinity chromatography columns

Affinity chromatography (AC) columns separate proteins on the basis of a reversible interaction between a protein (or a group of proteins) and a specific ligand attached to a chromatographic matrix. Affinity chromatography can be used whenever a suitable ligand is available. The AC column binds specific molecules from even large sample volumes very efficiently.

The following AC columns are supported by ÄKTAxpress:

Supported AC columns and volumes	Characteristics
HisTrap™ HP 1 and 5 ml	HiTrap™ column prepacked with Ni Sepharose™ High Performance, a Ni^{2+} precharged medium optimized for purification of His-tagged proteins.
HiTrap Chelating HP 1 and 5 ml	HiTrap column prepacked with Chelating Sepharose High Performance, to be charged with the metal of choice for purification of His-tagged proteins.
GSTrap™ HP 1 and 5 ml	HiTrap column prepacked with Glutathione Sepharose High Performance. For high resolution purification of GST-tagged proteins.
GSTrap FF 1 and 5 ml	HiTrap column prepacked with Glutathione Sepharose Fast Flow. For fast purification of GST-tagged proteins.

Desalting columns

Desalting (DS) columns separate molecules with large size differences. Desalting is a quick method for size separation and is often used to remove salts from proteins or to change buffers.

The following desalting columns are supported by ÄKTAxpress:

Supported DS columns and volumes	Characteristics
HiPrep™ 26/10 Desalting 53 ml	HiPrep column prepacked with Sephadex™ G-25 Fine. For fast buffer exchange and desalting of samples with a volume of ≤ 15 ml.
2 x HiTrap Desalting 2 × 5 ml, see also **4.2.1 Coupled columns** on page 69	HiTrap columns prepacked with Sephadex G-25 Superfine. For fast buffer exchange and desalting of samples with a volume of ≤ 3 ml.

Ion exchange columns

Ion exchange (IEX) columns separate proteins based on differences in charge. The separation is mediated by a reversible interaction between a charged protein and an oppositely charged chromatographic media.

Anion exchange columns

Anion exchange columns are used to separate *negatively* charged proteins.

The following anion exchange columns are supported by ÄKTAxpress:

Supported anion IEX columns and volumes	Characteristics
RESOURCE™ Q 1 and 6 ml	Columns prepacked with SOURCE™ 15Q. Fast purification with high resolution.
HiTrap Q HP 1 and 2 × 1 ml, see also **4.2.1 Coupled columns** on page 69	Columns prepacked with Q Sepharose High Performance for high resolution purification.
Mono Q™ 5/50 GL 1 ml	Column prepacked with MonoBeads™ for purification with the highest resolution.

Cation exchange columns

Cation exchange columns are used to separate *positively* charged proteins.

The following cation exchange columns are supported in ÄKTAxpress:

Supported cation IEX columns and volumes	Characteristics
RESOURCE S 1 and 6 ml	Columns prepacked with SOURCE 15S. Fast purification with high resolution.
HiTrap SP HP 1 and 2 × 1 ml, see also **4.2.1 Coupled columns** on page 69	Columns prepacked with SP Sepharose High Performance for high resolution purification.
Mono S™ 5/50 GL 1 ml	Column prepacked with MonoBeads for purification with the highest resolution.

Gel filtration columns

Gel filtration (GF) columns separate proteins with differences in molecular size. They are often used in a final polishing step. Gel filtration separation results in high quality samples of homogenous size. The method could also be suitable e.g. for the separation of protein monomers from protein dimers and trimers.

The following gel filtration columns are supported by ÄKTAxpress:

Supported GF columns and volumes	Characteristics
HiLoad™ 16/60 Superdex™ 75 prep grade 120 ml	HiLoad column prepacked with Superdex 75 prep grade. For high resolution polishing step for proteins of sizes 3–70 kDa and for sample volumes of 5 ml.
HiLoad 16/60 Superdex 200 prep grade 120 ml	Columns prepacked with Superdex 200 prep grade. For high resolution polishing step for proteins of sizes 10–600 kDa and for sample volumes of 5 ml.

Coupled columns

Some columns can be used in pairs:

2 × 1 ml HiTrap SP/Q HP	2 × 5 ml HiTrap Desalting
	

Further information

More information about the columns can be found in the Column list, e.g. flow rates and pressure limits used. Select Edit:Column list in the Method Editor. Click Advanced Parameters to view the ÄKTAxpress column parameters.

Note: *The Method Wizard does not support all columns in the Column list.*

For further information see the handbooks in **4.4 Documents for further information** on page 83.

4.2.2 Choosing columns

Introduction

This section describes how to choose columns. Information on what to consider before choosing columns as well as selection guides for the various supported protocols are included.

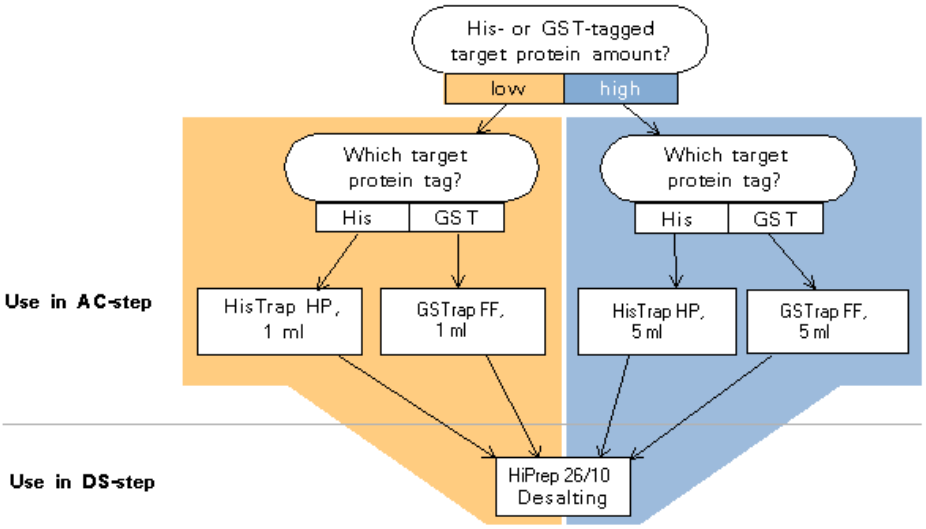
Consider when choosing columns

Questions to be considered before choosing columns:

- How much purified protein is needed after the final purification step?
- How much sample is to be loaded?
- What purity and resolution is required?

Choosing columns for AC-DS

Recommended column combinations when running an AC-DS protocol:



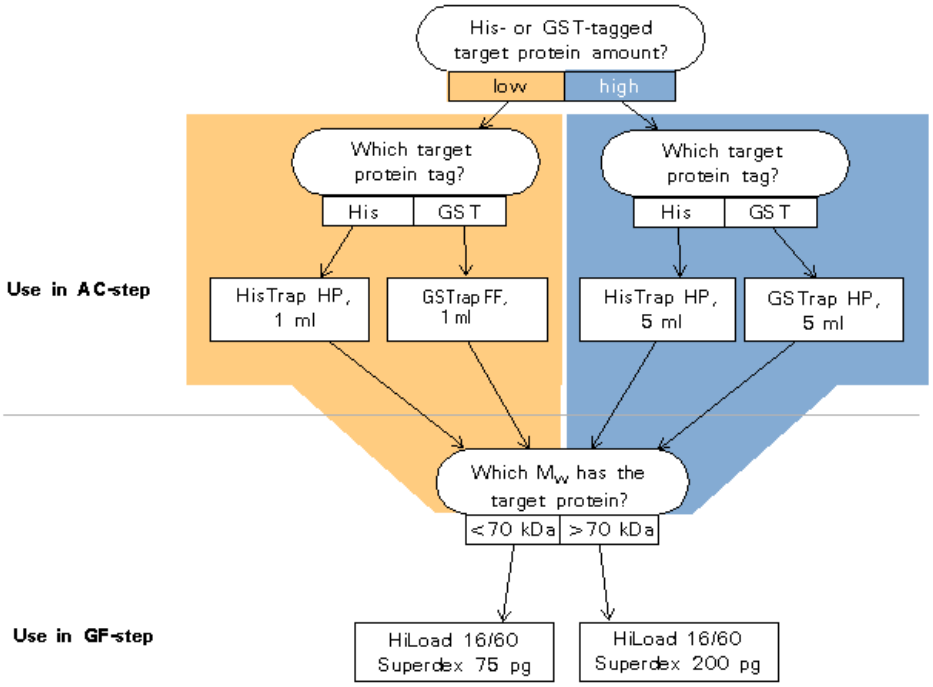
Comments on alternative column choices:

Step	Comments on alternative column choices
AC	Overloading of an affinity column can be performed to minimize non-specific binding (often results in a purer end product).
AC	If there is a low expressed target protein in a large sample volume, consider using a 5 ml HiTrap column to reduce the risk of column clogging or protein degradation.
DS	1 ml HiTrap affinity columns can be combined with 2 × HiTrap desalting columns. Band broadening effects in the system combined with a limited sample loading volume on the 2 × HiTrap desalting column might however reduce protein recovery.

Step	Comments on alternative column choices
DS	5 ml HiTrap affinity column should not be combined with 2 × HiTrap desalting columns since the eluted affinity peak might be larger than the volume possible to load on 2 × HiTrap desalting columns.

Choosing columns for AC-GF

Recommended column combinations when running an AC-GF protocol:

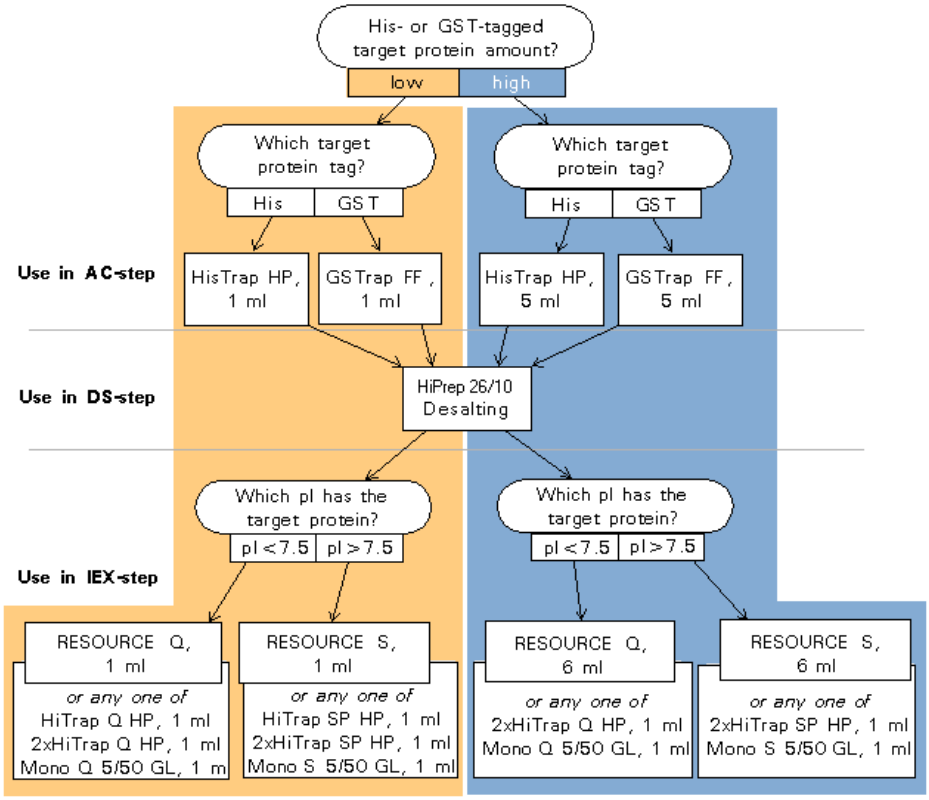


Comments on alternative column choices:

Step	Comments on alternative column choices
AC	Overloading of an affinity column can be performed to minimize non-specific binding (often results in a purer end product).
AC	If there is a low expressed target protein in a large sample volume, consider using a 5 ml HiTrap column to reduce the risk of column clogging or protein degradation.
GF	To get sufficient separation it is important to choose a gel filtration column with appropriate selectivity.

Choosing columns for AC-DS-IEX

Recommended column combinations when running an AC-DS-IEX protocol:



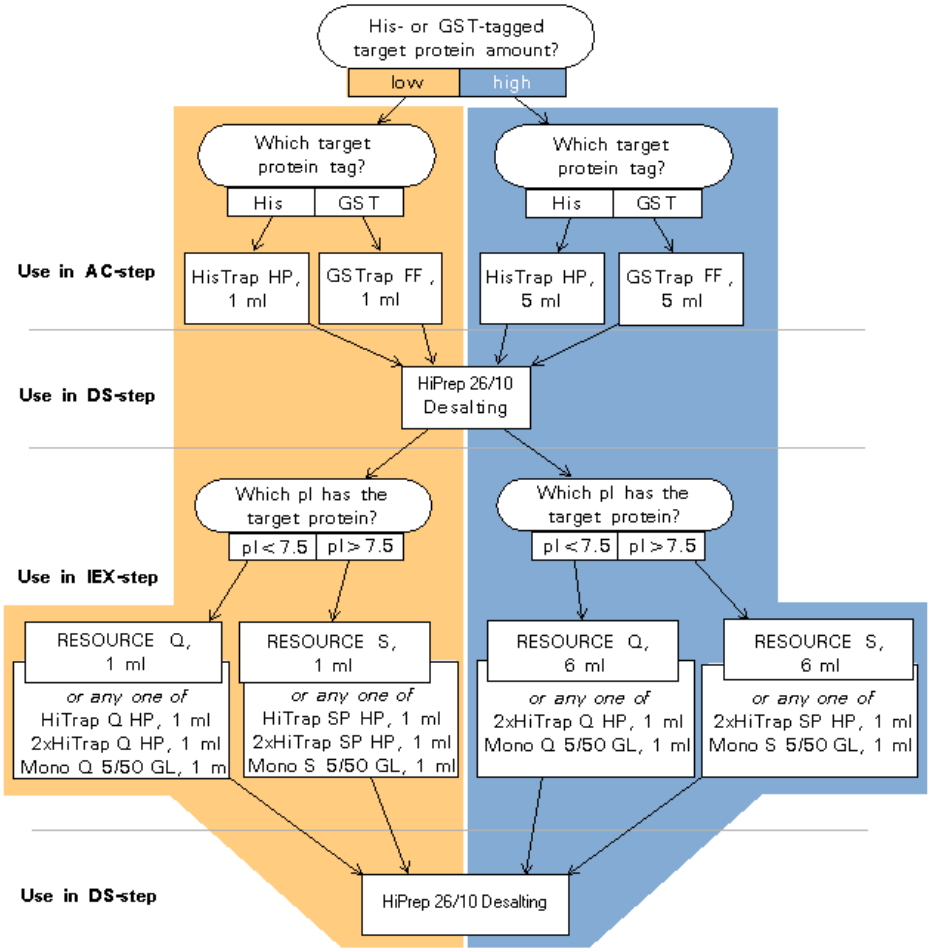
Comments on alternative column choices:

Step	Comments on alternative column choices
AC	Overloading of an affinity column can be performed to minimize non-specific binding (often results in a purer end product).
AC	If there is a low expressed target protein in a large sample volume, consider using a 5 ml HiTrap column to reduce the risk of column clogging or protein degradation.
DS	1 ml HiTrap affinity columns can be combined with 2 × HiTrap desalting columns. Band broadening effects in the system combined with a limited sample loading volume on the 2 × HiTrap desalting column might however reduce protein recovery.
DS	5 ml HiTrap affinity columns should not be combined with 2 × HiTrap desalting columns since the eluted affinity peak might be larger than the volume possible to load on the 2 × HiTrap desalting column.
IEX	A longer IEX column or a column with smaller bead size can be used to increase resolution.

Step	Comments on alternative column choices
IEX	Make sure to use an IEX column with sufficient capacity for the protein amount.

Choosing columns for AC-DS-IEX-DS

Recommended column combinations when running an AC-DS-IEX-DS protocol:



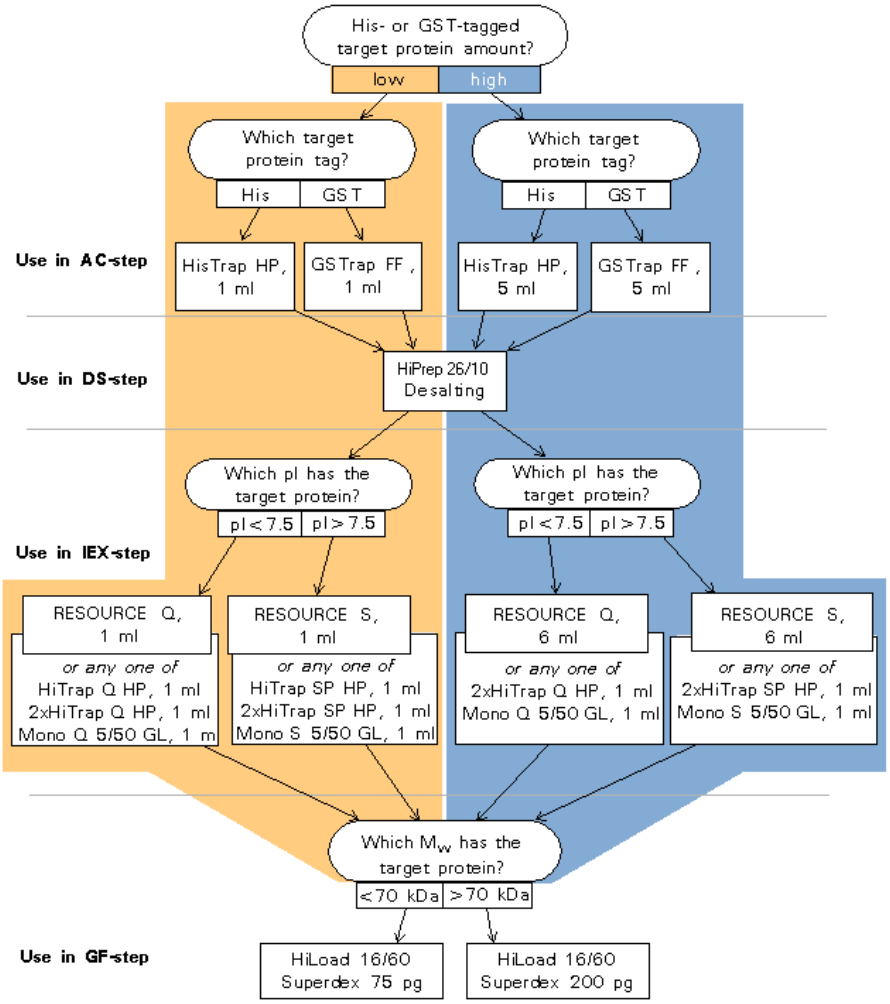
Comments on alternative column choices:

Step	Comments on alternative column choices
AC	Overloading of an affinity column can be performed to minimize non-specific binding (often results in a purer end product).
AC	If there is a low expressed target protein in a large sample volume, consider using a 5 ml HiTrap column to reduce the risk of column clogging or protein degradation.

Step	Comments on alternative column choices
DS	1 ml HiTrap affinity columns can be combined with 2 × HiTrap desalting columns. Band broadening effects in the system combined with a limited sample loading volume on the 2 × HiTrap desalting column might however reduce protein recovery.
DS	5 ml HiTrap affinity columns should not be combined with 2 × HiTrap desalting columns since the eluted affinity peak might be larger than the volume possible to load on the 2 × HiTrap desalting column.
IEX	A longer IEX column or a column with smaller bead size can be used to increase resolution.
IEX	Make sure to use an IEX column with sufficient capacity for the protein amount.
DS	Only the same desalting column can be used for all DS steps in this protocol.

Choosing columns for AC-DS-IEX-GF

Recommended column combinations when running an AC-DS-IEX-GF protocol:



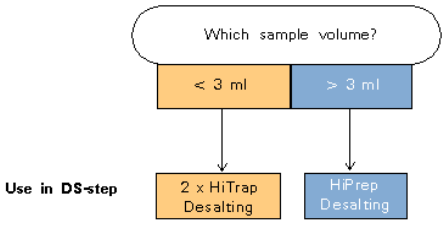
Comments on alternative column choices.

Step	Comments on alternative column choices
AC	Overloading of an affinity column can be performed to minimize non-specific binding (often results in a purer end product).
AC	If there is a low expressed target protein in a large sample volume, consider using a 5 ml HiTrap column to reduce the risk of column clogging or protein degradation.
DS	1 ml HiTrap affinity columns can be combined with 2 × HiTrap desaltingcolumns.Band broadening effects in the system combined with a limited sample loading volume on the 2 × HiTrap desalting column might however reduce protein recovery.

Step	Comments on alternative column choices
DS	5 ml HiTrap affinity columns should not be combined with 2 × HiTrap desalting columns since the eluted affinity peak might be larger than the volume possible to load on the 2 × HiTrap desalting column.
IEX	A longer IEX column or a column with smaller bead size can be used to increase resolution.
IEX	Make sure to use an IEX column with sufficient capacity for the protein amount.
GF	To get sufficient separation it is important to choose a gel filtration column with appropriate selectivity.

Choosing columns for DS

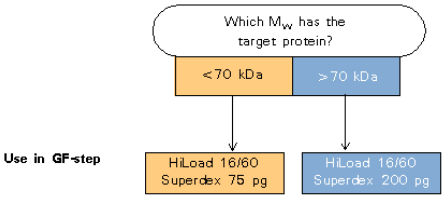
Recommended column choice when running a DS one step protocol with manual sample loading:



Note: *Sample loading volume is limited to the loop volume, i.e. 10 ml.*

Choosing columns for GF

Recommended column choice when running a GF one step protocol with manual sample loading:



Note: *Maximum sample loading volume is 5 ml.*

4.3 Buffer alternatives

About this section

This section contains general descriptions of different buffers and gives guidance on how to choose buffers for a protocol. See also buffer suggestions in the column instructions.

In this section

This section contains the following sub-sections

4.3.1 Description of buffers

Introduction

This section gives general guidance on how to choose buffers for different chromatography steps. See also buffer suggestions in the column instructions.

Affinity chromatography buffers

There are 3 steps in the affinity chromatography: binding, wash, and elution. In addition to these steps, affinity tag removal can also be performed on the AC column.

- **Binding buffer**

  The buffer for binding has to match the binding properties of the used affinity column.

- **Extra wash buffer (second wash)**

  The extra wash is used to wash out non-specifically bound impurities that do not come off with the normal binding buffer wash. The extra wash buffer should include a small amount of elution buffer to release more of the impurities from the column, but not enough to release the target protein.

  Note: *The extra wash is recommended when running HisTrap or HiTrap Chelating columns. The use of extra wash is not recommended when purifying GST-tagged proteins.*

- **Cleavage buffer**

  If affinity tag cleavage is performed, the binding buffer can often be used. If preferred, an alternative cleavage buffer can be used to condition the affinity column before injection of the protease.

- **Elution buffer**

  The elution buffer should be chosen to elute the target protein as specifically as possible. For ÄKTAxpress, step elution of the bound protein from the AC column is used to achieve an elution volume suitable for the next purification step.

Desalting chromatography buffers

Desalting can be performed in preparation for an ion exchange chromatography step or as a last buffer exchange step.

- **DS before IEX**

  If IEX is to be performed after DS, use the IEX binding buffer for the desalting step.

- **DS as last step**

  If DS is used as a final purification step, the buffer should be chosen to match the activity or application that will follow (e.g. crystallization, NMR-studies, functional studies or storage).

Ion exchange chromatography buffers

The choice of buffer and its pH for IEX chromatography depends on the pI of the target protein, and if an anion or a cation exchange column should be used.

Normally the pH of the buffer is selected to be at least 1 pH unit below or above the pI of the target protein.

- If using an **anion** exchange column (for example Resource Q), use a buffer with a **pH > pI** of target protein.
- If using a **cation** exchange column (for example Resource S), use a buffer with a **pH < pI** of target protein.

Gel filtration chromatography buffers

Gel filtration is used to perform a high resolution purification of the protein. The buffer should be chosen to match the activity or application that will follow (e.g. crystallization, NMR-studies, functional studies or storage).

4.3.2 Buffer suggestions

Introduction

This section contains buffer suggestions that can be used as a starting point for purification. For optimal behavior in a specific protein purification, optimization might be needed. See also buffer suggestions in the column instructions.

Guidelines

Several aspects need to be considered when choosing buffers. Guidance is provided in the instructions for each column and in the purification handbooks, see **4.2.1 Descriptions of columns** on page 66.

AC buffer suggestions for His-tagged proteins

Suggested buffers for AC runs with His-tagged proteins:

When performing...	suggested buffer
binding using HisTrap HP	50 mM Tris-HCl pH 7.5, 0.5 M NaCl, 40 mM imidazole
binding using HiTrap Chelating	50 mM Tris-HCl pH 7.5, 0.5 M NaCl, 20 mM imidazole
extra wash (second wash)	50 mM Tris-HCl pH 7.5, 0.5 M NaCl, 100 mM imidazole ***Note:*** *x% of the extra wash buffer is added to the binding buffer. This value can be changed in the Advanced Zone. See **5.5.4 Advanced Zone for Purify - Affinity** on page 120 for details.* *Example*: If using a binding buffer with 20 mM imidazole and 20% of extra wash buffer (100 mM imidazole), the total concentration of imidazole during the extra wash will be 40 mM.
cleavage using TEV protease on HisTrap HP or HiTrap Chelating HP	50 mM Tris-HCl pH 7.5, 0.5 M NaCl, 50 mM imidazole ***Note:*** *The imidazole concentration might need to be optimized, especially if a different His-tagged protease than TEV is used.*
step elution	50 mM Tris-HCl pH 7.5, 0.5 M NaCl, 500 mM imidazole

In the affinity tag removal protocols, the affinity columns can be equilibrated with either AC-binding/wash buffer, extra wash buffer, or an alternative "cleavage buffer" before protease injection.

AC buffer suggestions for GST-tagged proteins

Suggested buffers for AC runs with GST-tagged proteins:

When performing...	suggested buffer
binding using GSTrap HP or FF	50 mM Tris-HCl pH 7.5, 150 mM NaCl, 1 mM DTT
cleavage using PreScission on GSTrap HP or FF	50 mM Tris-HCl pH 7.5, 150 mM NaCl, 1 mM EDTA, 1 mM DTT
step elution	50 mM Tris-HCl, 10 mM reduced gluthathione, pH 8.0

In the affinity tag removal protocols, the affinity columns can be equilibrated with either AC-binding/wash buffer or an alternative "cleavage buffer" before protease injection.

DS buffer suggestions

Suggested buffers for DS runs:

If for example...	suggested buffer
preparing for AIEX	50 mM Tris-HCl pH 8.0
preparing for CIEX	20 mM MES pH 6.0
preparing for storage	include e.g. 10% glycerol in a suitable buffer (e.g. 50 mM Tris-HCl pH 7.5, 150 mM NaCl)

IEX buffer suggestions

Suggested buffers for IEX runs:

If for example...	suggested buffer
binding to AIEX	50 mM Tris-HCl pH 8.0
binding to CIEX	20 mM MES pH 6.0
elution from AIEX	50 mM Tris-HCl pH 8.0, 1 M NaCl
elution from CIEX	20 mM MES pH 6.0, 1 M NaCl

Note: *The chosen buffer and its pH will depend on the pI of the target protein.*

GF buffer suggestions

Suggested buffers for GF runs:

If for example...	suggested buffer
preparing for further studies	50 mM Tris-HCl pH 7.5, 150 mM NaCl
preparing for storage	include e.g. 10% glycerol in a suitable buffer (e.g. 50 mM Tris-HCl pH 7.5, 150 mM NaCl)

Further information

For further information, see method handbooks in **4.4 Documents For Further Information** on page 83 and product specifications.

4.4 Documents for further information

Introduction

This section includes a list of the different documents that can be used to find more information on protein separation and related topics.

Further information

Further information on protein separation can be found in the following documents:

Documents	Code number
Affinity Chromatography Handbook: Principles and methods	18-1022-29
Gel Filtration: Principles and methods	18-1022-18
GST Gene Fusion System Handbook	18-1157-58
Ion Exchange Chromatography: Principles and methods	18-1114-21
Protein Purification Handbook	18-1132-29
Recombinant Protein Handbook	18-1142-75

5 Method Wizard

About this chapter

This chapter describes the Method Wizard in UNICORN.

In this chapter

This chapter contains the following sections

5.1 Method Wizard introduction

Introduction

This section gives a general description of the Method Wizard in the Method Editor module and instructions for how to start the wizard.

The Method Wizard

The *Method Wizard* in Method Editor is a step-by-step guide for creating method plans. It consists of a number of pages with questions and instructions on how to create the method plan. The options on subsequent pages depends on the choices made on the initial page.

The Method Wizard can be divided in two different parts:

- The first part, where the main selections that define the method plan are made e.g. type of purfication protocol, column types, etc. All parameters have pre-optimized default values.
- The second part, Advanced Zone, where a number of parameter values in the method plan can be viewed and changed, if preferred.

  Note: *Avoid changing default parameter values in a method plan unless the result is clearly understood. Changing a parameter value might require adjustments of other parameter values as well.*

The method plan

A *Method plan* is a set of method instructions created by the Method Wizard, which define the frame of a run. Additional information is though needed before running the method plan on a specific system. See **Using a method plan** below. The Method Wizard can be used for creating three different types of method plans:

- Preparation of columns and the system.
- A protein purification run.
- Maintenance of columns and the system.

The first step when creating a method plan is to select the type of method plan to be created. Different options are then presented, depending on the chosen type. When all required selections are made, the method plan is saved by a user specified name.

Using a method plan

Before using a method plan in a run, some additional information must be added, e.g. selecting the separations systems to use, number of samples, name of the result files etc. This information will be requested in a wizard in the System Control module where the final preparation before starting the run is made. When the wizard is finished, a unique *method* for each of the systems is created and started. See **6.6.1 Starting a run using a method plan** on page 186.

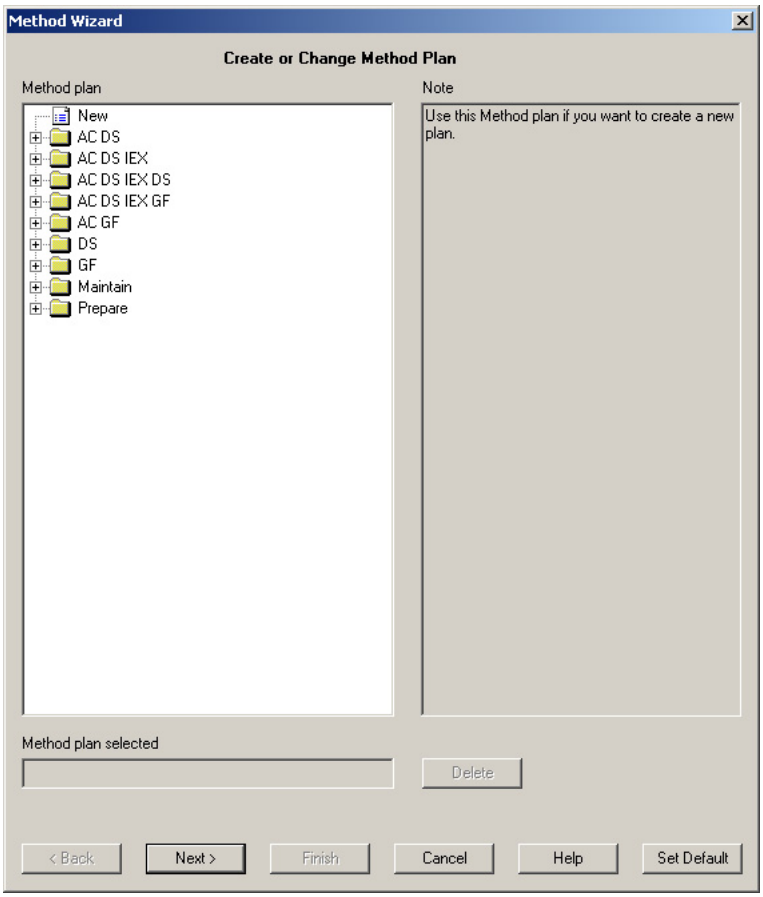
Opening the Method Wizard

To open the Method Wizard:

Step	Action
1	Click the **Method Editor** button, located in UNICORN task bar. *Result*: The Method Editor module appears.
2	Click the Method Wizard icon, located in the tool bar. Alternatively, choose **File:Method Wizard**.  *Result*: The Method Wizard appears.

The first page in the Method Wizard

The first page (shown below) contains a list of previously created and stored method plans and a New option for creating a new method plan. The first time the Method Wizard is used the list only contains the Installation test. The Note pane to the right gives a short summary of the chosen method plan.



The Method Wizard buttons

The table below describes the Method Wizard buttons.

Click...	To...
< Back	go back to the previous page.
Next >	go to the next page.
Finish	finish creating the method plan and save it.
Cancel	cancel the settings and close the wizard. Nothing is saved.
Help	open help texts for the active page.
Set Default	restore all settings to the default values. Enabled on the first page only.
Delete	delete a selected method plan or folder.

Changing selections

The major selections made in the first part of the Method Wizard will be locked and changes not allowed when:

- the Save As page is entered in the first part of the Method Wizard, *or*
- the Last Page in Advanced Zone is entered.

Note: *Entering the Main Selections page sets the parameters values on the subsequent pages to default.*

It is though always possible to change parameter values in Advanced Zone, parameters included in the major selections.

5.2 Creating a method plan

About this section

This section provides instructions for creating a new method plan using the Method Wizard.

In this section

This section contains the following sub-sections

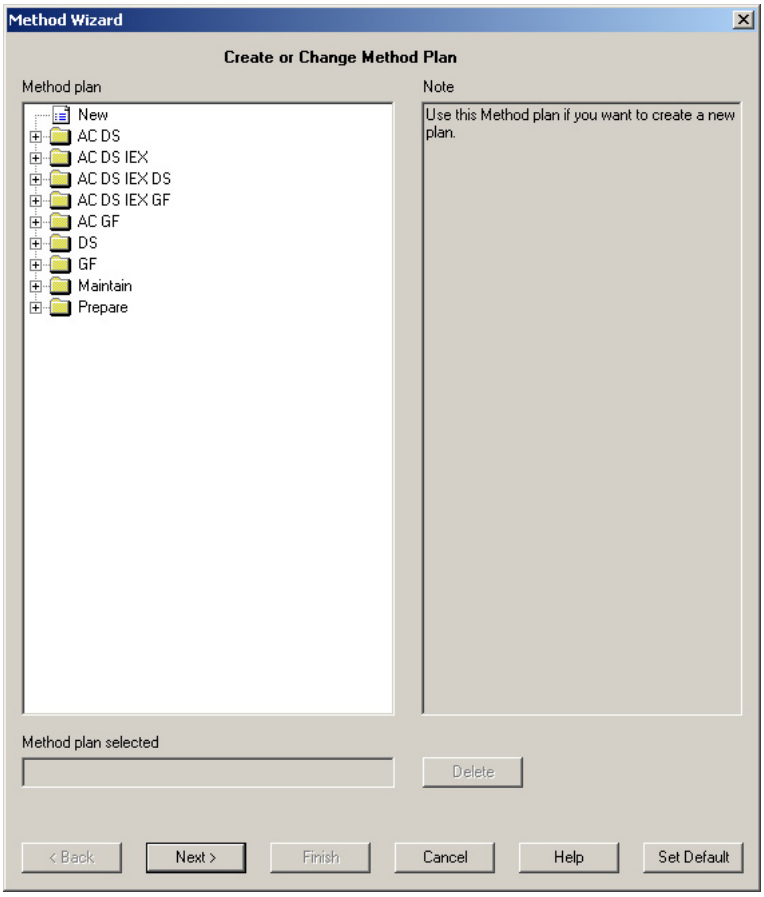
5.2.1 Creating a new method plan

Introduction

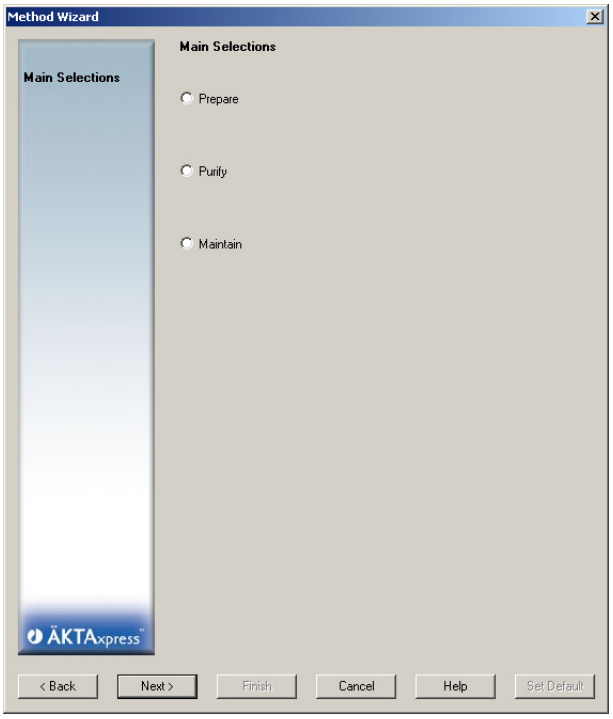
This section describes how to create a new method plan and the main selections available. It is also possible to open and edit an existing method plan and save it under a new name.

Creating a new method plan

The first page in the Method Wizard is the Create or Change Method Plan page. The Method Plan pane contains a list of saved method plans, if any, and a New option. The Note pane displays the contents in the selected method plan.



To create a new method plan:

Step	Action
1	In the **Method plan** list, select **New** and click **Next**. *Result*: The Main Selections page appears.  ***Note:*** *Always when entering the Main Selections page, the parameter values on the subsequent pages are set to default.*
2	Choose the requested type of method plan. The Method Wizard provides three main selections. The table below describes the method plan types that can be created from each selection.

Main selections

Option	Use to create a method plan for...
Prepare	Preparation of the system and/or the columns before the actual purification run. *Example of actions:* Filling the buffer and sample inlets with buffer. Equilibrating the columns. Instructions for the Prepare option are given in **5.2.2 Using the Prepare option** on page 92.

Option	Use to create a method plan for...
Purify	Purification operations. *Example of actions:* Performing different types of protein purification runs. Instructions for the Purify option are given in **5.2.3 Using the Purify option** on page 96.
Maintain	Maintenance of system and columns. *Example of actions:* Performs different types of cleaning operation and customized equilibrations. Run installation test. Instructions for the Maintain option are given in **5.2.4 Using the Maintain option** on page 102.

For more information on the options available on the current page, click **Help**.

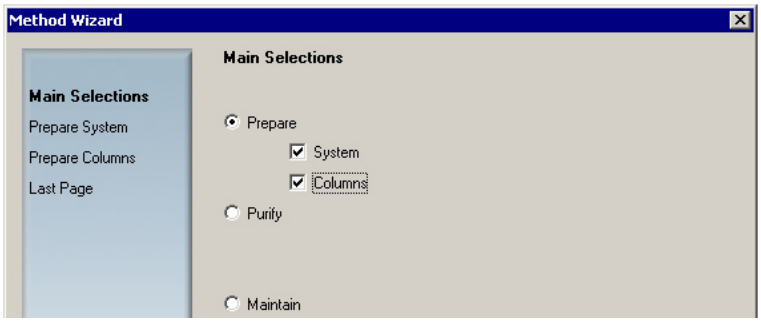
5.2.2 Using the Prepare option

Introduction

This section describes how to create a method plan for preparation of the systems, or the columns, before the actual purification run.

Selecting the type of preparation

To select the type of preparation:

Step	Action
1	On the **Main Selections** page, select **Prepare**. ***Note:*** *Always when entering the Main Selections page, the parameters values on the subsequent pages are set to default.*
2	Select **System** and/or **Columns**. The table below describes the options. 
3	Click **Next**.

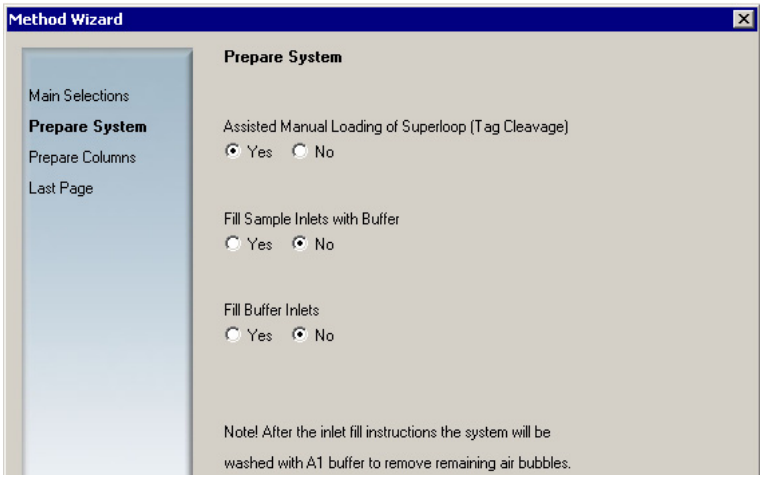
The table below describes the Prepare options.

To...	Select...
fill the buffer and sample inlets with buffer	**System** (Can also be performed within a Purify method plan. See **5.2.3 Using the Purify option** on page 96).
equilibrate the columns	**Columns** (Can also be performed within a Purify method plan. See **5.2.3 Using the Purify option** on page 96).
metal ion charge of affinity column(s)	**Columns**
perform a blank run to prepare the affinity, and/or the ion exchange column(s)	**Columns**

To...	Select...
prepare both system and columns	**System** and **Columns**

The Prepare System dialog

If selecting System, follow the instructions below. If only selecting the Columns option, go to step 1 in **5.2.2 The Prepare Columns dialog** on page 94 for further instructions.

Step	Action
1	Select the appropriate options on the **Prepare System** page. The table below describes the options. 
2	Click **Next**.

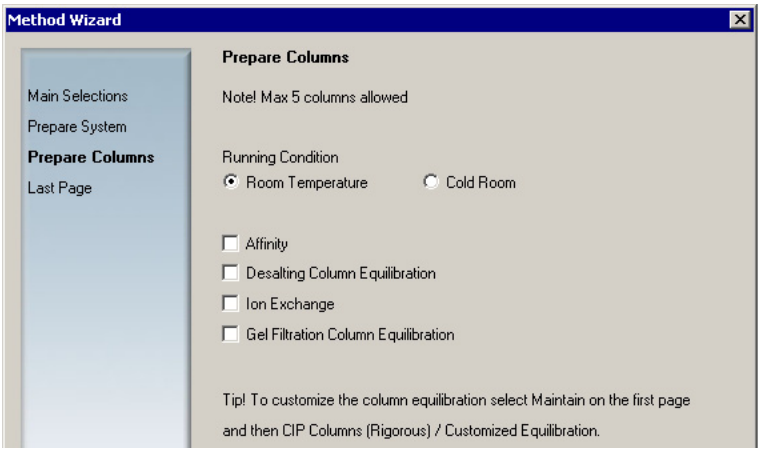
Selecting Prepare System options

The table below describes the Prepare System options and the related actions during the run.

To...	Choose... /related action
manually fill the superloop with protease solution through the fill port in the injection valve	**Yes** (default) under **Assisted Manual Loading of Superloop (Tag Cleavage)**. When starting a run, help texts appear to guide through the operation.
fill the sample inlets with buffer	**Yes** under **Fill Sample Inlets with Buffer**. Select which sample inlets to fill.
fill buffer inlets	**Yes** under **Fill Buffer Inlets**. Select which buffer inlets to fill.

The Prepare Columns dialog

If selecting Columns, follow the instructions below. If not, proceed to **5.2.5 Saving the Method plan** on page 106.

Step	Action
1	Specify **Running Condition** on the **Prepare Columns** page. 
2	Select type of column(s) to prepare. When selecting a column type, new options will appear related to the column. The options are described in the table below.
3	Click **Next**.

Selecting Prepare Column options

The table below describes the Prepare Column options and the related actions during the run.

Option	Description /related action
Column Equilibration (available for all columns)	Equilibrates the column(s). During the run, the column(s) will be equilibrated with 5 column volumes (CV) of the appropriate buffer, except for the gel filtration column which will be equilibrated with 2 CV. ***Note:*** *A more customized equilibration can be made using the Maintain option. See **5.2.4 Using the Maintain option** on page 102.*

Option	Description /related action
Ion Exchange Blank Run (ion exchange columns only)	Provides the ion exchanger with exchangeable counter ions. A blank run should be performed before first-time usage and after long-term storage. The column will be washed with: • 5 CV of buffer A • 5 CV of buffer B • 10 CV of buffer A
Metal Ion Charge (affinity columns only)	Metal ion charging of HiTrap Chelating HP and HisTrap HP columns. The inlet tubing has to be filled with metal ion solution before starting the run, manually or within a method plan. The column will be washed with: • 5 CV deionized water • 1 CV 0.1M metal ion solution • 5 CV deionized water The metal waste will be collected through F11.
Affinity Blank Run (affinity columns only)	Avoids leakage of loosely bound metal ions during the purification run. A blank run should always be performed after metal ion charging or stripping of a column. The column will be washed with: • 5 CV of buffer A • 5 CV of buffer B • 10 CV of buffer A

Finishing creating the method plan

Continue with the instructions below.

Step	Action
1	After selecting the requested preparation options, click **Next**. *Result*: The Last Page appears.
2	Proceed to **5.2.5 Saving the method plan** on page 106.

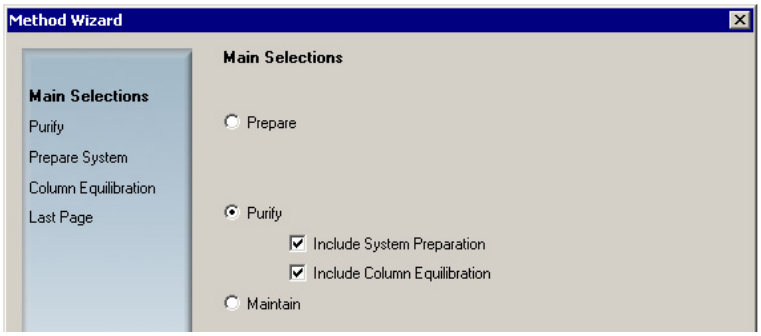
5.2.3 Using the Purify option

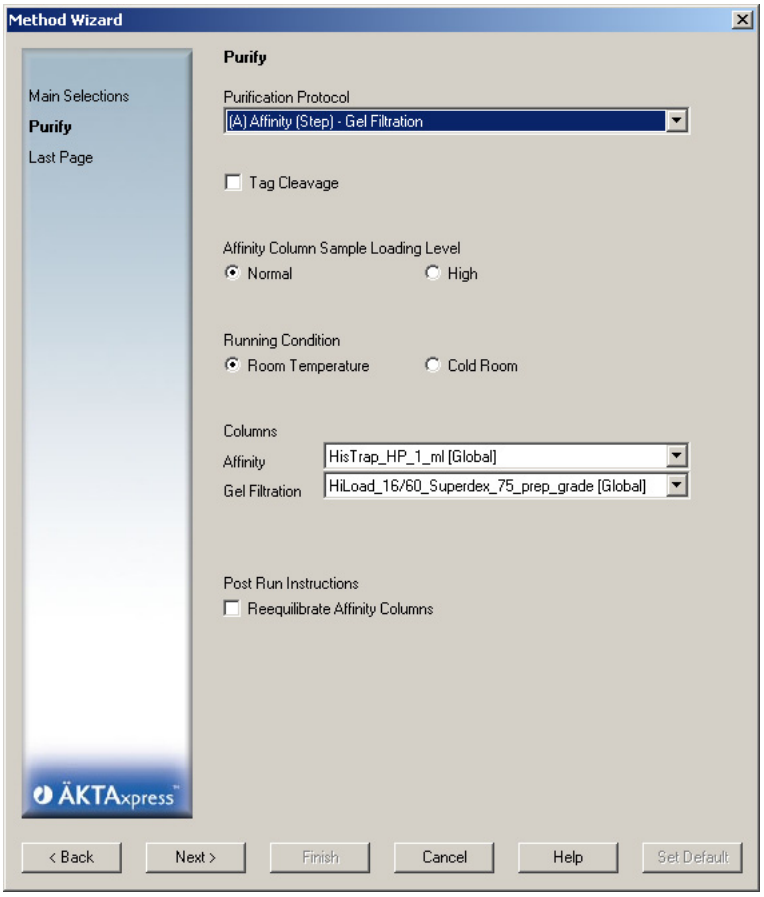
Introduction

This section describes how to create a method plan for a purification run.

Selecting the Purify option

To select a protocol for purification:

Step	Action
1	On the **Main Selections** page, select **Purify**. • **Include System Preparation** For assisted manual filling of the superloop for tag cleavage, to fill the sample inlets before purification, or to clean the sample inlets after sample loading. • **Include Column Equilibration** To equilibrate the columns before starting a purification run. Click **Next**. 

Step	Action
2	Select **Purification Protocol** and the appropriate options on the **Purify** page. The table below describes the options. 

The Purify page options

The table below describes the Purify dialog options and the related actions during the run.

To perform...	Select...	Description/related actions
tag cleavage (not available for Protocol F+G)	**Tag Cleavage**	Includes step for removing the affinity tag from the recombinant protein. See **4.1.4 Affinity tag removal** on page 62 for more information.

To perform...	Select...	Description/related actions
tag cleavage (not available for Protocol F+G)	appropriate **Incubation Time**. Information on factors affecting the cleavage time can be found on **4.1.5 Conditions for cleavage** on page 64.	The time required for the added protease to cut the tag from the protein.
tag cleavage (not available for Protocol F+G)	**Fill Columns with Cleavage Buffer**	Fills the affinity columns with cleavage buffer from inlet A7 before adding protease.
all types of purification runs, except Protocol F+G	appropriate **Affinity Column Sample Loading Level**	Select **High** when overloading the affinity column. It will raise the Level values in Watch commands used for peak detection in order to collect the best part of the peak. See *UNICORN ÄKTAxpress User Reference Manual*.
all types of purification runs	actual **Running Condition**	The actual running condition, room temperature or cold room, for the purification run. If selecting **Cold Room**, the flow rates will be decreased to avoid high pressure due to the higher viscosity of samples and buffers.
all types of purification runs	the **Column(s)** to be used. For information about appropriate columns see **4.2.2 Choosing Columns** on page 70.	Lists showing the available columns

To perform...	Select...	Description/related actions
equilibration of the affinity column(s) after the purification run	**Reequilibrate Affinity Columns**	Equilibrates the affinity column(s) after the purification run with 5 column volumes (CV) of affinity buffer A.
equilibration of the IEX column(s) after the purification run	**Reequilibrate Ion Exchange Column**	Equilibrates the ion exchange column after the purification run with 5 CV of appropriate IEX buffer A.

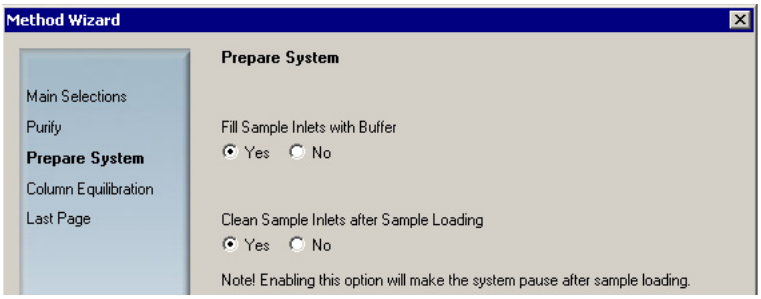
Continuing with, or finishing the method plan

The subsequent pages depend on the selections made on the previous pages. Follow the requested step below.

If selecting...	then...
Purify (only)	click **Next**. The **Last page** appears. Proceed to **5.2.5 Saving the method plan** on page 106.
Prepare System	click **Next**. The **Prepare System** page appears. Continue with the instructions in **5.2.3 Prepare system options** on page 100.
Column Equilibration	click **Next**. The **Column Equilibration** page appears. Continue with the instructions in **5.2.3 Column equilibration options** on page 101.

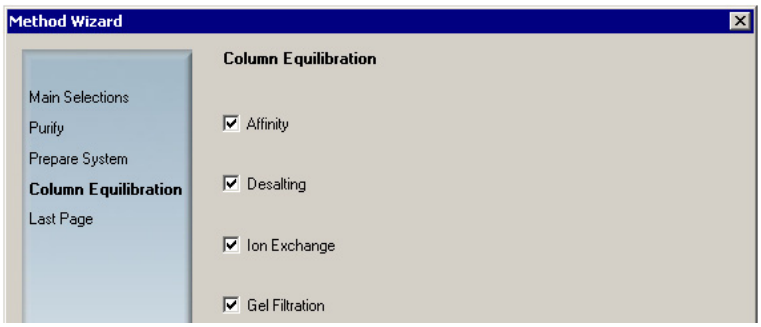
Prepare system options

The options on the **Prepare System** page depend on the choice of purification protocol. In the table below, identify the protocol used and follow the given instruction.

Step	Action
1	**Protocol A–E:**  • **Fill Sample Inlets with Buffer** To fill the sample inlets with affinity buffer A before immersing them in the samples. • **Clean Sample Inlets after Sample Loading** To clean all used sample inlets with a cleaning solution after sample loading. ***Note:*** *During and after these operations the run will Pause and messages appear to guide through the operations. The operator MUST click Continue to proceed the run.* **Protocol F and G:** • **Assisted Sample Loading** To assist when filling the loops with sample. ***Note:*** *When performing the filling, the system will pause and messages appear to guide through the operation.*
2	Click **Next**.

Column equilibration options

To select column equilibration options:

Step	Action
1	On the **Column Equilibration** page, select the type of column(s) to be equilibrated.  During a run, the affinity, ion exchange and desalting columns will be equilibrated with 5 CV, and the gel filtration columns with 2 CV of the appropriate buffer. ***Note:*** *The chosen column(s) will be equilibrated before the sample is loaded on the affinity column.*
2	Click **Next**.

Finishing the creating

When all selections are made, continue with **5.2.5 Saving the method plan** on page 106.

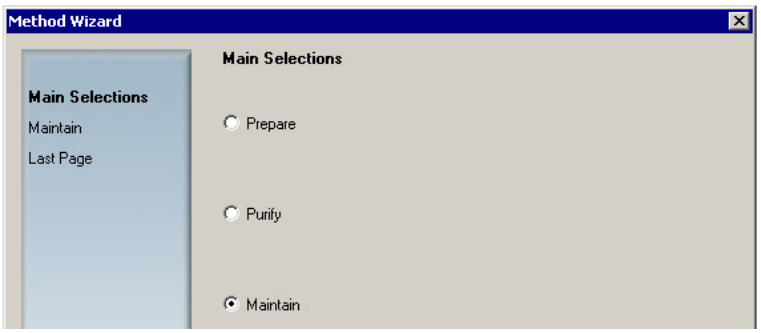
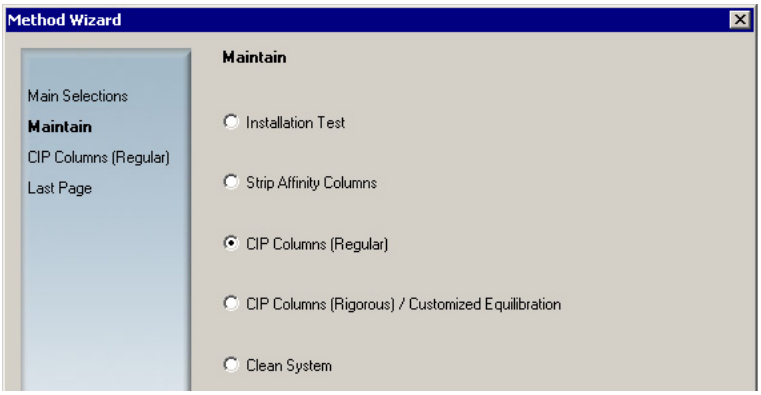
5.2.4 Using the Maintain option

Introduction

This section describes how to create a method plan for maintenance of the system and the columns. The method plan can, for example, consist of cleaning instructions or an installation test.

Creating a method plan for maintenance

To create a method plan for maintenance:

Step	Action
1	On the **Main Selections** page, select **Maintain**. Click **Next**.  ***Note:*** *When entering the Main Selections page, the parameters values on the subsequent pages are set to default.*
2	Select the requested type of maintenance procedure on the **Maintain** page. Click **Next**. 
3	See the description and follow the instructions for the chosen operation in the tables below.

Installation test options

The table below contains a description and instructions for the Installation test options.

Maintain operation	Description/Instruction
Installation Test	Tests the function of the pump and the switch valves. • Proceed to **5.2.4 Finishing the Maintain method plan** on page 105.

Strip affinity columns options

The table below contains a description and instruction for the Strip affinity columns options.

Maintain operation	Description/Instruction
Strip Affinity Columns	Strips metal ions off the affinity column(s) using 5 CV of strip buffer followed by 10 CV of deionized water. 1. Select type of column, number of columns, and running conditions. 2. Proceed to **5.2.4 Finishing the Maintain method plan** on page 105.

CIP Columns regular options

The table below contains a description and setting instructions for the CIP Columns Regular option.

Maintain operation	Description/Instruction
CIP Columns (Regular)	Cleans the chosen columns with two different cleaning solutions. The type of solutions and the amount for each column depend on the column choices. See the Summary page in the wizard when starting a run, or the instructions supplied with the column. 1. Select running conditions, and whether to wash the columns with water before the CIP or not. 2. Select columns and column positions (only the DS column). 3. Proceed to **5.2.4 Finishing the Maintain method plan** on page 105.

CIP Columns rigorous/ Customized equilibration options

The table below contains a description and instructions for the CIP Columns Rigorous and Customized equilibration option.

Maintain operation	Description/Instruction
CIP Columns (Rigorous) and Customized equilibration	• Cleans the chosen column with up to nine cleaning solutions, *or* • Equilibrates the column with an optional number of solutions. Recommended for removing ethanol from new columns and for adding ethanol to columns for storage. For the required amounts of solutions, see the Summary page in the wizard when starting a run. ***Note:*** *Only one column type can be included.* 1. Select column type and the number of columns. 2. Select **CIP** or **Equilibration**. 3. Select the number of steps. For each step, make the following selections (if there is more than one cleaning step, additional pages appear by clicking **Next**): A To perform a pump wash before the cleaning step, select **PumpWash**. B Enter an appropriate **Flow Rate** according to the instructions supplied with the column. C Select the buffer **Inlet** to be used. D Enter an appropriate **Volume** (in column volumes) according to the instructions supplied with the column. 4. Enter a pause time in **Pause before Next Step** if incubation of the column in the chosen solution is required. 5. Proceed to **5.2.4 Finishing the Maintain method plan** on page 105.

Clean system options

The table below contains a description and instructions for the Clean System option.

Maintain operation	Description/Instruction
Clean System	Cleans the chosen tubing, loops, and valves with one cleaning solution at a time. When running the method, the method will pause and a message appear when the inlets should be inserted into a new cleaning solution. The loops, column valve and outlets will be cleaned using inlet A1. 1. Select the **Sample InletsS1–S4** and the **Buffer Inlets A1–A8**, **B1** and **B2** to clean. 2. Select **Clean Outlets** to clean all outlets. A microplate must be placed in the fraction collector. 3. Select **Clean loop 1–4** to clean all four loops. If **Loop 5** is selected, it must be a capillary loop. 4. Select **Clean Column Valve** to clean all column positions. All positions require bypass tubing. 5. Select **Number of Cleaning Solutions** to use. 6. Proceed to **5.2.4 Finishing the Maintain method plan** on page 105.

Finishing the Maintain method plan

To finish the Maintain method plan:

Step	Action
1	When all selections are made, click **Next.** *Result*: The Last Page appears.
2	Proceed to **5.2.5 Saving the Method plan** on page 106.

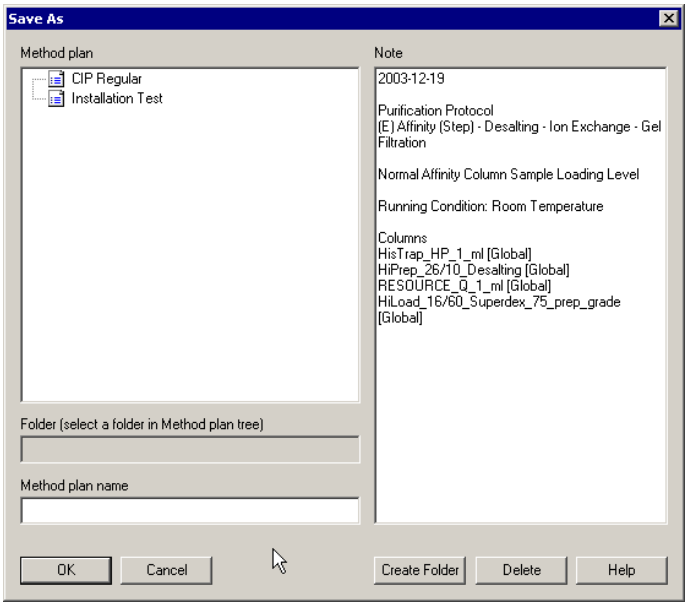
5.2.5 Saving the method plan

Introduction

This section describes how to save the method plan.

Saving the method plan

To save the method plan:

Step	Action
1	On the **Last Page** page: • click **Finish** to complete the method plan (go to step 2), or • click **Next** to enter the Advanced Zone for changing parameter values. See **5.5 Advanced zone** on page 109.
2	On the **Save As** page, select destination folder in the **Method plan** tree. • Click **Create Folder** to create a new folder, if preferred. In the dialog that appears, enter the name. Click **OK**. • Additional information can be typed in the **Notes** field. Useful for example for identifying old method plans by the Method Notes to reconstruct them after changing the strategy. 
3	Enter a name of the method plan and click **OK**. *Result*: The method plan is saved.

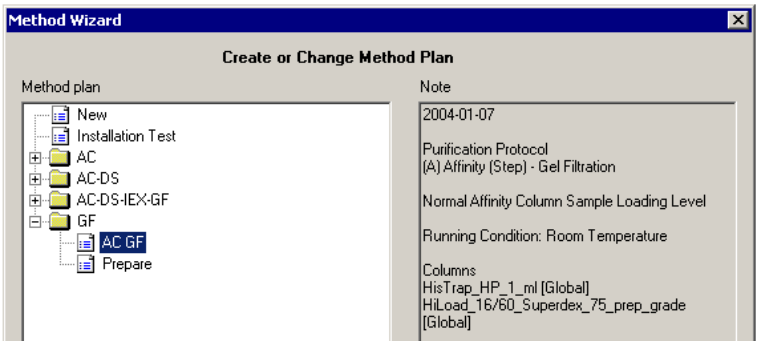
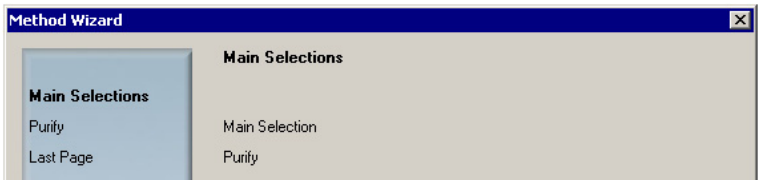
5.3 Editing a method plan

Introduction

This section describes how to edit an existing method plan.

Editing a method plan

To edit a method plan:

Step	Action
1	In the **Method plan** list in the Method Wizard, select the method plan to be edited. 
2	Click **Next**. *Result*: The Main Selections page appears. The parameters values cannot be edited in this part of the Method Wizard. 
3	Click **Next** repeatedly to enter Advanced Zone.
4	Make the appropriate selections and edit the desired parameter values as described in **5.5.1 Advanced Zone introduction** on page 110 and the subsequent sections.
5	Save the method plan as described in **5.2.5 Saving the method plan** on page 106. ***Note:*** *The changes can be written in the Notes field in the Save As dialog.*

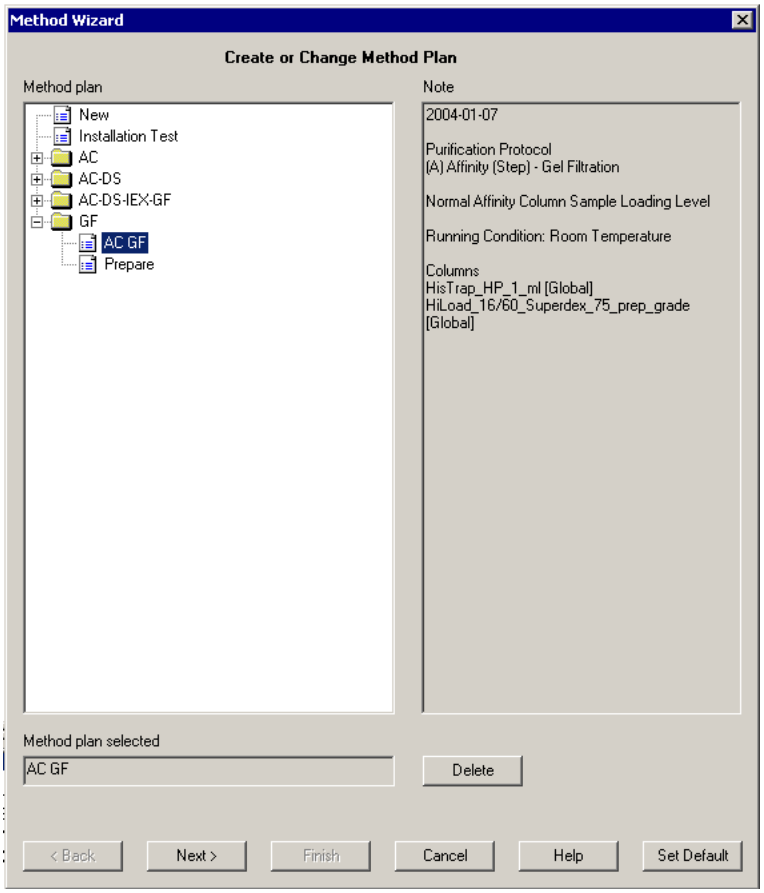
5.4 Deleting a method plan

Introduction

This section describes how to delete an existing method plan.

Deleting a method plan

To delete a method plan:

Step	Action
1	In the **Method plan** list in the Method Wizard, select the method plan to be deleted. 
2	Click **Delete**. *Result*: A confirmation dialog appears.
3	Click **Yes** in the dialog. *Result*: The method plan is deleted and disappears from the Method plan list.

5.5 Advanced Zone

About this section

This section describes how to change parameter values in the Advanced Zone in the Method Wizard.

In this section

This section contains the following sub-sections

5.5.1 Advanced Zone introduction

Introduction

This section describes parameters in Advanced Zone and how to change them.

The Advanced Zone

The Advanced Zone in the Method Wizard is used for viewing and changing parameter values in method plans. In new method plans the default values are shown. They are preoptimized, but can be changed to meet specific demands. Values in new method plans as well as in previously made method plans can be changed.

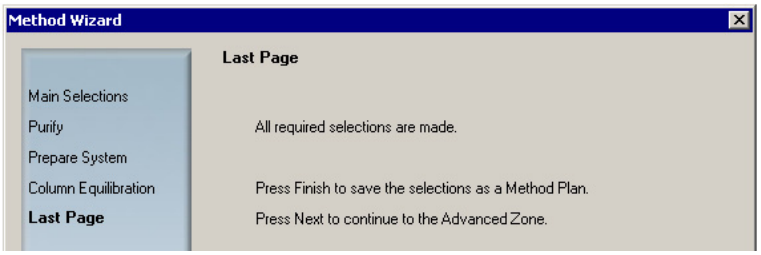
Note: *Avoid changing default parameter values in a method plan unless the result is clearly understood. Changing a parameter value might require adjustments of other parameter values as well.*

Advanced Zone content

The content in Advanced Zone depends on the parameters selected on the first page in Advanced Zone. Only pages that are related to these parameters will be shown. If a selection or parameter value in Advanced Zone needs to be changed, the Back and Next buttons can always be used to return to the Advanced Zone page in question. Entering the Last Page in Advanced Zone will though lock the main selections made in the first part of the Method Wizard.

Entering the Advanced Zone

To enter the Advanced Zone:

Step	Action
1	When all selections are made in the Method Wizard, the Last Page appears. • Click **Next**. 
2	On **Page 1**, select the parameters that should be changed. The following sections describe the main procedures for changing parameter values: • Advanced Zone for Prepare • Advanced Zone for Purify – *chromatographic technique* • Advanced Zone for Maintain

5.5.2 Advanced Zone for Prepare

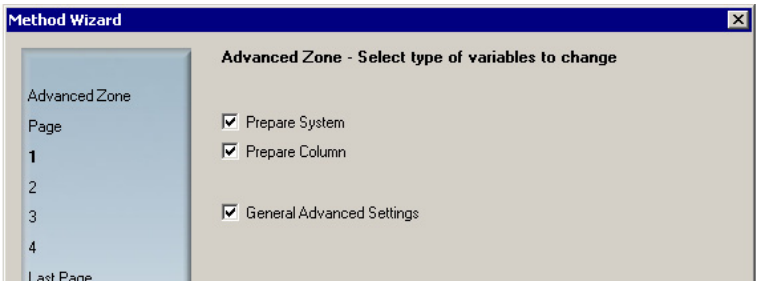
Introduction

This section describes the Advanced Zone for a Prepare method plan.

Note: *Make sure that the parameter values do not exceed the specification of the chosen column.*

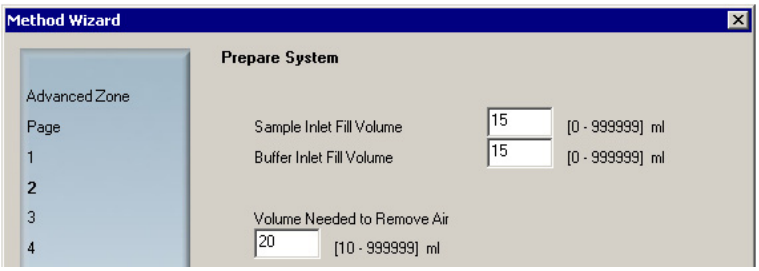
Selecting parameters

To select parameters:

Step	Action
1	Select the required options on **Page 1**. (Select **General Advanced Settings** to define a main folder for storing the results.) 
2	Click **Next**. *Result*: The next selected page appears.

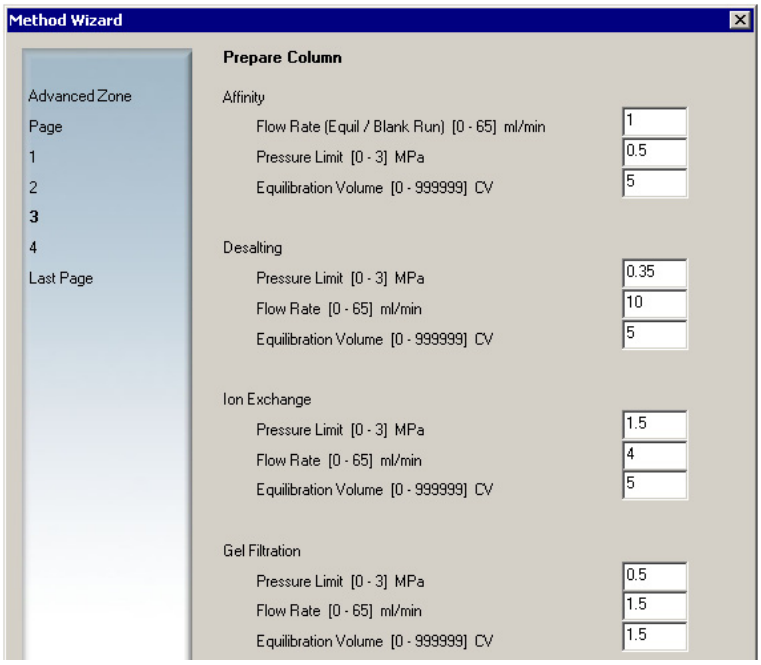
Changing Prepare System parameters

To change Prepare System parameters if the options have been selected in the first part of the Method Wizard:

Step	Action
1	Change the desired values on **Page 2**.  **Sample Inlet Fill Volume** Volume used for filling the sample inlet tubing. **Buffer Inlet Fill Volume** Volume used for filling the buffer inlet tubing with buffer before immersing the tubing in the samples. **Volume Needed to Remove Air** Volume used to remove air from the pump after the inlets have been filled.
2	Click **Next**. *Result*: The next selected page appears.

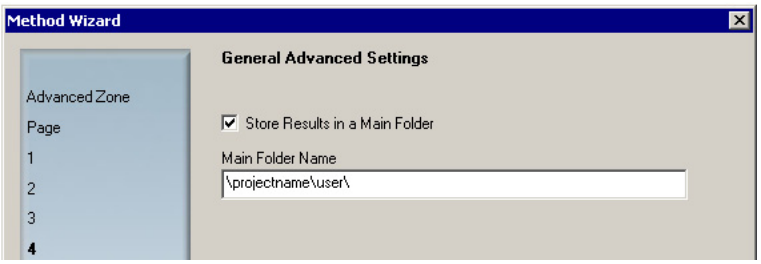
Changing Prepare Column parameters

To change Prepare Column parameters if the options have been selected in the first part of the Method Wizard:

Step	Action
1	Change the desired values on **Page 3**. ***Note:*** *The parameter available depend on the choices made earlier in the Method Wizard.*  **Pressure limit** Upper pressure limit when equilibrating a column. **Flow Rate** Flow rate when equilibrating a column. **Equilibration Volume** Volume used for equilibrating a column.
2	Click **Next**. *Result*: The next selected page appears.

Defining a folder for storing the results

To define a sub folder under the Home folder for storing the results :

Step	Action
1	Enter the desired folder name.  **Store Results in Main Folder** To store the results in a sub folder under the Home folder, fill in the name of the folder in the field. If the name of the new folder do not exist, the folder is created automatically.
2	Click **Next** to save the method plan. See **5.2.5 Saving the method plan** on page 106.

5.5.3 Advanced Zone for Purify

Introduction

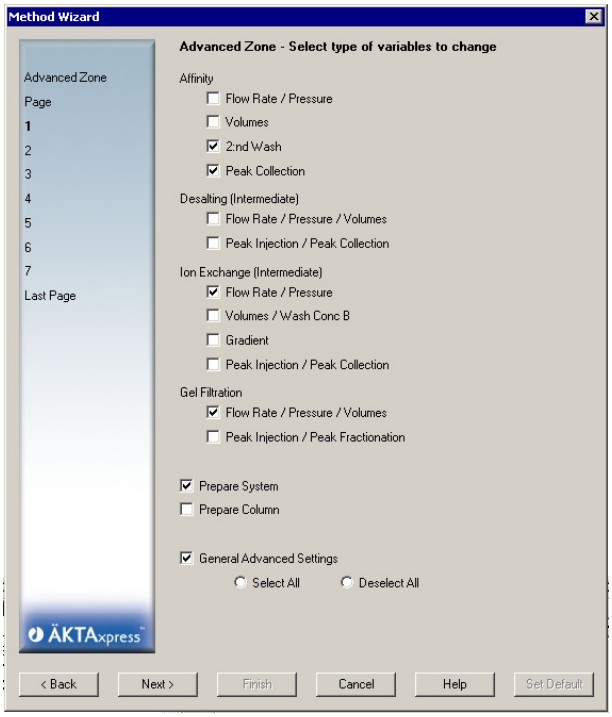
This section gives an introduction to the Advanced Zone for a Purify method plan. It also describes the principles of peak handling and how watch conditions are used for peak collection and fractionation.

Note: *Avoid changing default parameter values in a method plan unless the result is clearly understood. Changing a parameter value might require adjustments of other parameter values as well.*

Entering the Advanced Zone

The first page that appears in the Advanced Zone contains a list of parameters that can be changed in the current method plan. The method plan is based on the selections made in the first part of the Method Wizard.

To select parameters:

Step	Action
1	Select the required options on **Page 1**. 
2	Click **Next** to go through the subsequent pages.
3	On **Last Page** click **Next** to save the method plan. See **5.2.5 Saving the method plan** on page 106.

Changing Purify parameters

The pages appearing in the Advanced Zone corresponds to the selected options on Page 1. The following sections describe all options and parameters available for each of the chromatography techniques respectively. The options related to preparing the system and columns, and General Advanced Settings are described in separate sections. See the table below.

For parameters regarding...	See...
affinity	**5.5.4 Advanced Zone Purify - AC** on page 120
desalting	**5.5.5 Advanced Zone Purify - DS** on page 125
ion exchange	**5.5.6 Advanced Zone Purify - IEX** on page 128
gel filtration	**5.5.7 Advanced Zone Purify - GF** on page 132
preparing the system	**5.5.8 Advanced Zone Purify - Prepare System** on page 134
preparing the columns	**5.5.8 Advanced Zone Purify - Prepare Colums** on page 134
general advanced settings	**5.5.9 Advanced Zone Purify - General Adv. Settings** on page 136

Peak handling in ÄKTAxpress

All purification steps in the standard protocols in the Method Wizard include peak handling. In Advanced Zone some of the parameters that control peak handling can be changed. The parameters are divided in two categories:

- Peak collection
- Peak fractionation

The *peak collection* parameters are used in watch conditions to determine how peaks are collected between the purification steps. These peaks are collected in one or several of the five loops. One peak can also be divided into several loops depending upon the volume. The largest peak is then used in the next purification step. Peaks that are detected when all loops are occupied are collected in a separate outlet tube.

The *peak fractionation* parameters determine how peaks are collected at the final fractionation during the last purification step. These peaks, containing the final product of the purification run, are collected in the fraction collector. The collection in the microplate is performed in a serpentine manner i.e. first in A1, A2, ..., A11, A12 and then in B12, B11, ..., B2, B1, and so on.

Peak collection and watch conditions

The peak collection is controlled by a series of UV signal watch conditions included in the method. When a watch condition is met, a predefined action is issued, for example start collecting a peak. Another watch is then activated for detecting the peak maximum, and so on.

The table below describes the basic watch instructions available in the ÄKTAxpress Method Wizard. Other instructions are combinations of these:

Option	Explanation
Greater_Than	The signal exceeds a certain value.
Less_Than	The signal falls below a certain value.
Slope_Greater_Than	The slope of the the signal curve exceeds a specified value.
Less_Than_Or_Valley	The signal falls below a specified value or a valley is detected. A valley is detected only after a **Peak_Max** has been detected, and the valley is defined by a local minimum followed by an increase to 102% of the local minimum plus the **Delta_Peak** value.
Peak_Max	The signal falls to a specified fraction of the most recent peak maximum minus the **Delta_Peak** value.
Stable_Baseline	The signal is stable within the limits of the **Delta_Base** value for a specified time interval.
Stable_Plateau	The signal is stable within the limits of the **Delta_Plat** value for a specified time interval.

Note: *If the flow rate is changed, the shape of the peak will change as well. Hence, the slope values might need to be changed accordingly.*

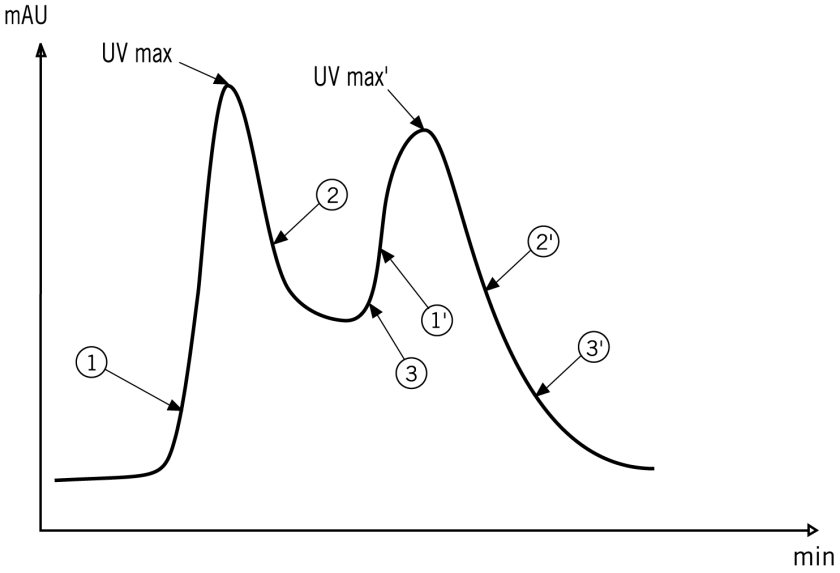
For more information on watch instructions, see *UNICORN User Reference Manual for ÄKTAxpress*.

Example of using watch combinations

This example shows how peak collection is performed in ÄKTAxpress using watch conditions. The following watch conditions are used:

1. Greater_Than **AND** Slope_Greater_Than
2. Peak_Max with Factor
3. Less_Than_**or**_Valley **OR** Stable_Plateau

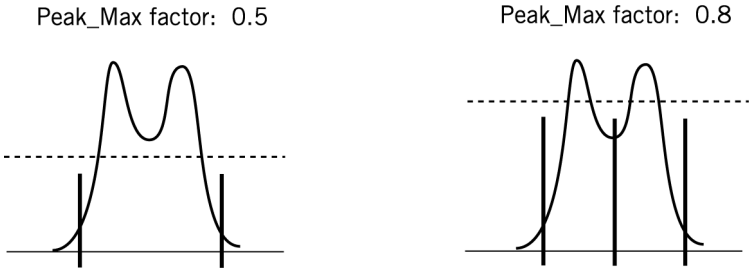
The illustration below shows where the watch conditions are met.



Example of using the Peak_Max factor

The Peak_Max factor is important for dectecting a valley since Peak_End cannot be detected unless Peak_Max has been detected.

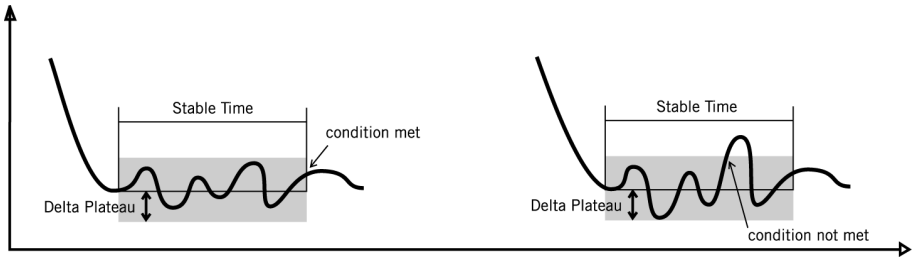
The illustration below shows how two different Peak_Max factors affects the detection of a Valley and collection of double peaks:



Example of using Stable_Plateau

The condition Stable_Plateau is met if the UV signal does not deviate by more than the Delta_Plat value during the time interval specified for the watch.

The illustration below shows an example of this:

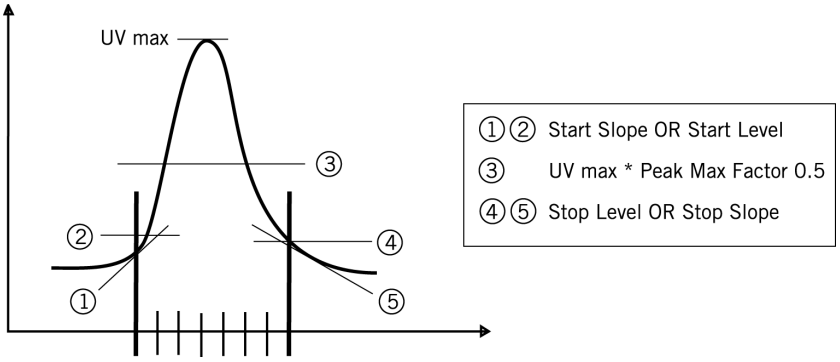


Peak fractionation parameters

The final peak fractionation does not involve any watch instructions. The fractionation simply starts when the signal level and/or the slope of the signal curve exceeds a defined value. During the fractionation, a specified maximum volume is collected in each well in the fraction collector.

The fractionation continues at least until the Minimum Peak Width time has elapsed. The fractionation stops when the signal level and/or the slope of the signal curve falls below a defined value. The Stop Slope condition cannot be met unless Peak_Max has been detected.

The illustration below describes this:



Note: *If the flow rate is changed, the shape of the peak will change as well. Hence, the slope values might need to be changed accordingly.*

Note: *When the fractionation of a peak is finished, it will in the chromatogram appear as the subsequent fraction volume is very large. This is because the position of the fraction collector is shown and only the delay volume for the next peak (if any) actually is collected in this well.*

5.5.4 Advanced Zone for Purify – Affinity

Introduction

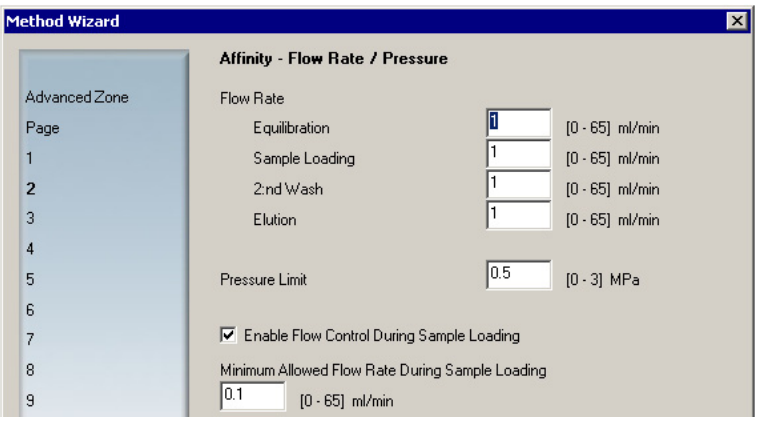
This section describes the parameters used in a standard affinity step during a purification run. Some of the default parameter values depend on the chosen column.

Note: *Make sure that the parameter values do not exceed the specification of the chosen column.*

Tag cleavage parameters are described separately since they appear only when tag cleavage is selected.

Flow rate/Pressure parameters

The first affinity page that appears contains options and parameters related to flow rate and pressure, as described below:



Equilibration Flow rate when equilibrating the affinity columns.

Sample Loading Sample loading flow rate.

2nd Wash Flow rate used in the second wash.

Elution Flow rate during the elution of the affinity columns.

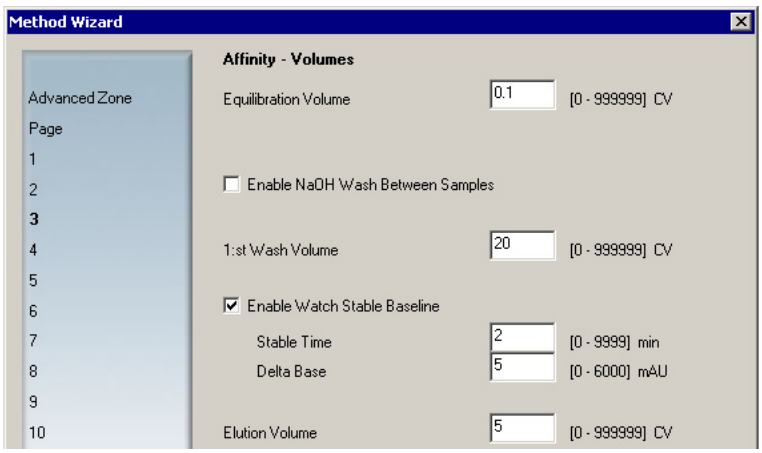
Pressure Limit Upper pressure limit during the affinity step.

Enable Flow Control During Sample Loading Automatic flow control when loading the sample. Prevents the pressure from exceeding the maximum limit by reducing the flow. If the flow falls below the **Minimum Allowed Flow rate**, an Alarm is issued and the system enters Pause mode.

See also **6.5.11 Using flow control during sample loading** on page 184.

Volume parameters

The volume options and parameters are described below:



Equilibration Volume The volume used for equilibrating the affinity column.

Enable NaOH Wash Between Samples To wash the pump, mixer, and injection valve with NaOH (inlet A6) followed by affinity buffer A between the sample loadings.

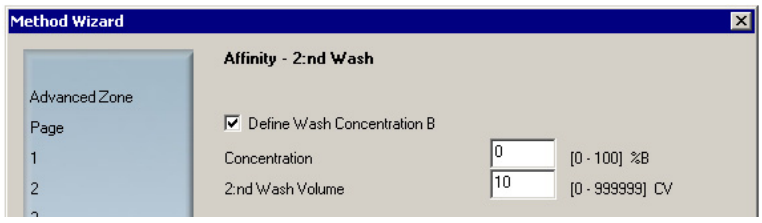
1:st Wash Volume The volume of affinity buffer A used for washing out unbound sample.

Enable Watch Stable Baseline To check the stability of the UV signal during a specified time interval. The Stable Baseline condition is met when the signal fluctuates less than the set **Delta Base** value during the set **Stable Time** interval. If Watch Stable Baseline is enabled, the first wash step will be completed when the baseline is stable.

Elution Volume The volume used for elution of the affinity column.

2:nd Wash parameters

The second wash is a harsher wash used to remove contaminants, loosely bound protein, residues etc. from the affinity column. The volume options and parameters are described below:

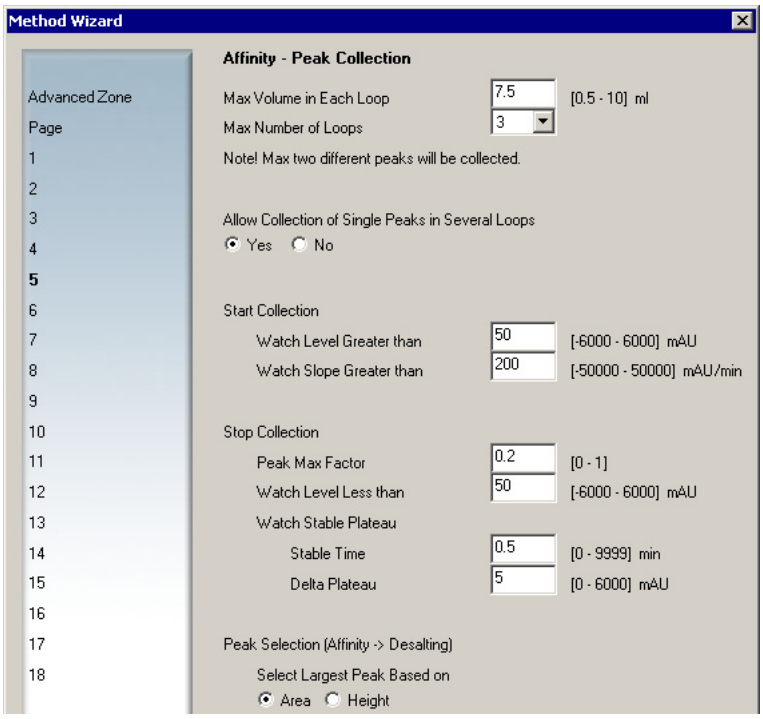


Define Wash Concentration B To wash out contaminants from the column(s). Choose **Concentration** of B to use in the wash step and the **2:nd Wash volume**.

Note: The default setting for the second wash is 0% B which means that affinity buffer A is used.

Peak collection parameters

The peak collection options and parameters are described below. The parameters define how the peaks are collected in the loops.



Max Volume in Each Loop The maximum volume allowed in each loop. Using volumes above 7.5 ml might cause sample loss due to band broadening.

Max Number of Loops The maximum number of loops used to collect peaks in the affinity step. Maximum two peaks will be collected in loops, but more than two loops might be required to collect large peaks.

Allow Collection of Single Peaks in Several Loops To collect single peaks, with a volume larger than Max Volume in Each Loop, in several loops.

Start Collection The peak collection starts when both the UV level exceeds the **Watch Level Greater than** value, **and** the UV slope exceeds the **Watch Slope Greater than** value.

Note: If the flow rate is changed, the shape of the peak will change as well. Hence, the slope values might need to be changed accordingly.

Stop Collection The Peak_Max condition occurs when the UV signal has fallen to a fraction specified by **Peak Max factor** of the most recent peak maximum.

The Peak end is detected when the Peak_Max condition is met *AND*:

- the signal falls below the **Watch Level Less than** value, *OR*
- a Valley is detected, *OR*
- a Watch StablePlateau condition is met. The condition is met when the signal is stable within the limits of the **Delta Plateau** value during the **Stable Time** interval.

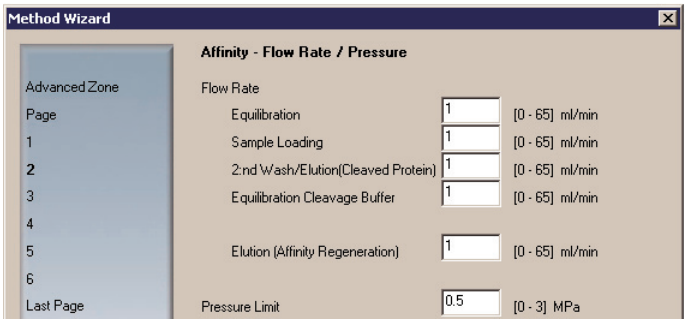
Peak Selection Select Largest Peak Based on either peak height or peak area to be transferred to the next column. Area is the default value.

For more information on peak handling and watch conditions, see **5.5.3 Peak handling and watch conditions** on page 116.

Tag cleavage parameters

The tag cleavage parameters are shown only when tag cleavage is selected. In addition, a few other standard parameters are used differently.

Flow rate/Pressure parameters



Equilibration Flow rate when equilibrating the affinity columns.

Sample Loading Sample loading *and* protease injection flow rate.

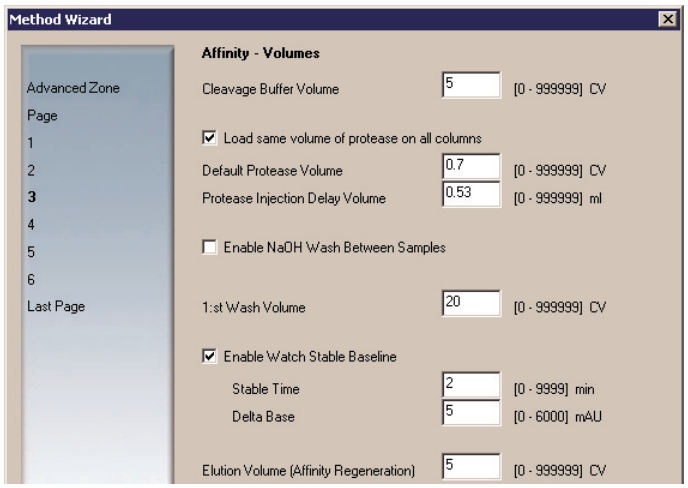
2:nd Wash/Elution(Cleaved Protein) Flow rate when washing out the unbound sample from the column(s) before the protease is injected onto the column(s). Also the flow rate used when eluting the cleaved protein after protease incubation.

Equilibration Cleavage Buffer Flow rate when loading the cleavage buffer onto the column before loading the protease solution.

Elution (Affinity Regeneration) The flow rate used to regenerate the affinity column, i.e. elution of tags, tagged protease, and uncleaved protein using affinity elution buffer.

Pressure Limit Upper pressure limit during the affinity step.

Volume parameters



Cleavage Buffer Volume Cleavage buffer volume applied before loading the protease solution.

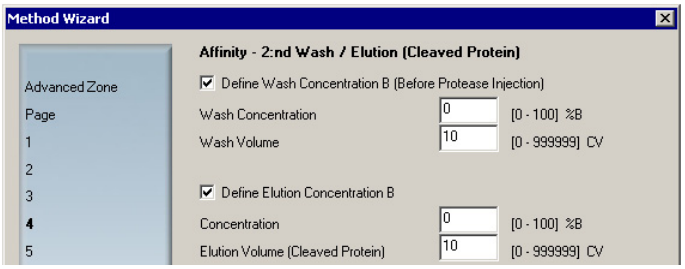
Load same volume of protease on all columns To load the same protease volume on all columns, as entered in **Default Protease Volume**. To use separate volumes for different columns, deselect this option. The separate volumes have to be entered in the System Control wizard before starting the run. The **Protease Injection Delay Volume** is the volume between the loop valve and the column valve.

Enable NaOH Wash Between Samples To wash the pump, mixer, and injection valve with NaOH (inlet A6) followed by affinity buffer A between the sample loadings.

Enable Watch Stable Baseline To check the stability of the UV signal during a specified time interval. The Stable Baseline condition is met when the signal fluctuates less than the set **Delta Base** value during the set **Stable Time** interval. If Watch Stable Baseline is enabled, the first wash step will be completed when the baseline is stable.

Elution Volume (Affinity regeneration) The volume of affinity elution buffer used to regenerate the affinity column, i.e. elution of tags, tagged protease, and uncleaved protein.

2:nd Wash parameters



Define Wash Concentration B (Before Protease Injection) To wash out the unbound sample from the column(s) before the protease is injected onto the column(s). Choose **Wash Concentration** %B in the wash solution and the **Wash volume**.

Define Elution Concentration B Choose **Concentration** %B used during the elution of the cleaved protein. **Elution Volume (Cleaved Protein)** is the buffer volume used.

For more information on tag cleavage, see **4.1 Protocols and affinity tag removal** on page 54.

5.5.5 Advanced Zone for Purify – Desalting

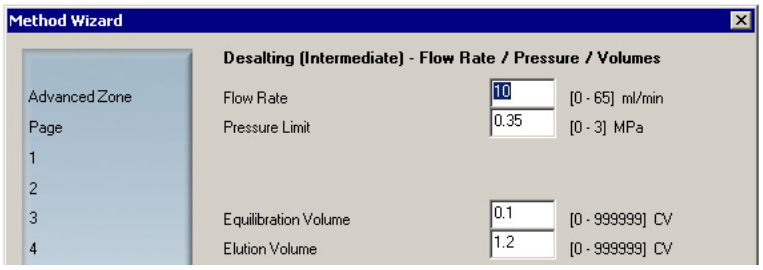
Introduction

This section describes the parameters used in the desalting step for the protocols B–F. Some of the default parameter values depend on the chosen column.

Note: *Make sure that the parameter values do not exceed the specification of the chosen column.*

Flow rate/Pressure/Volumes parameters

The first desalting page that appears contains options and parameters related to flow rate, pressure, and volumes.



Flow Rate Flow rate during the desalting step.

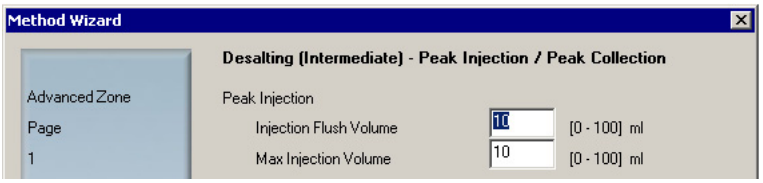
Pressure Limit Upper pressure limit during the desalting step.

Equilibration volume Volume used when equilibrating the desalting column.

Elution Volume Volume used during the elution of the desalting column.

Peak injection parameters

The peak injection options and parameters:



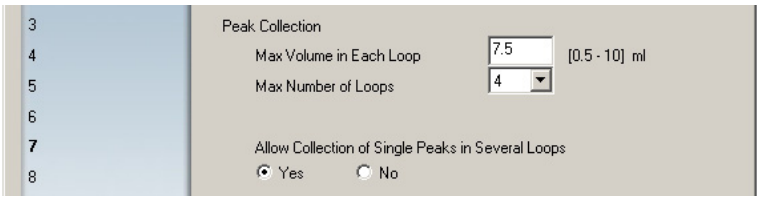
Injection Flush Volume The extra volume besides the peak volume used to flush the loops when loading the sample onto the desalting column.

Max Injection Volume The maximum volume to be injected onto the desalting column.

Note: *The maximum injection volume is set up to make sure that the peak volume + flush volume do not exceed the maximum sample loading volume of the column (10 ml for the HiPrep Desalting column).*

Peak collection parameters

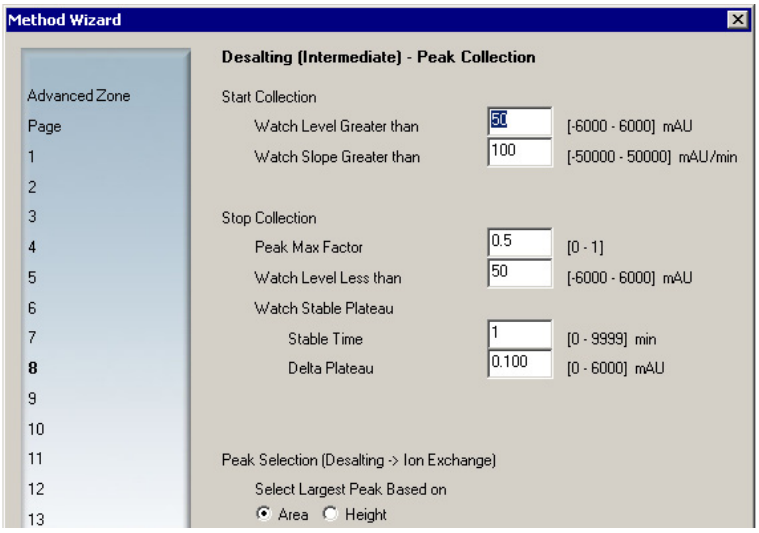
The peak collection options and parameters for the protocols C–E are described below. The parameters define how the peaks are collected in the loops.



Max Volume in Each Loop The maximum volume allowed in each loop. Using volumes above 7.5 ml might cause sample loss due to band broadening.

Max Number of Loops The maximum number of loops used to collect peaks in the desalting step.

Allow Collection of Single Peaks in Several Loops To collect single peaks, with a volume larger than Max Volume in Each Loop, in several loops.



Start Collection The peak collection starts when both the UV level exceeds the **Watch Level Greater than** value, AND the UV slope exceeds the **Watch Slope Greater than** value.

Note: If the flow rate is changed, the shape of the peak will change as well. Hence, the slope values might need to be changed accordingly.

Stop Collection The Peak_Max condition occurs when the UV signal has fallen to a fraction specified by **Peak Max factor** of the most recent peak maximum.

The Peak end is detected when the Peak_Max condition is met *AND*:

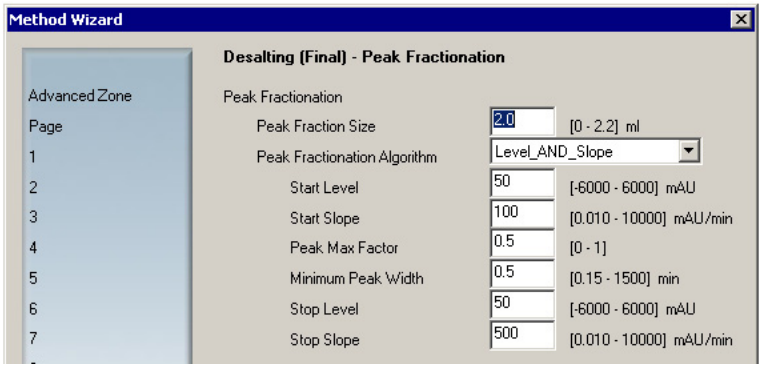
- the signal falls below the **Watch Level Less than** value, *OR*
- a Valley is detected, *OR*
- a Watch StablePlateau condition is met. The condition is met when the signal is stable within the limits of the **Delta Plateau** value during the **Stable Time** interval.

Peak SelectionSelect Largest Peak Based on either peak height or peak area to be transferred to the next column. Area is the default value.

For more information on peak handling and watch conditions, see **5.5.3 Peak handling and watch conditions** on page 116.

Peak fractionation

The peak fractionation options and parameters for the protocols B, D and F are described below. The parameters define the final fractionation and how the final peaks are collected in the fraction collector.



Peak Fraction Size The maximum volume of each peak fraction.

Peak Fractionation Algorithm Choose to start and stop the peak fractionation based on the signal curve:

- **Level**
- **Slope**
- **Level_AND_Slope**
- **Level_OR_Slope**

AND means that both conditions have to be met, OR means that only one condition has to be met.

The fractionation start values are set by **Start Level** and **Start Slope**, and the stop values by **Stop Level** and **Stop Slope**. Different start and stop values for level and slope can be set.

The Stop Slope condition cannot be met unless the Peak_Max condition is met. Stop Level can be met before Peak_Max. The Peak_Max condition occurs when the UV signal has fallen to a fraction specified by **Peak Max factor** of the most recent peak maximum.

Note: If the flow rate is changed, the shape of the peak will change as well. Hence, the slope values might need to be changed accordingly.

Minimum Peak Width sets the minimum fractionation time of a single peak. The fractionation of the current peak continues at least until the Minimum Peak Width is reached, even if the signal is fluctuating.

5.5.6 Advanced Zone for Purify – Ion exchange

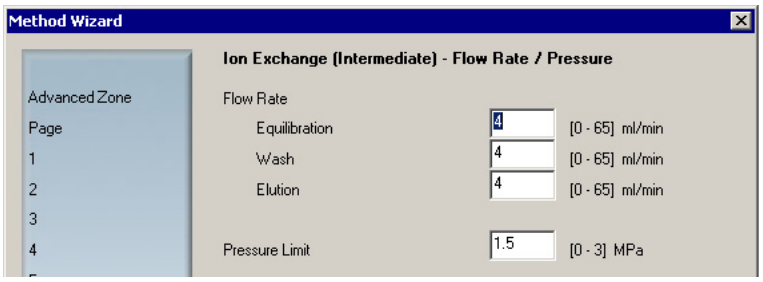
Introduction

This section describes the parameters used in the ion exchange steps for the protocols C–E. Some of the default parameter values depend on the chosen column.

Note: Make sure that the parameter values do not exceed the specification of the chosen column.

Flow rate/Pressure parameters

The first ion exchange page that appears contains options and parameters related to flow rate and pressure, as described below:



Equilibration Flow rate when equilibrating the ion exchange column.

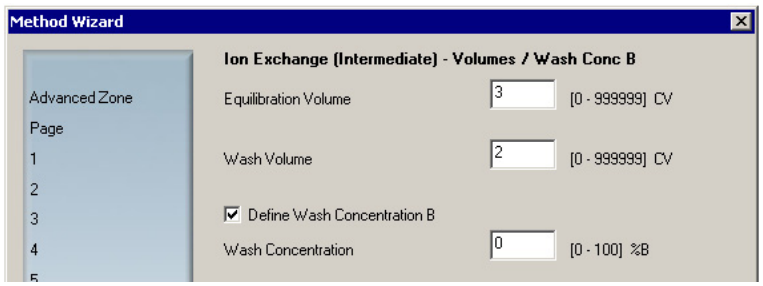
Wash Flow rate when washing out unbound sample from the ion exchange column after sample injection.

Elution Flow rate during the elution.

Pressure Limit Upper pressure limit during the ion exchange step.

Volumes/Wash Conc B parameters

The volume and wash options and parameters are described below:



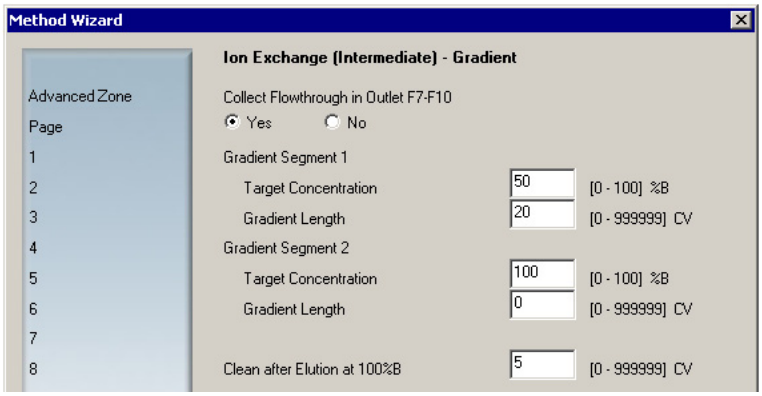
Equilibration Volume Volume used for equilibrating the ion exchange column.

Wash Volume Volume used for washing out unbound sample from the ion exchange column after sample injection.

Define Wash Concentration B Concentration %B of ion exchange buffer B2 for washing out the unbound sample.

Gradient parameters

The gradient options and parameters are described below.



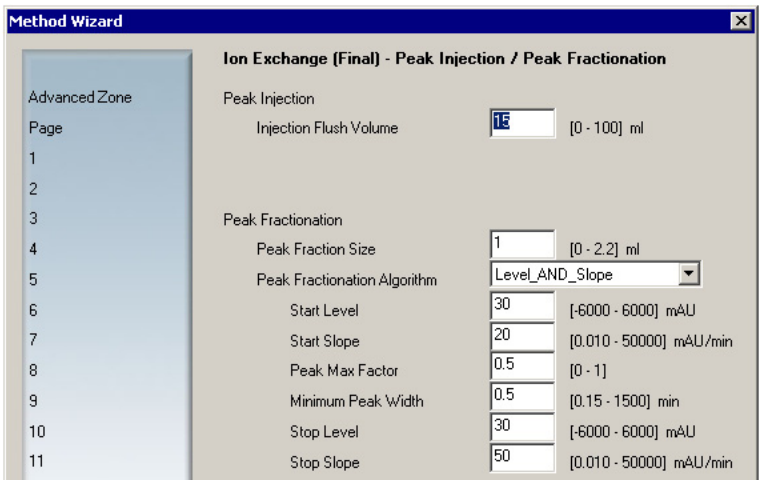
Collect Flowthrough in Outlet F7–F10 To collect the wash volume in outlets F7–F10. Sample 1 in F7, sample 2 in F8, etc.

Gradient Segment 1 and **2** The **Target Concentration** and the **Gradient Length** are used to define two different gradient segments.

Clean after elution at 100%B Volume of buffer B2 used to wash the column after the gradient segments.

Peak injection parameters

The peak injection options and parameters are described below:



Injection Flush Volume The extra volume besides the peak volume used to flush the loops when loading the sample onto the ion exchange column.

Peak fractionation

The peak fractionation options and parameters for protocol C are described below. The parameters define the final fractionation.

Peak Fraction Size The maximum volume of each peak fraction.

Peak Fractionation Algorithm Choose to start and stop the peak fractionation based on the signal curve:

- **Level**
- **Slope**
- **Level_AND_Slope**
- **Level_OR_Slope**

AND means that both conditions have to be met, OR means that only one condition has to be met.

The fractionation start values are set by **Start Level** and **Start Slope**, and the stop values by **Stop Level** and **Stop Slope**. Different start and stop values for level and slope can be set.

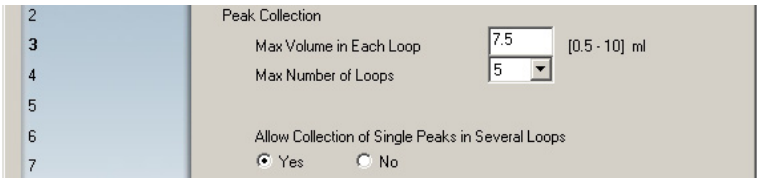
The Stop Slope condition cannot be met unless the Peak_Max condition is met. Stop Level can be met before Peak_Max. The Peak_Max condition occurs when the UV signal has fallen to a fraction specified by **Peak Max factor** of the most recent peak maximum.

Note: *If the flow rate is changed, the shape of the peak will change as well. Hence, the slope values might need to be changed accordingly.*

Minimum Peak Width sets the minimum fractionation time of a single peak. The fractionation of the current peak continues at least until the Minimum Peak Width is reached, even if the signal is fluctuating.

Peak collection

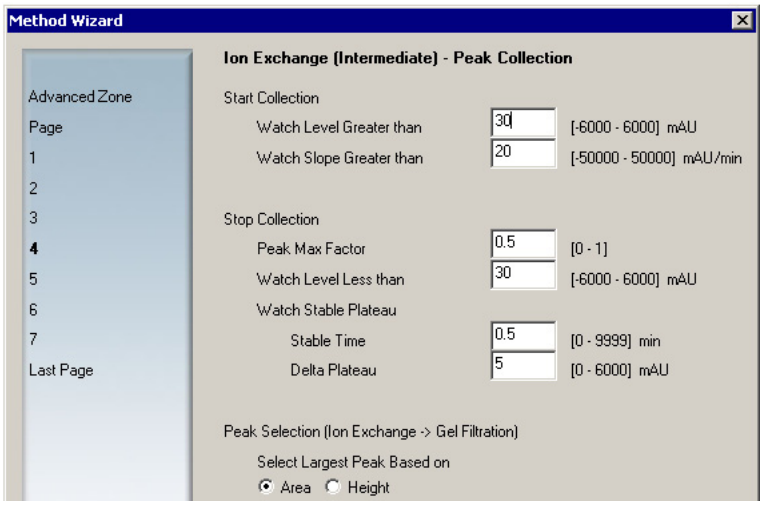
The peak collection options and parameters for the protocols D and E are described below. The parameters define how the peaks are collected in the loops.



Max Volume in Each Loop The maximum volume allowed in each loop. Using volumes above 7.5 ml might cause sample loss due to band broadening.

Max Number of Loops The maximum number of loops used to collect peaks in the ion exchange step.

Allow Collection of Single Peaks in Several Loops Collect single peaks in several loops. To be used when the peak volume is larger than the value in Max Volume in Each Loop.



Start Collection The peak collection starts when both the UV level exceeds the **Watch Level Greater than** value, AND the UV slope exceeds the **Watch Slope Greater than** value.

Note: If the flow rate is changed, the shape of the peak will change as well. Hence, the slope values might need to be changed accordingly.

Stop Collection The Peak_Max condition occurs when the UV signal has fallen to a fraction specified by **Peak Max factor** of the most recent peak maximum.

The Peak end is detected when the Peak_Max condition is met *AND*:

- the signal falls below the **Watch Level Less than** value, *OR*
- a Valley is detected, *OR*
- a Watch StablePlateau condition is met. The condition is met when the signal is stable within the limits of the **Delta Plateau** value during the **Stable Time** interval

Peak SelectionSelect Largest Peak Based on either peak height or peak area to be transferred to the next column. Area is the default value.

For more information on peak handling and watch conditions, see **5.5.3 Peak handling and watch conditions** on page 116.

5.5.7 Advanced Zone for Purify – Gel filtration

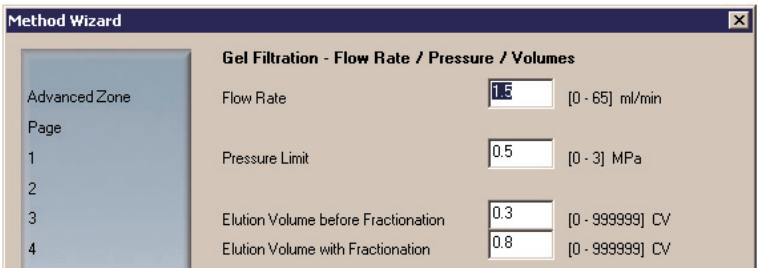
Introduction

This section describes the parameters used in the gel filtration steps for the protocols A, E and G. Some of the default parameter values depend on the chosen column.

Note: Make sure that the parameter values do not exceed the specification of the chosen column.

Flow rate/Pressure/Volumes parameters

The first gel filtration page that appears contains options and parameters related to flow rate, pressure, and volumes.



Flow Rate Flow rate during the gel filtration.

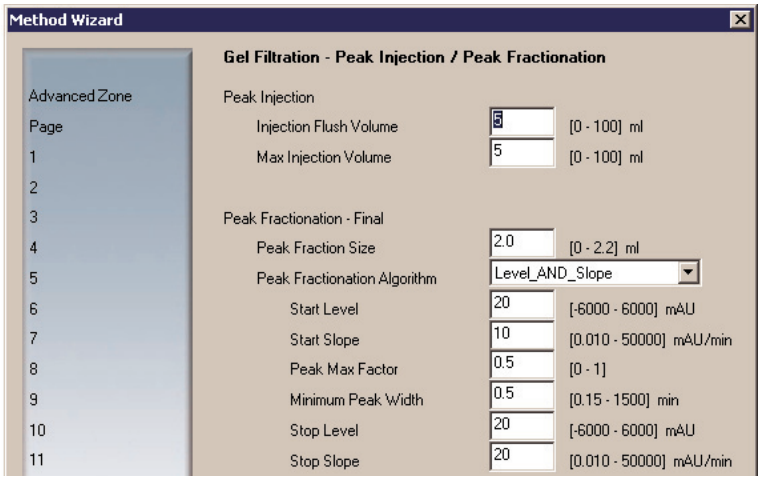
Pressure Limit Upper pressure limit during the gel filtration.

Elution Volume before Fractionation Volume used for elution of the gel filtration column before the peak fractionation starts.

Elution Volume with Fractionation Volume used for elution of the gel filtration column after the peak fractionation has started.

Peak injection parameters

The peak injection options and parameters for the protocols A and E are described below:



Injection Flush Volume The extra volume besides the peak volume used to flush the loops when loading the sample onto the gel filtration column.

Max Injection Volume The maximum volume to be injected onto the gel filtration column. The maximum volume is set up to make sure that the peak volume + flush volume do not exceed the maximum sample loading volume of the column.

Peak fractionation

The peak fractionation options and parameters for the protocols A and E are described below. The parameters define the final fractionation, how the final peaks are collected in the fraction collector.

Peak Fraction Size The maximum volume of each peak fraction.

Peak Fractionation Algorithm Choose to start and stop the peak fractionation based on the signal curve:

- **Level**
- **Slope**
- **Level_AND_Slope**
- **Level_OR_Slope**

AND means that both conditions have to be met, OR means that only one condition has to be met.

The fractionation start values are set by **Start Level** and **Start Slope**, and the stop values by **Stop Level** and **Stop Slope**. Different start and stop values for level and slope can be set.

The Stop Slope condition cannot be met unless the Peak_Max condition is met. Stop Level can be met before Peak_Max. The Peak_Max condition occurs when the UV signal has fallen to a fraction specified by **Peak Max factor** of the most recent peak maximum.

Note: *If the flow rate is changed, the shape of the peak will change as well. Hence, the slope values might need to be changed accordingly.*

Minimum Peak Width sets the minimum fractionation time of a single peak. The fractionation of the current peak continues at least until the Minimum Peak Width is reached, even if the signal is fluctuating.

For more information on peak handling and watch conditions, see **5.5.3 Peak handling and watch conditions** on page 116.

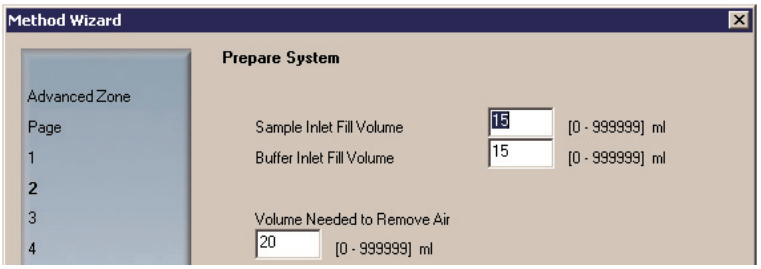
5.5.8 Advanced Zone for Purify – Prepare system and columns

Introduction

This section describes the parameters used in the system and column preparation steps before a purification run.

Prepare system parameters

The Prepare System page contains options and parameters related to system preparation before and after the sample loading.



Sample Inlet Fill Volume Volume used for filling the sample inlet tubing with buffer before immersing the tubing in the samples.

Note: The system will Pause during the run and a message appear requesting the inlet tubing to be moved from the buffers to the samples.

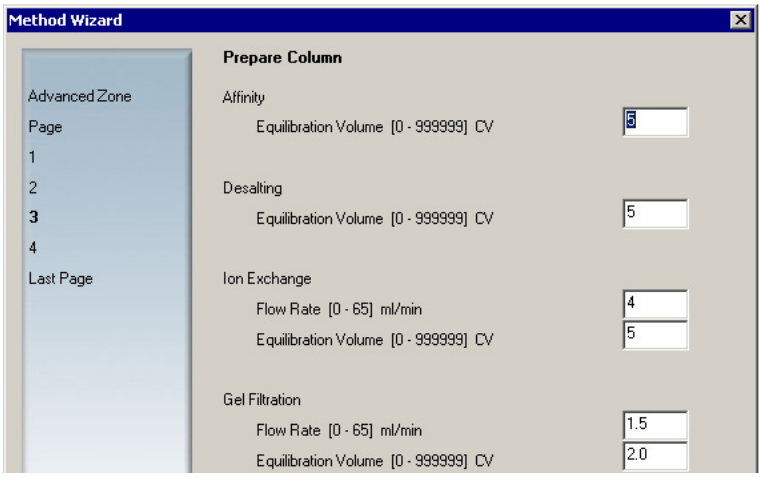
Sample Inlet Clean Volume Volume used for cleaning the sample inlet tubing after loading the sample onto the affinity columns. The system will Pause during the run when all samples are loaded onto the columns and a message appear requesting the inlet tubing to be moved to the cleaning solution. After the cleaning, the system will Pause. The run then has to be manually continued.

Note: The system will Pause during the run when all samples are loaded onto the columns. A message will appear requesting the inlet tubing to be moved to the cleaning solution. After the cleaning, the system will Pause. ***The run then has to be manually continued.***

Volume Needed to Remove Air The volume used to remove air from the pump after the inlets have been filled.

Prepare column parameters

The Prepare Column page contains options and parameters related to column preparation before the sample loading.



Equilibration Volume Volume used for equilibrating a column. Optional for all columns.

Flow Rate Flow rate when equilibrating a column. Optional for ion exchange and gel filtration columns only.

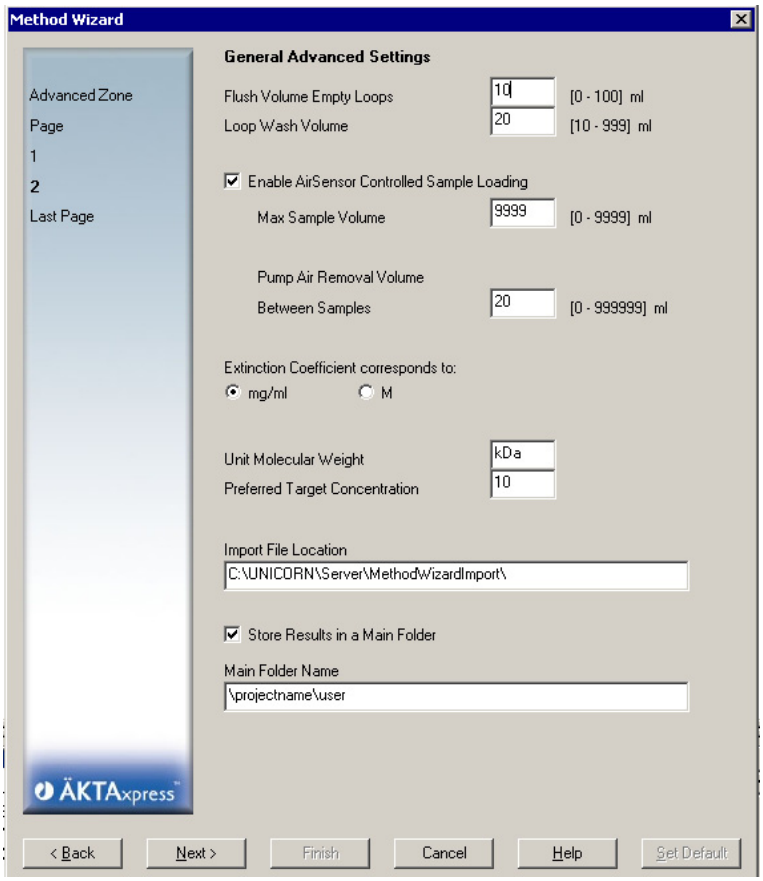
5.5.9 Advanced Zone for Purify – General advanced settings

Introduction

This section describes the options and parameters available on the General advanced settings page in all protocols. The content of the page differs depending on the protocol used.

The General Advanced Settings page

The General Advanced Settings page contains a number of parameters and miscellanous options.



Empty loops parameters

The options and parameters for emptying loops:

Flush Volume Empty Loops The volume used to flush the excess and discarded sample from the loops to the outlets F7–F10.

Loop Wash Volume The volume used to wash the loops.

Air sensor controlled sample loading parameters

The options and parameters defining air sensor controlled sample loading:

Enable AirSensor Controlled Sample Loading Used to provide loading of the entire sample volume. The sample loading will stop when the sample supply is empty and air enters the air sensor.

See also **6.5.10 Using air detection** on page 182.

Max Sample Volume The maximum allowed sample volume when air sensor controlled sample loading is enabled.

Pump Air Removal Volume Between Samples The volume used to remove air from the pump between sample loadings.

Miscellaneous options

Extinction Coefficient corresponds to Choose either **mg/ml** or **M**:

- **mg/ml**: When starting a run with the wizard in System Control, the absorbance of 1 mg/ml solution of the purified protein at 280 nm in a 1 cm cuvette must be entered. When using Pool Fraction in Evaluation, the unit will be given in mg/ml and and the amount of protein in mg.
- **M**: When starting a run with the wizard in System Control, the Molar extinction coefficient 1/(M*cm) of the purified protein at 280 nm in a 1 cm cuvette must be entered. When using Pool Fraction in Evaluation, the unit will be given in M and and the amount of protein in mmoles.

Note: *The extinction coefficient is used for calculating the protein amount and concentration when using Pool Fraction in Evaluation.*

Unit Molecular Weight The unit of the protein molecular weight that will be shown e.g. during the evaluation.

Preferred Target Concentration The target concentration of the protein. When pooling the fractions in Evaluation, the software calculates how each pool should be concentrated or diluted in order to get the set target concentration.

Import File Location The path to the folder where import file is stored. The import file might contain information about, e.g. extinction coefficient and molecular weight of the proteins to be used. For more information on the import file, see **10.3 Import file format** on page 331.

Store Results in Main Folder By default the results are stored directly under the Home folder. To store the results in a sub folder under the Home folder, fill in the name of the folder in the field. If the name of the folder does not exist, the folder is created automatically.

5.5.10 Advanced Zone for Maintain

Introduction

This section describes the Advanced Zone for a Maintain method plan.

The following method plans allow parameter values to be changed:

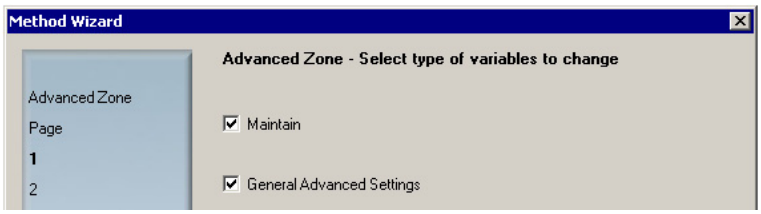
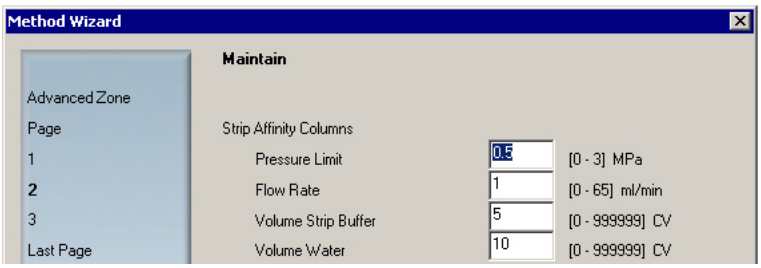
- Stripping Affinity Columns
- Clean System

All method plans allow defining a main folder for storing the results.

Note: *Make sure that the parameter values do not exceed the specification of the chosen column.*

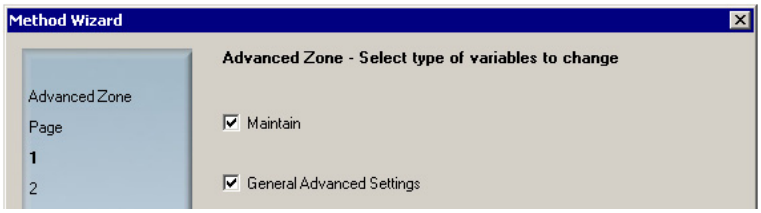
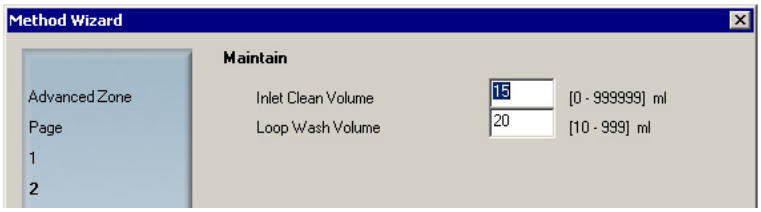
Stripping affinity columns

To change parameter values in a Stripping Affinity columns method plan:

Step	Action
1	Select the required options on **Page 1** and click **Next**. (Select **General Advance Settings** to define a main folder for storing the results.) 
2	Change the desired values on **Page 2** (if **Maintain** was selected) and click **Next**.  **Pressure limit** Upper pressure limit during the stripping procedure. **Flow Rate** Flow rate during the stripping procedure. **Volume Strip Buffer** Volume of stripping buffer to be used. **Volume Water** Volume of water used for washing after the stripping procedure.

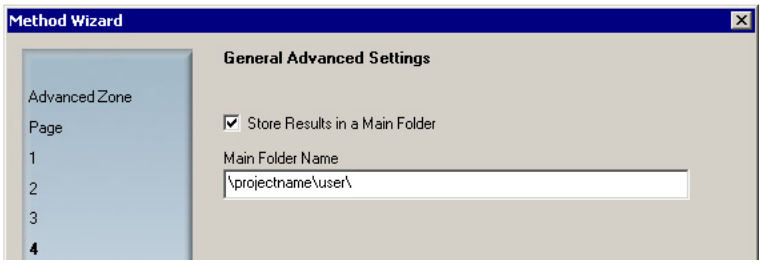
Clean system

To change parameter values in a Clean System method plan:

Step	Action
1	Select the required options on **Page 1** and click **Next**. (Select **General Advance Settings** to define a main folder for storing the results.) 
2	Change the desired values on **Page 2** (if **Maintain** was selected) and click **Next**.  **Inlet Clean Volume** Volume of cleaning solution to be used for each selected inlet. **Loop Wash Volume** Volume of cleaning solution to be used for washing each selected loop.

Defining a folder for storing the results

To define a sub folder under the Home folder for storing the results:

Step	Action
1	Enter the desired folder name on the page.  **Store Results in Main Folder** To store the results in a sub folder under the Home folder, fill in the name of the folder in the field. If the name of the folder does not exist, the folder is created automatically.
2	Click **Next** to save the method plan. See **5.2.5 Saving the method plan** on page 106.

5.5.11 Hints on optimizing a method plan

Introduction

This section contains hints and directions for how to optimize method plans for troublefree operation of ÄKTAxpress. Most of the adjustments are made in Advanced Zone in the Method Wizard.

Peak volume

Peak volumes for peaks eluted from affinity and ion exchange columns should be as small as possible. It will increase the possibility to:

- load the entire peak volume onto any desalting or gel filtration column in the next step since their maximum loading volumes are limited.
- optimize the recovery in the ion exchange step by making sure that the entire peak volume is collected when eluted in the ion exchange gradient. Only one loop is available for each peak, so peak volumes larger than 7.5 ml will cause sample loss.

To minimize the peak volume:

- Choose a suitable column type and size.
- Adjust the gradient slope. A steeper gradient will narrow the peaks.

Second wash of affinity chelating column

A second wash of affinity chelating columns can be used to wash out loosely bound proteins. The second wash is an additional step before the elution of the target protein. For a powerful wash, the imizadole concentration %B should be set to above 0%B. If no imidazole is used (%B=0), the volume used in this step should be decreased to minimize the run time.

Note: *Do not raise the imidazole concentration too much as it might cause the target protein to come off as well.*

Note: *The second wash is by default not included for GSTrap columns.*

Flow rates and pressure limits

No warning will be issued if the flow rate or pressure limit are set higher than the values recommended for the columns used.

Note: *Changing the flow rate will change the slope of an eluting peak.*

Peak detection and collection

Peak detection and collection

The default values for detecting peak start and peak end are set to match typical peaks eluting from the supported columns. Different values are used for normal and high sample loading. The slope and levels values though might need to be adjusted if other parameters are changed.

- The slope of a peak is changed if:
  - the flow rate is changed, or
  - the gradient slope is changed.
- The default level values might need adjustment if:
  - small peaks are expected, or
  - the peaks are broadened due to e.g. a more flat gradient.

Note: *If the level values are decreased for very large or broad peaks, the peak volume collected might be too big for the loop available or exceed the loading volume of the next column.*

Protease injection volume

This applies to protocols that include tag cleavage.

The default value for the protease injection is 0.7 CV. If using a larger value, the target protein might be lost since it will pass through the column before peak collection is activated.

6 Operation

About this chapter

This chapter describes the normal work flow when operating ÄKTAxpress, from starting the system, creating a method plan, and preparing the system to cleaning the system and columns after the run.

In this chapter

This chapter contains the following sections

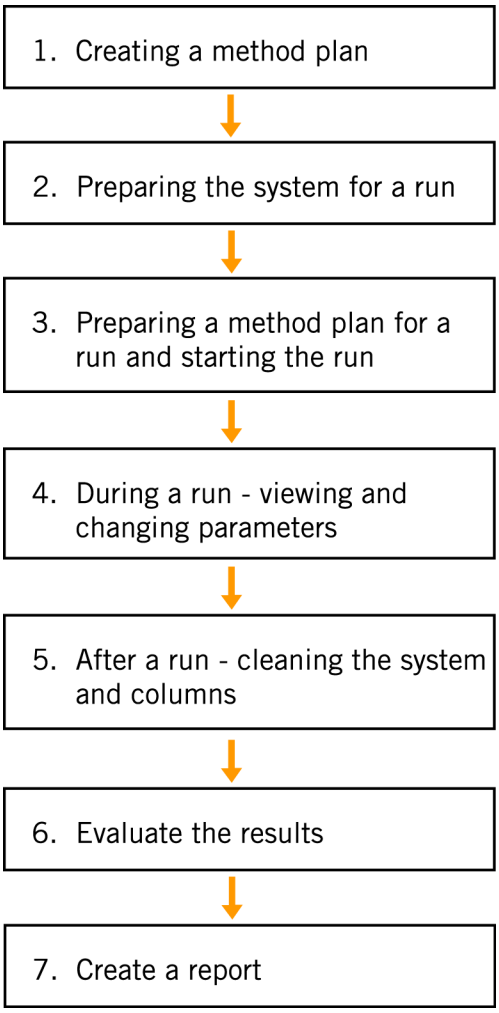
6.1 Operation overview

Introduction

This section gives an overview of the operation of ÄKTAxpress.

Work flow

The typical work flow in ÄKTAxpress after turning on a system and connecting it can be divided into a number of steps. Step 1–5 are described in separate sections in this chapter. Step 6 and 7 are described in chapter **7 Evaluation** on page 208.



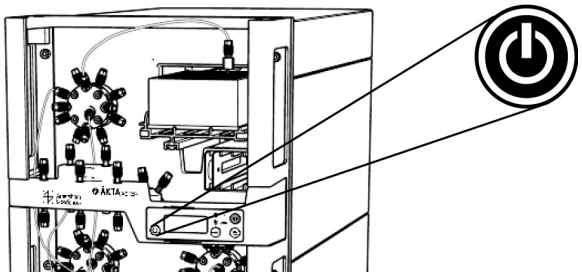
6.2 Starting the system

Introduction

This section describes how to turn on ÄKTAxpress including the computer equipment, and how to log on to UNICORN.

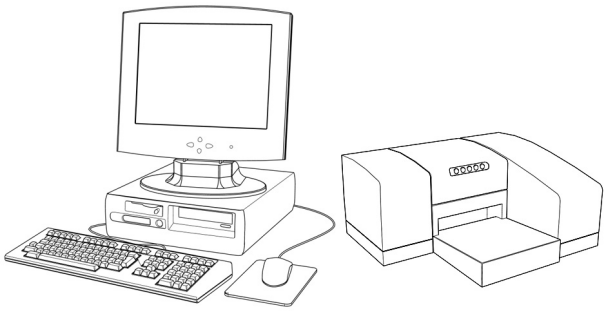
Turning on separation systems

To turn on the separation systems:

Step	Action
1	**If the system unit number is shown in the display:**  The system is set in **Idle** mode. No action is required, because the system was turned on when the AC cable was inserted. Two segments might flash to indicate no communication with the UNICORN computer. **If only one segment is on in the display:**  The system is set in **Standby** mode. Turn on the system by pressing the **On** button on the front panel.  *Result*: The display indicates **Idle** mode and the system identity (1–12) is shown. 
2	Check that the system identity is correct. The separation system should have a unique identity within the interval **1–12** which might have been set from factory. If the identity is *incorrect* it has to be set, see the *Installation Guide*.

Turn on the computer

To turn on the UNICORN computer:

Step	Action
1	Turn on the monitor, printer and computer according to the manufacturer's instructions. Wait for the computer to start up. 
2	Verify that the power indicator on the USB/CAN-converter is *on* when the computer has been turned on.
3	Log on to Windows.
4	When the Windows desktop appears, start UNICORN by double-clicking on the UNICORN shortcut icon. 
5	Select User name **default** and enter **default** as password. Click **OK**.  *Result*: UNICORN starts with the UNICORN Manager on top. ***Note:*** *See the UNICORN user documentation for instructions about how to create new users.*

6.3 Connecting a system

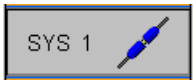
Introduction

This section describes how to connect to a separation system from UNICORN.

Up to twelve separation systems can be connected in the System Control module.

Connecting a separation system

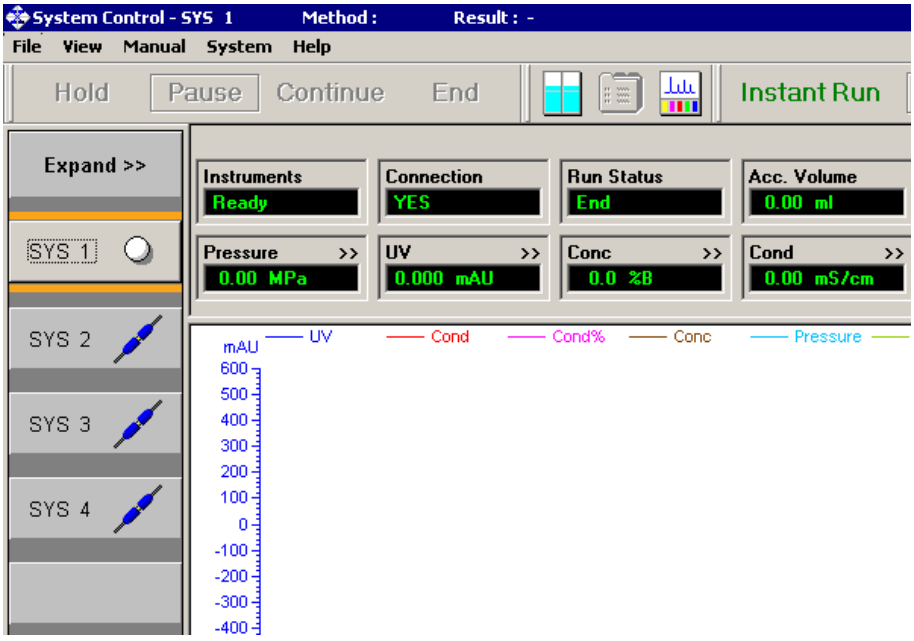
The separation systems that are available in UNICORN are shown in the vertical bar at the left-hand side of System Control. Disconnected systems are identified by a blue connector symbol.



Note: *To add more systems in UNICORN, see ÄKTAxpress Installation Guide.*

- Left-click the symbol of the system to be connected.

  Result: The system is connected and the symbol changes to a white status indicator. The run data, curves and logbook for the system is shown.



Status indicator colors

The table below shows how the indicator colors relate to the run status.

Indicator color	Run status
White	**End**
Green	**Run** or **Manual**
Yellow	**Hold**
Red	**Pause**

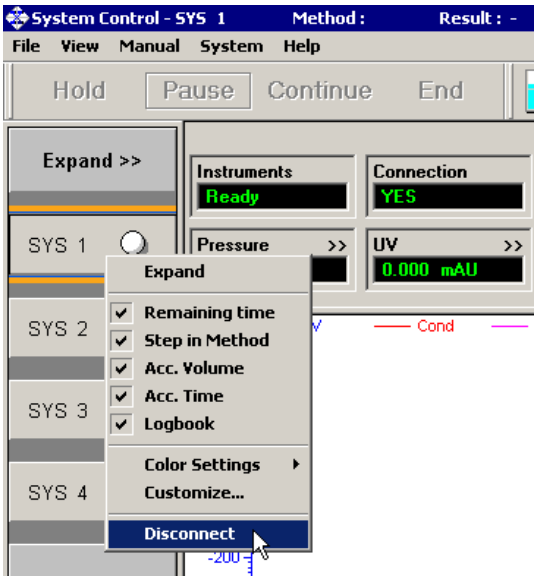
Error indication

When a warning or an alarm is issued from a system, the background of the system symbol starts flashing and the background color turns yellow.

Disconnecting a separation system

- Right-click the symbol of the system to be disconnected and select **Disconnect**.

  Result: The system is disconnected and the symbol changes.



Changing system display

To display the status for the another separation system, left-click the system symbol of the system on the left.

6.4 Creating a method plan

Introduction

This section gives a brief description on how to use the *Method Wizard* to create a new method plan. More information on creating a method plan can be found in **5.2.1 Creating a new method plan** on page 89.

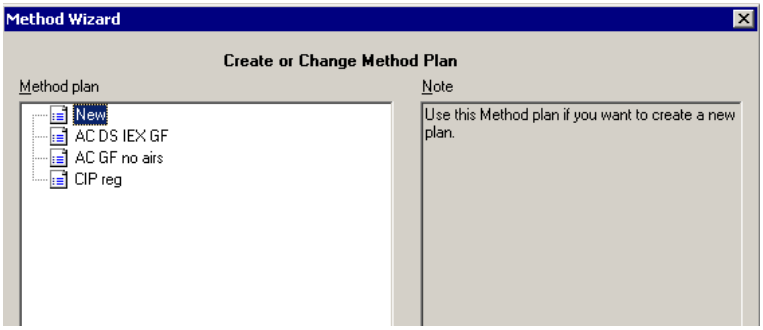
Method plan types

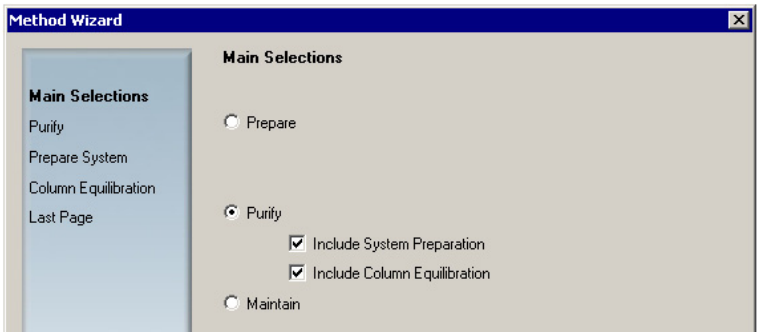
There are three main types of method plan available:

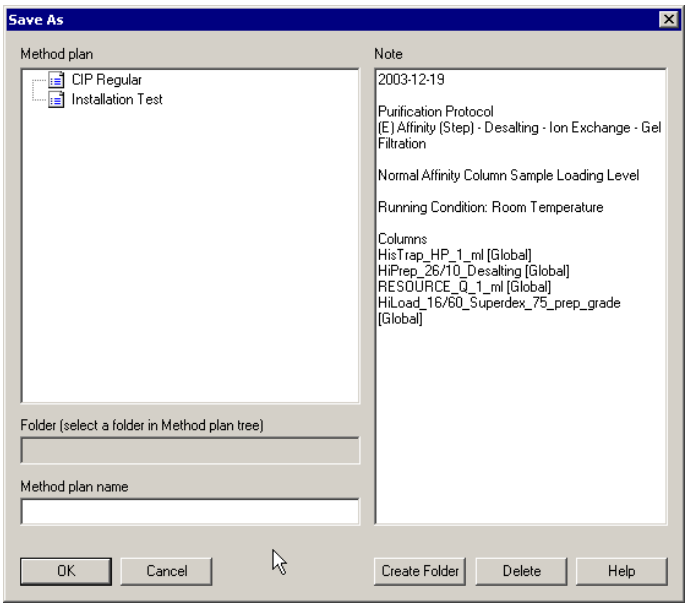
- Preparation of the system and columns.
- Purification of protein.
- Maintenance of the system and columns.

Creating a method plan

To create a method plan:

Step	Action
1	Click the **Method Wizard** icon in the **Method Editor** module.  *Result*: The **Method Wizard** dialog box appears.
2	• Select **New** to create a new method plan where all parameters have optimized default values provided by the Method Wizard, *or* • Select an existing method plan. Some of the parameter values can be changed in Advanced Zone.  ***Note:*** *For information on the current page, click* **Help**.
3	Click **Next**. *Result*: The **Main Selections** page appears.

Step	Action
4	Select the main type of method plan to be created and click **Next**.  ***Note:*** *Selecting Include System Preparation and then Fill Sample Inlets with Buffer on the Prepare System page is recommended.*
5	On each new page, select the appropriate parameter values and click **Next** to continue.
6	On the **Last Page**: • Click **Finish** to save the selections as a method plan, *or* (*Result*: The **Save As** page appears.) • Click **Next** to enter the Advanced zone if any parameter values needs to be changed. See **5.5 Advanced zone** on page 109. ***Note:*** *Do not change any values in the Advanced Zone unless the consequences are fully understood.*

Step	Action
7	• On the **Save As** page, select destination folder in the Method plan tree or create a new one, if needed. • Type any additional information in the **Notes** field.  ***Note:*** *It is not possible to move method plans between folders.*
8	Type the name of the method plan and click **OK**. *Result*: The method plan is saved and can now be started in the System Control module.

6.5 Preparing the system for a run

About this section

This section describes how to prepare the separation system for a run.

In this section

This section contains the following sub-sections

6.5.1 Preparing buffers and solutions

Introduction

This section describes where the inlet tubing from buffers and solutions should be connected and where the containers should be placed.

Liquid quality recommendation

For best purification results, use deionized water and high purity chemicals. Filtering of liquids through a 0.45 µm filter and degassing the liquids is recommended.

Note: *Do not use organic solvents in buffers. The mixer is designed for use with water based solutions only. If organic solvents are used, incorrect gradients or incomplete mixing might occur.*

Preparing the containers

- Prepare required buffers according to the chosen purification protocol and fill the containers.
- Place the containers on a suitable shelf under the bench.

Inlet supply for a purification run

Equilibration, binding, elution, and washing buffers, as well as cleaning solutions, are introduced into the system through the inlet tubing A1–A8, B1 and B2.

Running a purification method

The table below shows the standard buffer and solution supply for a normal purification run. See also **4 Methodology** on page 53.

- Immerse the inlet tubing in the containers according to the table (also shown on the Summary page in the Method Wizard in System Control).

Inlet tubing	Buffer or solution
A1	Affinity binding buffer (A)
A2	Ion exchange binding buffer (A) / desalting buffer
A3	Affinity elution buffer (B)
A4	Gel filtration/Desalting buffer (for final purification step)
A5	–
A6	0.5 M NaOH (if NaOH wash between samples is chosen)
A7	Cleavage buffer (tag removal only)
A8	–
B1	Affinity wash buffer (for additional wash)
B2	Ion exchange elution buffer (B)

Note: *The affinity elution buffer is fed from inlet A3, not B1. The step gradient made with A3 buffer will therefore not be shown in the chromatogram because it only shows the concentration of buffer B.*

Inlet supply for preparation and maintenance

The liquid supply differs from the purification methods when:

- stripping metal ions from affinity columns,
- charging new or stripped affinity columns with new metal ions, or
- running Cleaning in place (CIP) method plans.

Stripping and charging affinity columns

The table below shows the standard buffer and solution supply for stripping and charging affinity columns.

- Immerse the inlet tubing in the containers according to the table (also shown on the Summary page in the Method Wizard in System Control).

Inlet tubing	Buffer or solution
A1	Affinity binding buffer (A)
A2	–
A3	Affinity elution buffer (B)
A4	–
A5	Water
A6	–
A7	Metal ion charging solution (e.g. 0.1 M $NiSO_4$)
A8	Metal stripping solution (e.g. His-Affinity A buffer with 50 mM EDTA)
B1	–
B2	–

Note: *Inlet tubing A7 (metal ion charging solution) must be manually filled before starting the charging run.*

Note: *The waste from the metal ion charging and stripping runs is collected through outlet tubing F11.*

Cleaning in place

The table below shows the standard buffer and solution supply for performing Cleaning in place (CIP).

- Immerse the inlet tubing in the containers according to the table (also shown on the Summary page in the Method Wizard in System Control).

Inlet tubing	Buffer or solution
A1	GST A buffer
A2	Ion exchange binding buffer (A)
A3	30% isopropanol
A4	0.2 M NaOH
A5	Water
A6	0.5 M NaOH
A7	1.0 M NaOH
A8	6 M Guanidine HCl / 1% TritonX100
B1	1 M NaCl
B2	2 M NaCl

Purging the pump and the inlet tubing

If there is air in the flow path, the pump and the inlet tubing to be used must be filled manually before starting a run. See **6.5.3 Purging the pump and inlet tubing** on page 156.

Vent opening on container caps

If the containers have caps, each cap must have a vent opening to prevent a vacuum from forming as buffer or solution is extracted.

6.5.2 Preparing outlet and waste tubing

Introduction

This section describes where the outlet and waste tubing should be placed.

Preparing the tubing

- Immerse the tubing from the *outlet valve* in appropriate flasks or containers as shown in the table below (also shown on the Summary page in the Method Wizard in System Control).

Outlet valve port	Description
F1	Waste (used for e.g. equilibration and loop wash)
F2	Connected to the fraction collector
F3	Flow through from loading sample 1
F4	Flow through from loading sample 2
F5	Flow through from loading sample 3
F6	Flow through from loading sample 4
F7	Nonselected peaks from sample 1
F8	Nonselected peaks from sample 2
F9	Nonselected peaks from sample 3
F10	Nonselected peaks from sample 4
F11	Metal ion waste
F12	Connected to injection valve, port 7, to redirect intermediate peaks for storage in loops.

Note: *The optional flask holder can be used for storing samples and flasks holding F3–F11.*

- Immerse the waste tubing from the *injection valve* in appropriate flasks or containers as shown in the table below.

Injection valve port	Description
2	Waste 2 (used for manual sample injection)
3	Waste 3 (used for e.g. system wash and intermediate loop collection)

6.5.3 Purging the pump and inlet tubing

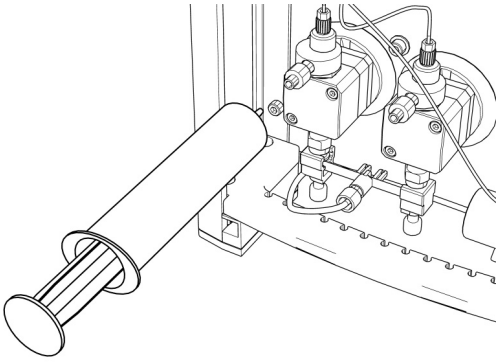
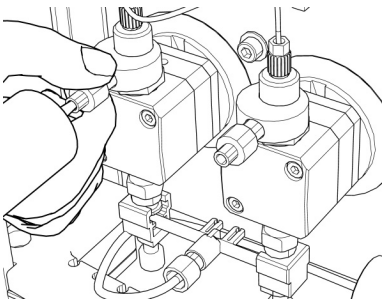
Overview

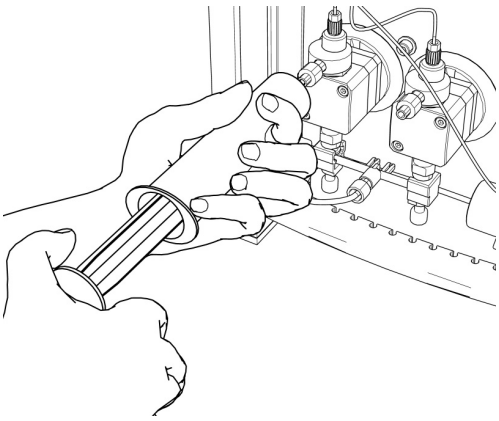
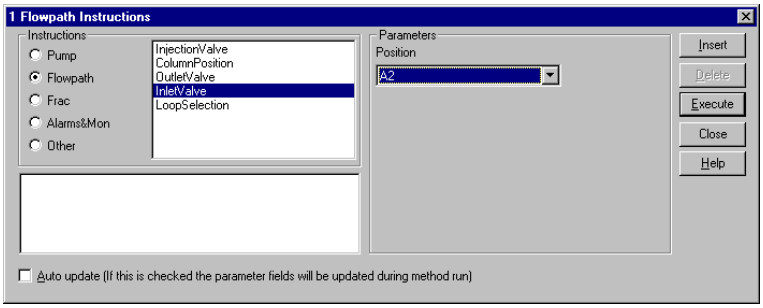
Before starting a run, all inlet tubing that will be used must be filled to remove air bubbles. In addition, both pump heads must be purged with methanol if the system has been left unused for a week or longer, or the pump has been run dry.

> **CAUTION!**
> To protect the piston seals in the pump, the pump must never be run with air in the inlet tubing.

Filling inlet tubing A1–A8 and S1–S4

To fill the inlet tubing **A1–A8** and **S1–S4**:

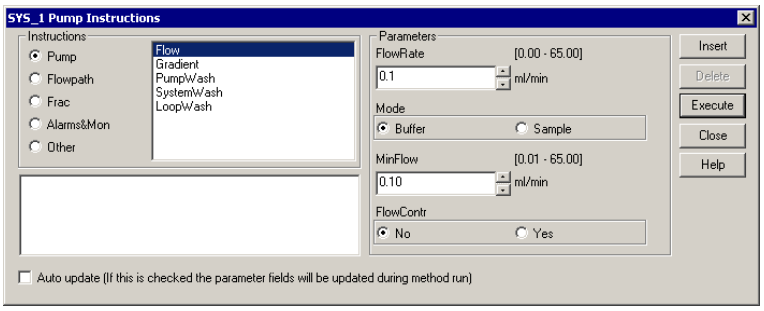
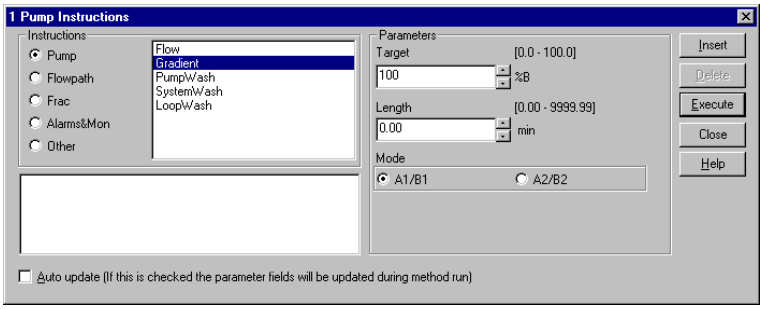
Step	Action
1	Check that the inlet tubing to be filled is properly immersed in the correct containers/flasks/tubes.
2	Connect a male Luer syringe, of at least 20 ml, to the left purge valve. Two syringes are included in the accessory kit supplied with the system. 
3	Turn the left purge valve counter clockwise half a turn to open it. 

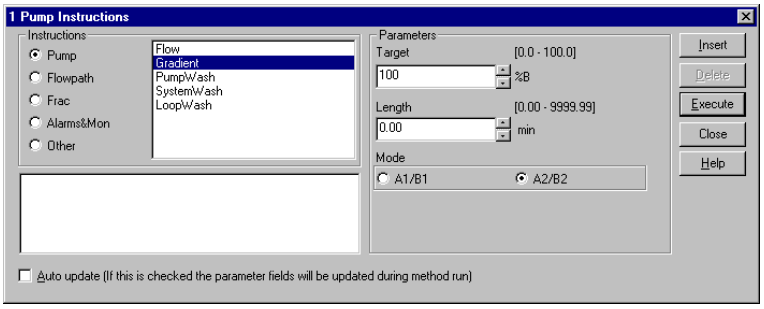
Step	Action
4	Slowly draw buffer A1 with the syringe. When fluid starts entering the syringe, close the purge valve. Check that there is no visible air left in the **A1** tubing.  ***Note:*** *If air in the other pump head is suspected, draw buffer A1 through that pump head as well.*
5	If required, remove the syringe, empty it and insert it in the purge valve again before continuing.
6	To fill the **A2** inlet tubing or any other inlet tubing to be used, first switch the valves: • Start UNICORN and select **System Control: Manual: Flowpath**. • Select **InletValve** and **A2** (or whichever inlet tubing to be filled. • Click **Execute** to set the valves to the correct positions. 
7	Repeat step 3–5 to fill the inlet tubing.

Filling the inlet tubing B1 and B2

To fill the inlet tubing **B1** and **B2**:

Step	Action
1	Check that the inlet tubing **B1** and **B2** are properly immersed in the correct flasks.

Step	Action
2	Start the pump at a small flow rate: • Start UNICORN and select **System Control:Manual:Pump**. • Select a flow rate of 0.1 ml/min.  • Click **Execute** to start the flow.
3	To fill the **B1** inlet tubing the inlet valve first has to be set: • Select **System Control:Manual:Pump**. • Select **Gradient** and **Target 100%B** and **Mode A1/B1**. • Click **Execute** to set the valve to position **B1**. 
4	Connect a male Luer syringe, of at least 20 ml, to the left purge valve.
5	Turn the left purge valve counter clockwise half a turn to open it.
6	Slowly draw eluent B1 with the syringe. When the **B1** tubing is full up to the pump head and eluent starts entering the syringe, close the purge valve. Check that there is no visible air left in the **B1** tubing.
7	If required, remove the syringe, empty it and insert it in the purge valve again before continuing.

Step	Action
8	To fill the **B2** inlet tubing, first switch the inlet valve: • Select **System Control: Manual:Pump**. • Select **Gradient** and **Target 0%B** and **Mode A1/B1**. • Click **Execute**. • Select **Gradient** and **Target 100%B and Mode A2/B2**. • Click **Execute** to set the valve to position **B2**. 
9	Repeat step 5–7 to fill inlet tubing **B2**.
10	Stop the pump by clicking **END** in System Control.

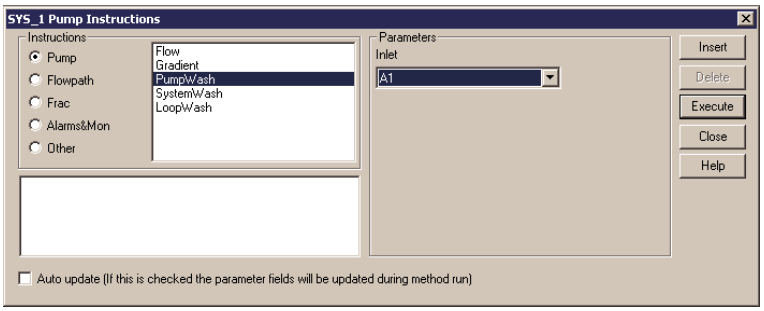
Purging the pump and system

Air remaining in the system may be removed by purging the pump and system by manually running Pump Wash or System Wash.

Note: *When performing a run using a Prepare, Purification or Maintain method plan, an initial system wash will be included in the system.*

To purge the pump and/or system:

Step	Action
1	Carefully immerse the inlet tubing **A1** in a flask containing the buffer to be used.

Step	Action
2	Run a Pump Wash or a System Wash: • Start UNICORN and select **System Control:Manual:Pump**. • Select **PumpWash** and inlet **A1**, or **SystemWash**.  • Click **Execute**.

Purging the pump with methanol

Both pump heads must be purged with methanol if:

- The system has been left unused for a week or longer, or
- The pump has been run dry.

The purging will maintain the pumping capacity and protect the pump piston seals.

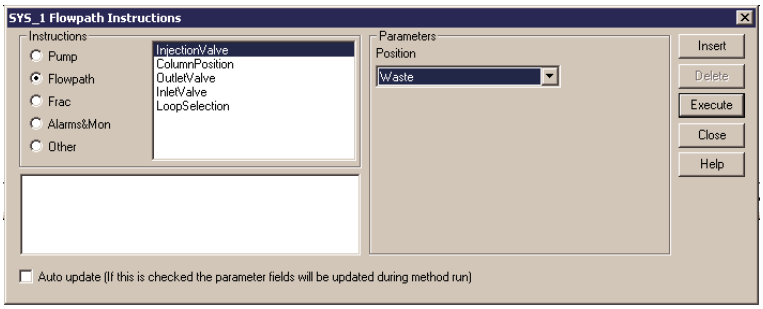
Note: *All inlet tubing that will be used in the next purification run should be filled manually before purging the pump.*

The purging procedure in short:

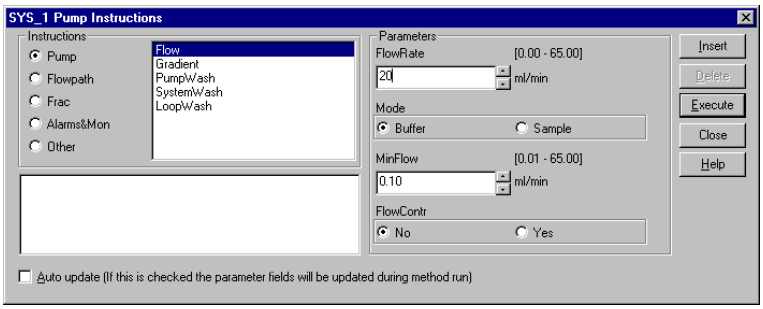
1. Flush out buffer using deionized water.
2. Purge the pump using methanol.
3. Flush out methanol using deionized water.

Preparation

Step	Action
1	Prepare two flasks containing 200 ml of methanol and 300 ml of deionized water respectively.
2	Carefully immerse the inlet tubing **A1** in the flask containing at least 300 ml of deionized water.
3	Put both **Waste** tubings from the injection valve into a waste flask.

Step	Action
4	Set the injection valve to position Waste: • Start UNICORN and select **System Control:Manual:Flowpath**. • Select **InjectionValve** and position **Waste**.  • Click **Execute**.

Purging procedure

Step	Action
1	Run the pump at 20 ml/min for 2 minutes: • Select **System Control:Manual:Pump**. • Select a flow rate of 20 ml/min.  • Click **Execute** to start the flow. Run the pump for 2 minutes. • Stop the pump by clicking **Pause** in System Control.
2	Carefully move the inlet tubing **A1** to the flask containing about 200 ml of methanol.
3	Run the pump at 50 ml/min for 2 minutes. • Set the flow rate to 50 ml/min and click **Execute**. • Click **Continue** to start the flow. Run the pump for 2 minutes. • Stop the pump by clicking **Pause**.

Step	Action
4	Run the pump at 20 ml/min for 1 minute: • Set the flow rate to 20 ml/min and click **Execute**. • Click **Continue** to start the flow. Run the pump for 1 minute. • Stop the pump by clicking **Pause**.
5	Carefully move the inlet tubing **A1** back to the flask containing water.
6	Run the pump at 10 ml/min for 10 minutes: • Set the flow rate to 10 ml/min and click **Execute**. • Click **Continue** to start the flow. Run the pump for 10 minutes. • Stop the pump by clicking **End**.
7	Carefully move the inlet tubing **A1** to the flask containing the buffer that will be used during the next purification run. ***Note:*** *Remember to fill inlet tubing A1 with the buffer to be used, as described previuosly in this section.*

6.5.4 Connecting columns and tubing

Introduction

This section describes how to connect columns and tubing that are used in a purification run.

Cautions

> **CAUTION!**
> Tighten the columns properly in the column block to avoid leakage. Over-tightening might though rupture the column connectors.

> **CAUTION!**
> Make sure that no parts, e.g. tubing or columns, are positioned in front of the fraction collector, hindering the ejection of the microplate. The movement of the plate might destroy columns and tubing positioned in front of the fraction collector.

Attaching the columns for a purification run

- Attach the columns to the column block or the column holder for a purification run according to the table below. A detailed specification can be found on the Summary page in the Method Wizard in System Control.

  See also the connection guide in this section for how to use unions with the columns.

Position	Column
Column block	
Port 1	Affinity column 1
Port 2	Affinity column 2
Port 3	Affinity column 3 or desalting column
Port 4	Affinity column 4 or ion exchange column
Port 5	Gel filtration or desalting column
Column holder	
Left-hand side	Gel filtration column and/or HiPrep desalting column
Right-hand side	Superloop (see also **6.5.8 Performing automatic tag removal** on page 175) and tube for pump rinsing solution.

Note: *The number of affinity columns equals the number of samples.*

Note: *Insert stop plugs or non-used connectors into empty ports in the column block to prevent dirt from entering the flow path.*

Note: *Some applications require two columns of the same type connected in series. This is marked "×2" in the column list in the Method Wizard.*

Connecting the column tubing

- Connect the tubing from the column valve to the columns according to the table below. To avoid introducing air into the column, run the pump at a low flow rate (e.g. 0.3 ml/min) when connecting the fitting (also when connecting an online filter to the gel filtration inlet tubing).

  See also the connection guide below for how to use unions to connect the tubing to the columns.

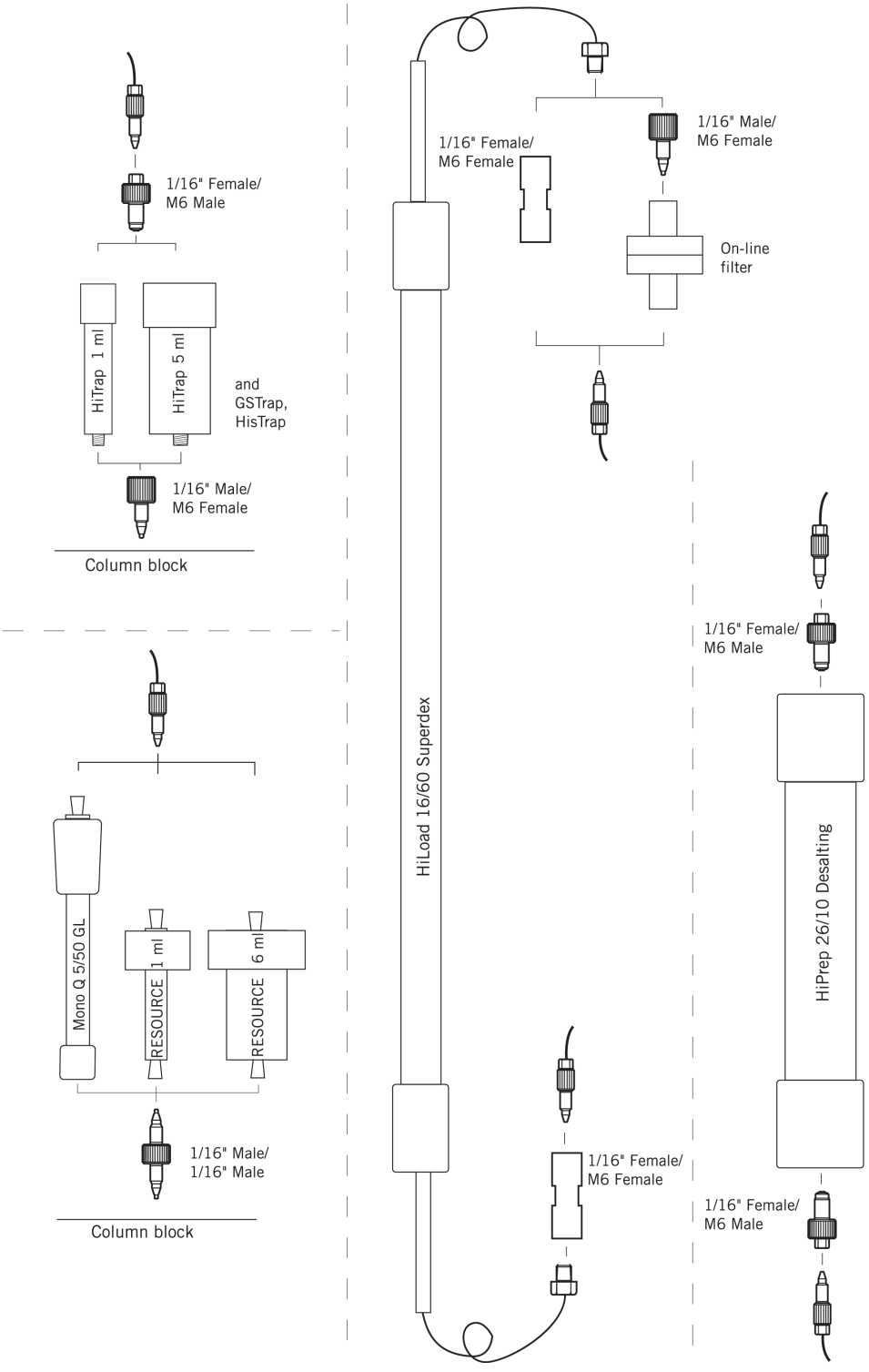
Column valve port	Connect to ...
Bypass	Bypass
IN 1	Column block, upper port 1
IN 2	Column block, upper port 2
IN 3	Column block, upper port 3 or in column holder
IN 4	Column block, upper port 4
IN 5	Column block, upper port 5 or in column holder
OUT 1	Column block, lower port 1
OUT 2	Column block, lower port 2
OUT 3	Column block, lower port 3 or in column holder
OUT 4	Column block, lower port 4
OUT 5	Column block, lower port 5 or in column holder

Note: *No column should be connected to the bypass ports.*

Connection guide

The connection guide below describes how to use the unions supplied with the system to connect tubing with the columns.

Note: *The gel filtration and HiPrep Desalting columns might require longer tubing. A tubing especially made for connecting these columns is supplied with the system.*


1/16" Female/
M6 Male
HiTrap 1 ml
HiTrap 5 ml
and
GSTrap,
HisTrap
1/16" Male/
M6 Female
Column block
Mono Q 5/50 GL
1 ml
RESOURCE
6 ml
RESOURCE
1/16" Male/
1/16" Male
Column block
1/16" Female/
M6 Female
1/16" Male/
M6 Female
On-line
filter
HiLoad 16/60 Superdex
1/16" Female/
M6 Female
1/16" Female/
M6 Male
HiPrep 26/10 Desalting
1/16" Female/
M6 Male

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6.5.5 Conditioning columns

Introduction

This section describes how to perform metal ion charging, blank runs, and equilibration of the columns used in ÄKTAxpress.

Metal ion charging of affinity columns

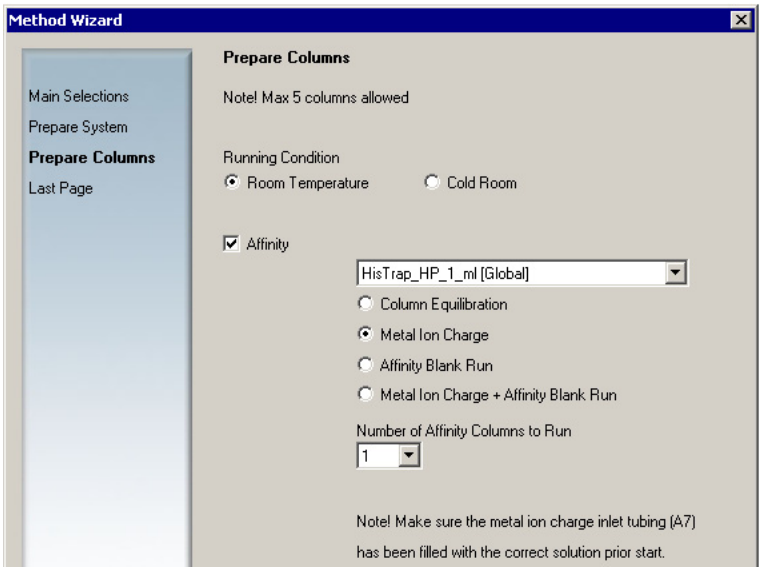
New or stripped HiTrap Chelating columns and stripped HisTrap affinity columns must be charged with metal ions. The software allows up to five columns to be charged automatically.

During the procedure each column is flushed with 5 column volumes (CV) of deionized water, 1 CV of metal salt solution, and finally with 5 CV of deionized water. The waste is collected through outlet F11.

Creating a method plan for metal ion charging

To create a method plan for metal ion charging:

Step	Action
1	Click the **Method Wizard** icon in the **Method Editor**.  *Result*: The Method Wizard dialog box appears.
2	Select **New** method plan in the **Method plan** field and click **Next**.
3	Click **Prepare** and then **Columns** on the **Main Selections** page. Click **Next**.

Step	Action
4	On the **Prepare Columns** page, make the following selections: • Click **Affinity**. • Select column type. • Click **Metal Ion Charge**. • Select the number of affinity columns. Click **Next**. 
5	On **Last page** click **Finish** and save the metod plan.

Note: *The Metal Ion Charge inlet tubing (A7) has to be filled manually before starting the run. See* ***6.5.3 Purging the pump and inlet tubing*** *on page 156.*

Note: *The Method Wizard also provides a combined method plan that includes metal ion charging automatically followed by a blank run.*

Starting a run using a method plan for metal ion charging

The table below describes how to start a run using the method plan. See also the standard procedure described in **6.6.1 Starting a run using a method plan** on page 186.

Step	Action
1	Click **Instant Run** in **System Control**. *Result*: The Method Wizard dialog box appears.
2	Select the required method plan in the **Method plan** field and click **Next**.

Step	Action
3	On each new page, select the appropriate system and type the requested information. Click **Next** to continue.
4	On the **Summary** page, check that the tubing and liquid supply fulfills the requirements listed on the page. ***Note:*** *It is recommended to print the Summary page since it will not be saved.*
5	Click **Run** on the last page.
6	Click **Continue** to start the run.

Blank run on affinity columns

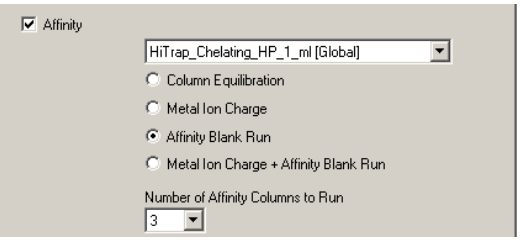
Before the first use of an affinity column (GSTrap FF or newly charged HiTrap Chelating or HisTrap), a blank run should be performed. This results in a well-conditioned and equilibrated column.

During the procedure each column is flushed with 5 CV of affinity buffer A, 5 CV of buffer B, and finally with 10 CV of buffer A.

Creating a method plan for an affinity blank run

The procedure for creating a method plan for an affinity blank run is the same as the metal ion charging procedure, except for the following changes on the **Prepare Columns** page:

- Click **Affinity Blank Run**.



Note: *The Method Wizard also provides a combined method plan that includes metal ion charging automatically followed by a blank run.*

Starting a run using a method plan for an affinity blank run.

Follow the description for the metal ion charging procedure, or see the standard procedure described in **6.6.1 Starting a run using a method plan** on page 186.

Blank run on ion exchange columns

Before the first use of an ion exchange column (e.g. HiTrap SP or RESOURCE Q) or after long-term storage, a blank run should be peformaed. The purpose is to provide the ion exchange column with exchangeable counter ions.

During the procedure each column is flushed with 5 CV of ion exchange buffer A, 5 CV of buffer B, and finally with 10 CV of buffer A.

Creating a method plan for an ion exchange blank run

The procedure for creating a method plan for an ion exchange blank run is the same as the metal ion charging procedure, except for the following changes on the **Prepare Columns** page:

- Click **Ion Exchange**.
- Select column type.
- Click **Ion Exchange Blank Run**.

Starting a run using a method plan for an ion exchange blank run.

Follow the description for the metal ion charging procedure, or see the standard procedure described in **6.6.1 Starting a run using a method plan** on page 186.

Equilibrating columns

The purpose of equilibrating a column is to remove ethanol and to equilibrate the column with buffer.

Note: *When using a HiLoad (gel filtration) or HiPrep (desalting) column for the first time or when changing buffer, the column must be equilibrated.*

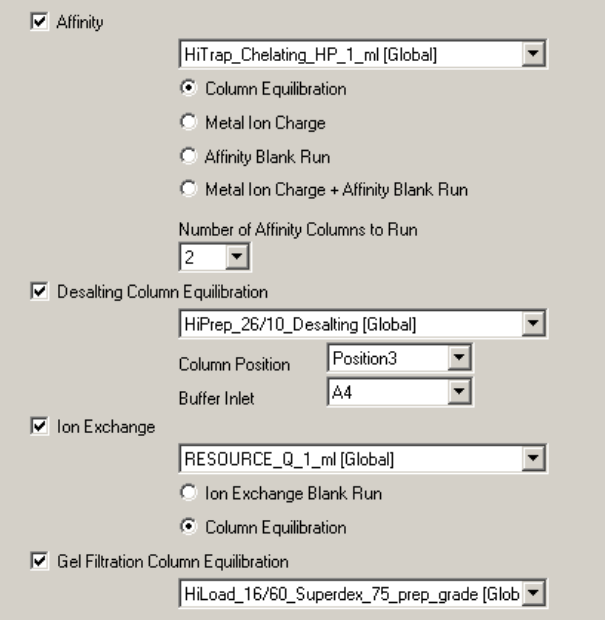
During the equilibration the column is flushed with 5 CV of the appropriate buffer, except for the gel filtration column which is flushed with 1.5 CV.

Creating a method plan for equilibration

The procedure for creating a method plan for equilibration is the same as the metal ion charging procedure, except for changes on the **Prepare Columns** page according to the following illustration:

- Click the check buttons to select the column types you want to equilibrate. In the illustration all four column types are selected.
- Besides columns, the required **Column Position** and **Buffer Inlet** should be selected for the desalting column.

Note: *If the gel filtration column is filled with ethanol, it must be washed with water before equilibration with gel filtration buffer. To do it automatically, create a Customized Equilibration method plan for two solutions in Maintain in the Method Wizard.*



Starting a run using a method plan for an equilibration run.

Follow the description for the metal ion charging procedure, or see the standard procedure described in **6.6.1 Starting a run using a method plan** on page 186.

6.5.6 Preparing samples

Introduction

This section describes how to prepare the samples.

Preparing samples

To prepare the samples:

Step	Action
1	Prepare the samples and clarify them using centrifugation and/or filtration through a 0.45 µm filter. The tube holder is used for storing tubes with small sample volumes. The optional flask holder can be used for sample flasks with larger volumes.
2	Immerse the sample inlet tubing in affinity buffer A.
3	Fill the sample inlet tubing with affinity buffer A. See **6.5.3 Purging the pump and inlet tubing** on page 156. (Performed automatically during the run if selected in the method plan.)
4	Gently move the sample inlet tubing to the sample flasks or tubes; tubing **S1** to sample 1, tubing **S2** to sample 2, etc. Make sure that no air enters the tubing. (If automatic filling is selected in the method plan, a dialog will appear requesting the sample inlet tubing to be moved.)

Filling the sample inlet tubing

The sample inlet tubing must be filled with buffer before starting a run to prevent air from entering the flow path. To perform it manually, see **6.5.3 Purging the pump and inlet tubing** on page 156. It is also possible to do it automatically during the system preparation by selecting it in the method plan.

Setting the sample volume

All purification protocols have air detection enabled by default during the sample application. The sample application stops automatically when the sample supply is empty and air detected.

The sample volumes can also be set manually, if preferred. The air detection will then be disabled during sample loading. See **6.5.10 Using air detection.** on page 182

Manual purification protocols

There are two purification protocols in the Method Wizard which require manual sample loading:

- Protocol F: Desalting (Manual Sample Loading)
- Protocol G: Gel Filtration (Manual Sample Loading)

When running these protocols, the sample is injected with a syringe through the fill port **MANUAL INJECTION** in the injection valve into the loops in the loop valve. Up to four loops can be filled. Dialog boxes will automatically appear during the run with instructions for filling the loop. See also **6.5.8 Performing affinity tag removal** on page 175 for a description of the procedure.

Note: *The sample volume must be lower than the maximum sample loading volume of the desalting or gel filtration column used. In addition, the sample volume must always be lower than the loop volume, which is 10 ml. Observe that the sample volume should be entered when starting the method plan in System Control as well. Example: The HiPrep Desalting column alone can take up to 15 ml, but when used in ÄKTAxpress, the maximum sample loading volume is 10 ml.*

6.5.7 Preparing the fraction collector

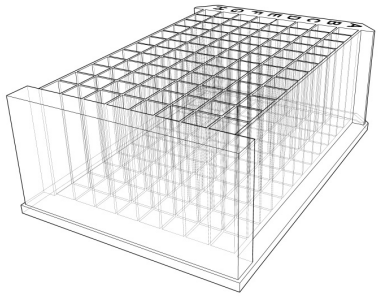
Introduction

This section describes how to load a microplate in the fraction collector.

Microplate requirements

The microplates for the fraction collector in ÄKTAxpress must fulfill the following requirements:

- *Deep well plate* with 96 wells, height 45 mm
- *Square* well design (not cylindrical wells)



The following manufacturer's microplates are tested and approved by Amersham Biosciences to be used with ÄKTAxpress:

- Greiner 780201
- Eppendorf Z605662
- Nunc P7616
- Corning A9347

Installing a microplate

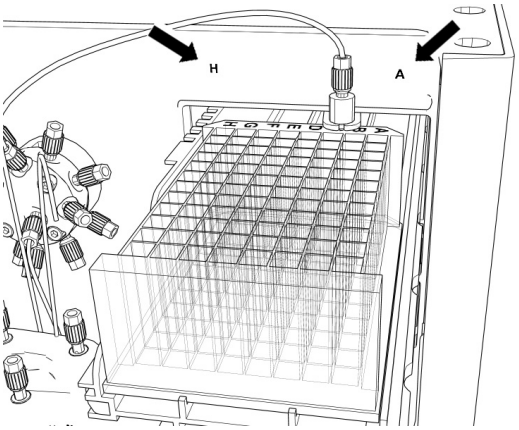
WARNING!

Do not put any body parts in front of the fraction collector. The microplate can be ejected both manually and automatically by the system.

WARNING!

Ensure that no parts, e.g. tubing or columns are positioned in front of the fraction collector, hindering the ejection of the microplate. The movement of the plate might destroy columns or tubing positioned in front of the fraction collector.

Follow the instruction in the table below to install a microplate.

Step	Action
1	Press the **Eject** button to eject the fraction collector plate, if necessary. 
2	Place the microplate on the sled and check that the labelling **H** and **A** match the labelling on the system. 
3	Check that the gap between the end of the tubing and the microplate is 1–2 mm.
3	Press the **Eject** button to load the collector plate, if necessary. 

6.5.8 Preparing the system for automatic affinity tag removal

Introduction

This section describes how to prepare the system for automatic affinity tag removal in ÄKTAxpress system.

Protocols for affinity tag removal

Automatic affinity tag removal can be combined with all ÄKTAxpress multi step protocols.

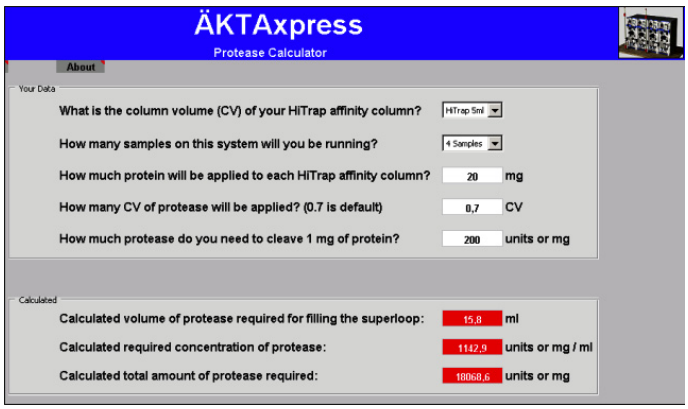
Adding affinity tag removal to a method plan

To add automatic tag removal to a method plan:

Step	Action
1	On the **Purify** page in the **Method Wizard**, select **Tag Cleavage** to add an affinity tag removal step to the method plan.
2	Choose **Incubation Time** and select **Fill Columns with Cleavage buffer** if a specific cleavage buffer, other than the ordinary affinity binding buffer, should be used.
3	Calculate the volume and concentration of protease needed. See **6.5.8 Example on protease calculation** on page 176.
4	During the run the software will give guidance to filling the superloop. The filling is performed manually with a syringe in the injection valve. See **6.5.8 Filling the superloop** on page 179. The rest of the tag cleavage procedure is performed automatically.

Example on protease calculation

The protease volume and concentration can be *automatically calculated* by using the **Protease Calculator** sheet found on the UNICORN CD.



The following example describes how to perform a *manual calculation*.

Conditions

- Protein type: His_6-tagged with TEV-protease cleavage site
- Number of samples: 4
- Estimated amount of protein: 20 mg
- Affinity column: HisTrap HP, 5 ml
- Protease: TEV protease, 200 units/mg target protein
- Delay volumes:
  - 0.73 ml for the first column only (loop valve to column valve plus delay volume for assisted manual loading of superloop)
  - 0.27 ml for all columns (column valve to column)

Protease volume

The default protease volume in the Method Wizard is 0.7 CV (can be changed).

Volume needed per column: 0.7 × 5 ml = 3.5 ml

Total volume needed: 3.5 ml × 4 columns + 0.27 ml × 4 columns + 0.73 ml = 15.8 ml

Protease concentration

Amount of protease per column: 20 mg × 200 units = 4000 units

Protease concentration: 4000 units / 3.5 ml =1143 units/ml

Conclusion

For this example, fill the superloop with 15.8 ml of 1143 units/ml TEV protease.

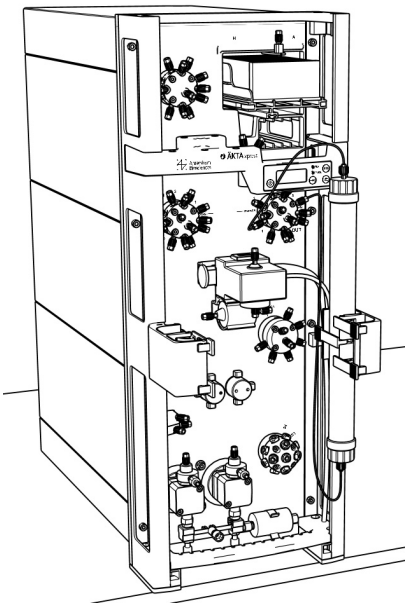
Note: *When injecting the protease solution into the superloop, the protease will be diluted with buffer corresponding to the volume in the capillaries between the injection valve and the superloop. In this calculation example and in the Protease Calculator sheet this dilution is overlooked. When using small protease volumes, e.g. one sample with 1 ml affinity column, the dilution might have to be considered when calculating the protease concentration.*

Note: *Try to keep the glycerol concentration low in the protease solution, since high glycerol concentration affects TEV activity negatively.*

Connecting the superloop

Follow the instructions below to connect the superloop:

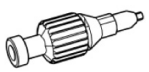
> **CAUTION!**
>
> Make sure that no parts, e.g. tubing or columns are positioned in front of the fraction collector, hindering the ejection of the microplate. The movement of the plate might destroy columns or tubing positioned in front of the fraction collector.

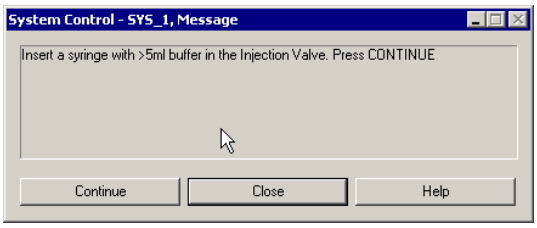
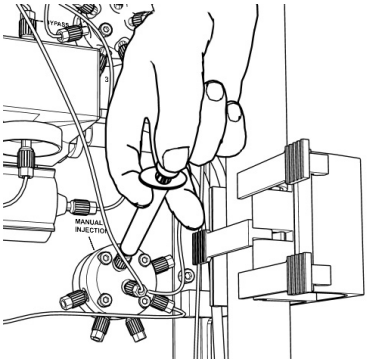
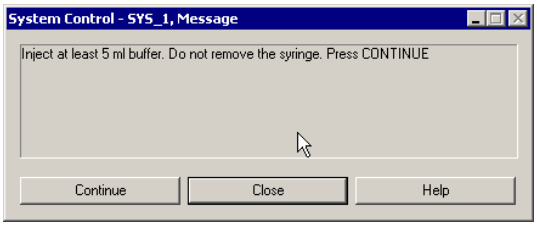
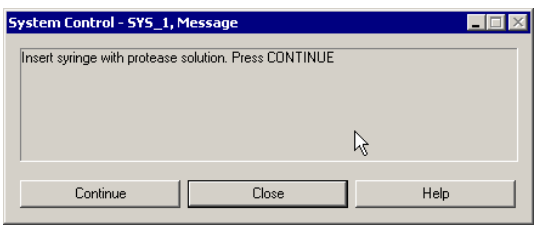
Step	Action
1	Attach the superloop to the column holder on the right-hand side. 
2	Remove the loop connected between port **OUT 5** and **IN 5** in the loop valve. Use the fingertight key if necessary.
2	Connect the tubing from the top of the superloop to the loop valve, port **OUT 5**.
3	Connect the tubing from the bottom of the superloop to the loop valve, port **IN 5**.

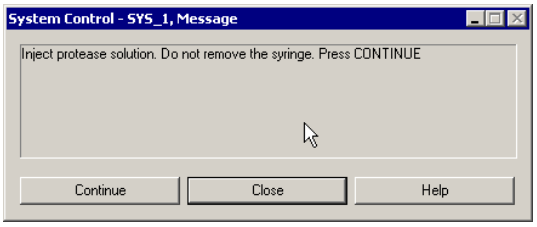
Filling the superloop

The superloop should be manually filled with protease solution. The Method Wizard contains an option for assisted manual loading of the superloop in preparation and purification runs. Dialog boxes will then automatically appear when starting the run, requesting the superloop to be filled.

To fill the superloop:

Step	Action
1	Connect a Luer fill port to the injection valve, port **MANUAL INJECTION**. 
2	Fill a syringe with at least 5 ml of affinity buffer A or the buffer used in the protease solution.

Step	Action
3	When starting a run, the system enters **Pause** mode. When pressing Continue, a dialog box appears requesting the syringe to be fitted. • Fit the syringe in the fill port and click **Continue**. Do not inject the buffer before clicking Continue! 
4	A new dialog box appears requesting the buffer to be injected.  • Inject the buffer and click **Continue**. Do not remove the syringe before clicking Continue! 
5	A new dialog box appears requesting a syringe with protease solution to be fitted. • Remove the syringe from the fill port. • Fill the syringe with the requested amount of protease solution, re-fit it in the fill port, and click **Continue**. Do not inject the protease before clicking Continue! 

Step	Action
6	A new dialog box appears requesting the protease solution to be injected. • Inject the protease solution and click **Continue** to proceed the run. Do not remove the syringe! The protease will automatically be loaded onto the affinity columns during the purification run. 

Filling the superloop manually

The protease can also be injected directly into the superloop before starting the run by connecting the syringe to the lower port of the superloop.

It is also possible to connect the superloop to the loop valve as usual and manually setting the injection valve and loop valve to the correct positions.

To set the valve positions manually:

Step	Action
1	Select **Manual:Flowpath** in **System Control**.
3	Set **LoopSelection** to **LP5** and click **Execute**.
2	• Before fitting or removing the syringe, set **InjectionValve** to **Inject** and click **Execute**. • To inject liquid into the superloop, set **InjectionValve** to **Load** and click **Execute**.

Using a column heater

If a system is used in a cold room, it is possible to raise the temperature in the affinity column by using a column heater. The efficiency of some proteases increases with increased temperature, thus decreasing the incubation time.

Note: *Carefully raise the temperature if the protein is temperature sensitive.*

The column heater is not controlled from ÄKTAxpress but can be physically placed on top of the system. Extra long capillaries might be needed between the column valve and the affinity column used. The protease volume then must be changed in the Advanced Zone in the Method Wizard, considering the new delay volume.

6.5.9 Checking the tubing

Introduction

This section describes how to check the tubing *before* a run.

Checking the tubing

The tubing must be checked for:

- Air bubbles
- Leakage

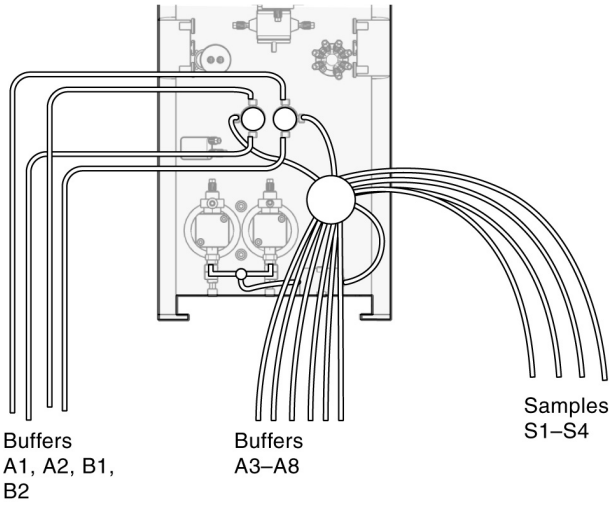
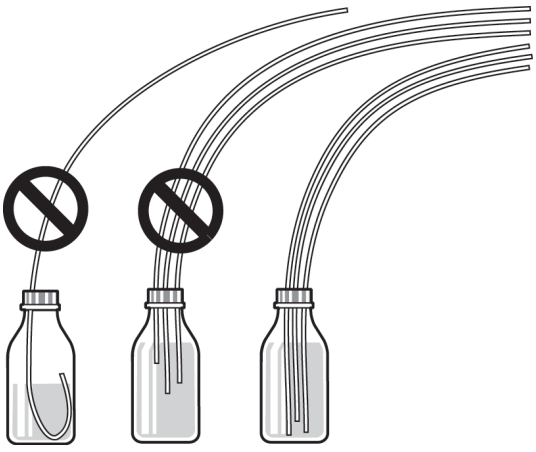
If there are air bubbles or leakage, the run might be adversely affected.

To check the tubing follow the procedure below:

> **CAUTION!**
> To protect the piston seals in the pump, the pump should not run with air in the inlet tubing. Follow the instructions for purging the pump.

> **CAUTION!**
> Before the start of each run, ensure that there is an adequate supply of eluent in the reservoirs. Never allow the pump to run dry, because this will affect the lifetime of the piston seals.

Step	Action
1	*Leakage inspection* Inspect the tubing for leakage. Pay special attention to the tubing connectors that are related to columns. If leakage is found, tighten the connectors or replace the tubing if necessary, see **8.6.2 Replacing tubing** on page 249.

Step	Action
2	*Air bubbles inspection* Inspect the inlet tubing for air bubbles. All tubing up to the pump heads should be inspected.  If air bubbles are found, perform manual purging of the system, see **6.5.3 Purging the pump and inlet tubing** on page 156.
3	*Buffer tubing* Check that the tubing is fully immersed in the buffer containers. Fix the tubing in the cap, for example by: • Fitting the inlet filter weights on the tubing. • Running the tubing through the rubber cap included in the accessory kit. 

6.5.10 Using air detection

Introduction

This section describes the automatic air detection in ÄKTAxpress.

About air detection

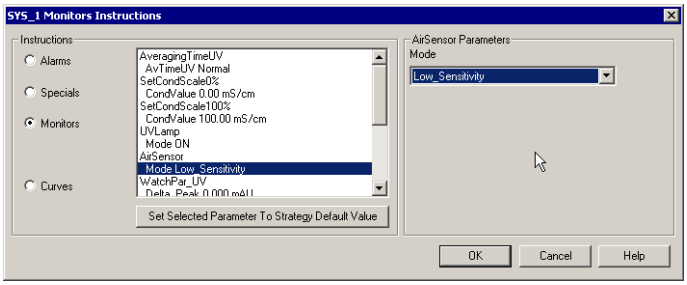
The separation system is equipped with an air sensor, which is located between the inlet valve and the pump.

Automatic air detection is used to:

- prevent air from entering the flow path during the purification, and
- detect when the sample supply is empty when applying sample.

Air sensor sensitivity

- Check that the air sensorsensitivity on each system used is set to **Low_Sensitivity** by selecting **System:Settings** in **System Control** and then clicking **Monitors**. The system must be in End mode.



Air detection at buffer application

Air detection during buffer application is used to prevent air from reaching the column. By default, air detection is always enabled during buffer transport in a method plan made in the Method Wizard.

When air is detected, for example, due to empty buffer supply or a loose tubing end, the system enters Pause mode. After correcting the error, for example by running a wash to remove the air, the run can proceed by clicking **Continue**.

Note: *If manually running PumpWash, SystemWash, or LoopWash after air detection, the system will automatically go to the same mode as before the air detection.*

Air detection at sample loading

Air detection during sample loading makes it possible to apply the entire sample volume and still prevent air from reaching the column. By default, air detection is always enabled during sample application in a method plan made in the Method Wizard.

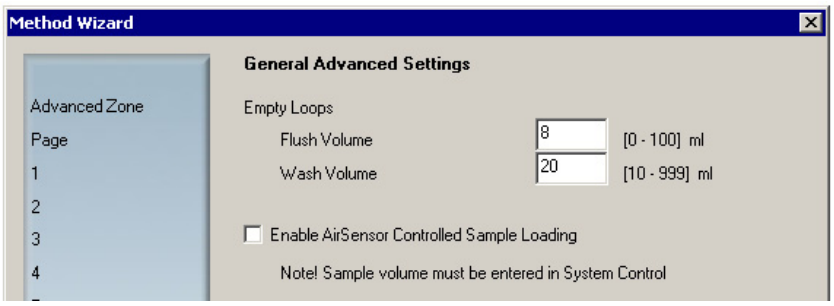
When air is detected during sample loading, the inlet valve switches to port A1 The pump then uses a few ml of buffer A1 to apply sample that is left in the tubing onto the column. The pump then flushes the air out with affinity buffer A1 at 20 ml/min. The flush volume can be changed on the General Advanced Settings page in Advanced Zone in the Method Wizard.

The sample loss when using air detection is approximately 0.5 ml.

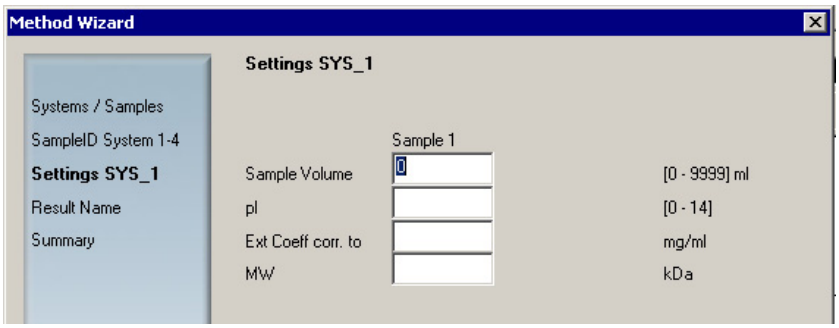
Setting a fixed sample volume

It is also possible to set a fixed sample loading volume.

- To set a fixed volume, the air sensor option must first be cleared in the method plan. This is done on the **General Advanced Settings** page in **Advanced Zone** in the **Method Wizard**.



- When the method plan is started in System Control, the sample volume has to be typed on the **Settings** page in the **Method Wizard**.



Note: *Make sure that the sample supply is sufficient for the given sample volume. Air might otherwise enter the column.*

6.5.11 Using flow control during sample loading

Introduction

This section describes the flow control function in ÄKTAxpress.

About flow control

The flow control is used to avoid exceeding the maximum pressure limit.

When flow control is used, the flow is automatically decreased when the pressure approaches the set maximum limit. After a short while, when the pressure has decreased, the flow slowly increases towards the set flow rate, and so on.

If the flow rate falls below the set minimum limit (0.1 ml/min is default), the system will issue an Alarm and enter Pause mode.

Enabling/disabling flow control

The flow control function is activated throughout the run in all purification protocols. The flow control can though be disabled during the sample loading in the affinity step. To disable flow control in a method plan, go to the **Affinity - Flow rate/Pressure** page in **Advanced Zone** in the **Method Wizard**.

6.6 Starting a run

About this section

This section describes how to start a run using a method plan and how to run a system manually.

In this section

This section contains the following sub-sections

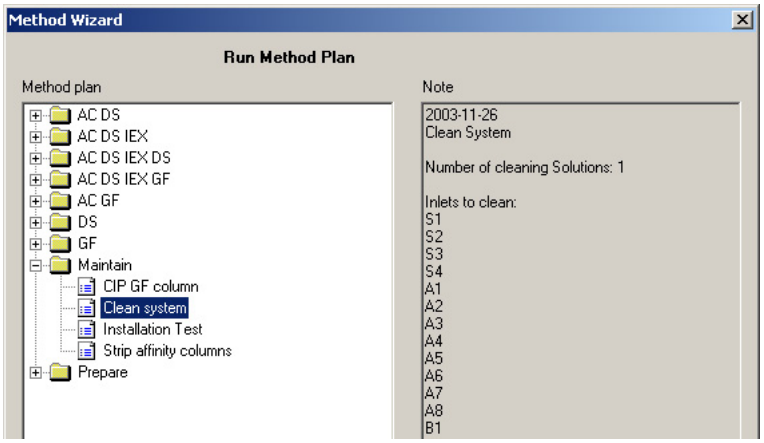
6.6.1 Starting a run using a method plan

Introduction

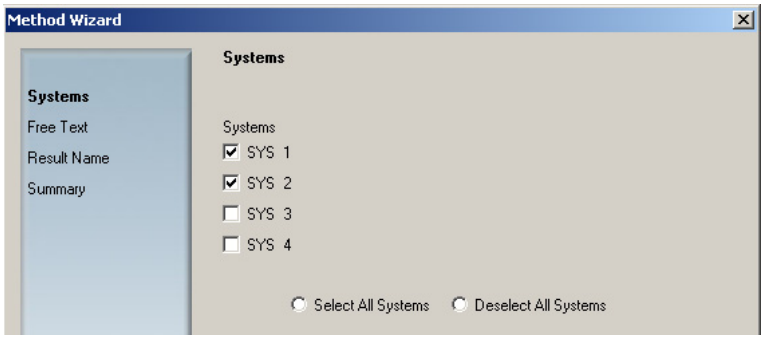
This section describes how to start a run using a saved method plan.

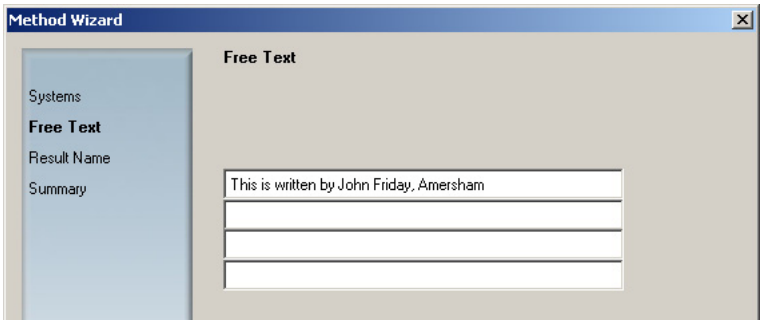
Selecting a method plan

Follow the steps below to select a method plan.

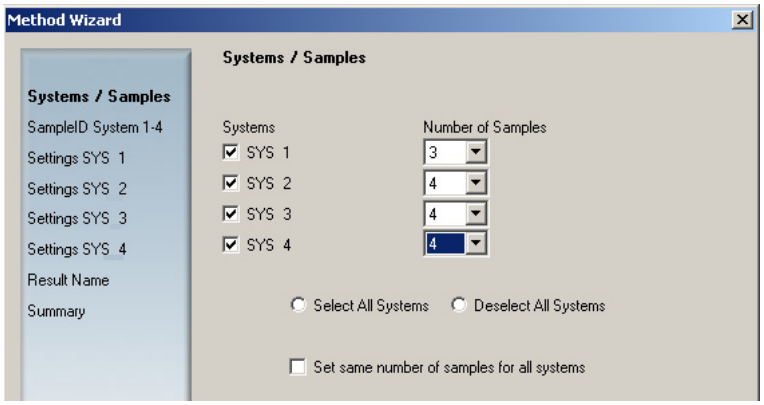
Step	Action
1	Click the **System Control** button, located on the task bar. *Result*: The System Control module appears.
2	Click the **Instant Run** button, located on the tool bar. Alternatively, select **File:Instant Run**.  *Result*: The Method Wizard in System Control appears.
3	Select the requested method plan in the **Method plan** list. Click **Next**. 

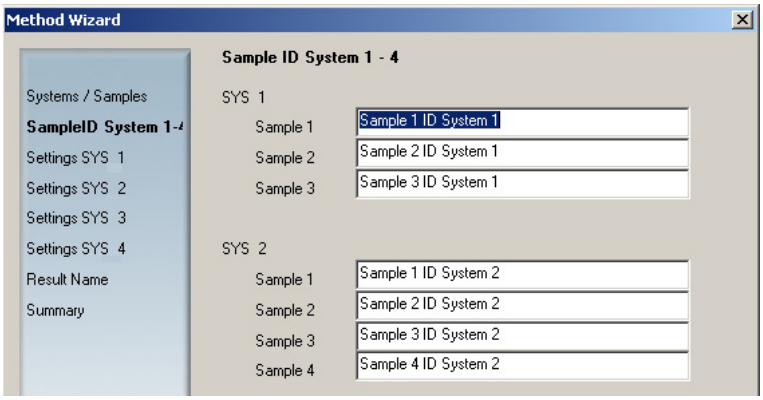
If selecting a Prepare or a Maintain method plan:

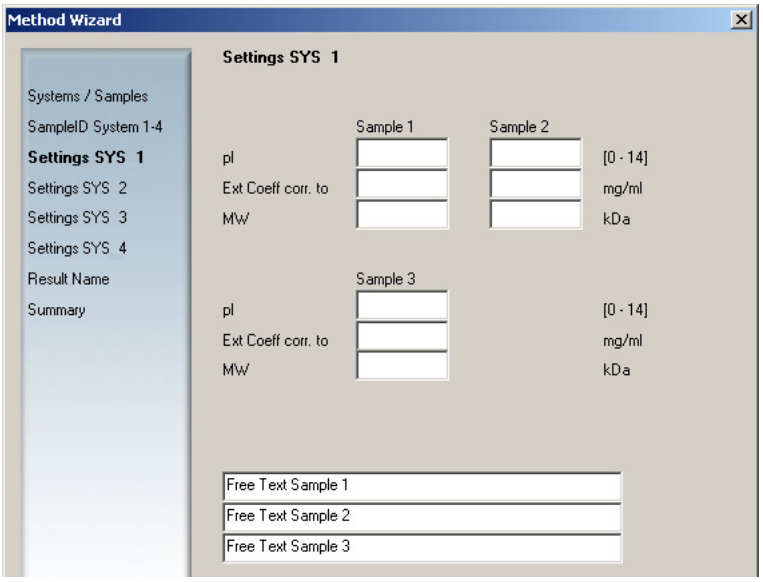
Step	Action
4	Select on which **System**(s) the method plan shall be used. It can be used on any or all selected systems. Click **Next**. 

Step	Action
5	Enter any optional text, for example, running data. Click **Next**. The text can be viewed later in the result file as Notes in Evaluation by selecting View:Documentation. 
6	Proceed to **6.6.1 Final setup and starting the run** on page 191.

If selecting a Purify method plan:

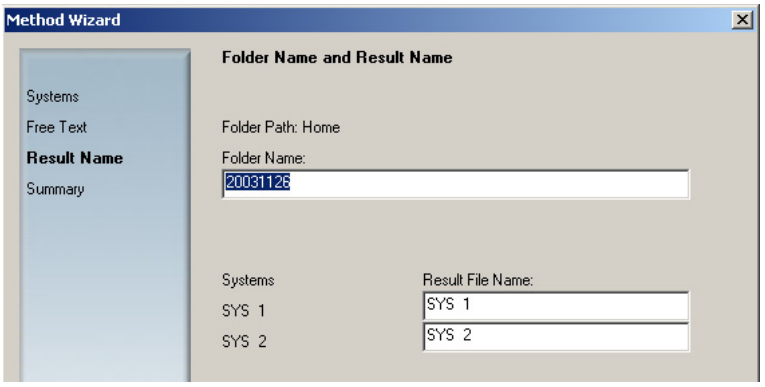
Step	Action
4	• Select on which **System**(s) the method plan shall be used. • Select the **Number of Samples** for each system(s). • Click **Next**. 

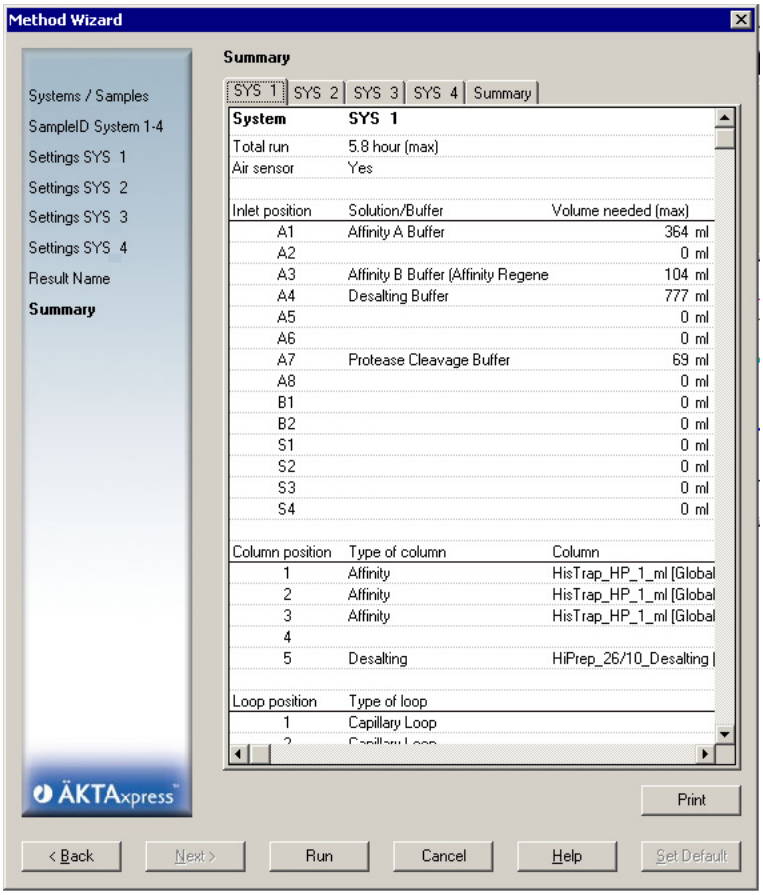
Step	Action
5	Enter identification name(s) for the sample(s). A bar code reader can also be used. An import file with sample information can be linked to this sample ID. See **10.3 Import file format** on page 331. Click **Next**. 

Step	Action
6	For each sample: • Enter the isoelectric point in **pI**, the extinction coefficient for the protein in **Ext Coeff**, and the molecular weight of the protein in **MW**. The extinction coefficient is the absorbance of 1 mg/ml solution of the purified protein at 280 nm in a 1 cm cuvette. ***Note:*** *If an import file exists containing these data for the proteins to be used, the fields might automatically be filled. The path to the import file folder must be specified in General Advanced Settings in Advanced Zone in the Method Wizard in Method Editor. For more information on the import file, see* ***10.3 Import file format*** *on page 331.* ***Note:*** *The import of data is done only once each time this wizard is used. To perform a new import of data, click Set Default on the first page, or click cancel and then Instant Run.* See also **5.5.9 Advanced Zone for Purify - General advanced settings** on page 136. • Enter optional text, for example running data, in the text box. It will appear in the pool table and in the result file in Documentation:Notes:Method Notes in Evaluation.  • Click **Next**.
7	Proceed to **6.6.1 Final setup and starting the run** on page 191.

Final setup and starting the run

For final setup and starting the run:

Step	Action
1	If required, edit the folder path and names of the result files. Click **Next**. By default, the result files will be saved in Home folder\Date\System name 001. If there are several result files created on the same date, the files will be named 001, 002 and so on after the identical name. If Main Folder is chosen in General Advanced Settings in the Method Wizard, the result file will be saved in Home folder\Main folder\Date\System name 001. 

Step	Action
2	The Summary page specifies the method requirements. The tabs for each system specifies the required columns, loops, buffers, solutions, etc. and where they should be connected. The total requirement of buffers and solutions is listed on the Summary tab. If required, make a printout of the list by clicking **Print**. Note that the list will not be saved. 
3	Click **Run** to start the run on the selected systems. *Result*: The systems will enter Pause mode.
4	Make a final check of the system setup using the list from the Summary page. Click **Continue** or **Continue all** in **System Control** or press the Continue button on the system to continue the method. 

6.6.2 Starting a manual run

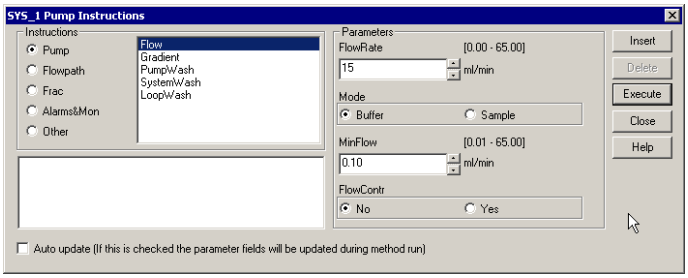
Introduction

This section describes how to run a system manually. For more information see the UNICORN user documentation.

Manual control

Besides running method plans on the systems, it is also possible to control the system manually, for example starting and stopping the pump, switching valve positions, etc.

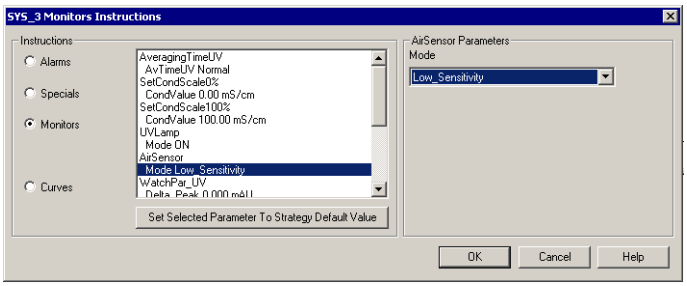
Follow the steps below to control the system manually.

Step	Action
1	Select **Manual:Pump** in **System Control**. A dialog box containing manual instructions appear. 
2	• Select an option in the **Instructions** field and an instruction. • Select parameter or set a suitable value in the **Parameters** field and click **Execute** to start the instruction.
3	For information about the instructions in a dialog box, click **Help**.

System settings

Each system has a set of default system settings which can be changed.

- To change a system setting, select **System:Settings** in **System Control**. The system must be in End mode. Make the appropriate changes on parameter values and click **OK**.



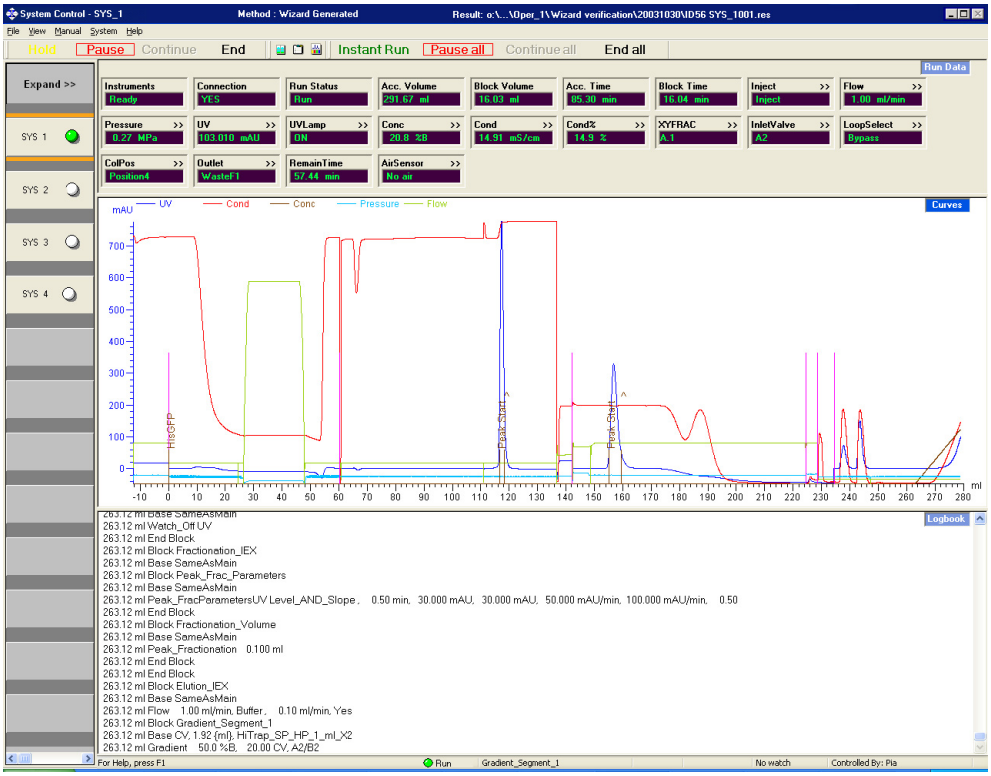
6.7 During a run

Overview

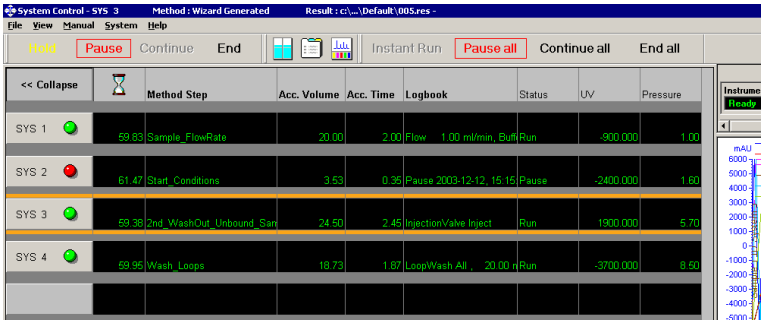
This section describes how to view the progress of the run and change parameters during a run.

Viewing the run

The progress of the run can be viewed in detail in System Control. By clicking the separation system symbol on the left, the current status for the particular system can be displayed.



By clicking **Expand** in the left-hand, upper corner, more information on the current status of each system is displayed. By clicking **Collapse** the information disappears.



Up to three view panes, Run Data, Curves and Logbook can be displayed showing different aspects of the run.

- The **Run Data** view pane displays the current values for selected run parameters.
- The **Curves** view pane displays the monitor signal values graphically.
- The **Logbook** view pane shows the actions as the run proceeds. All actions and unexpected conditons are logged, with date, time and current user name. The log book provides a complete history of the run and is saved in the result file.

To customize the view panes, right-click in the respective view pane and select **Properties**. For more information about customizing the view panes, see the UNICORN user documentation.

Ending the run

To stop the run on a system before it is finished:

Step	Action
1	Check that the correct system is selected on the screen.
2	Click **End** above the **Run data** view pane.

Status indicator colors

The table below shows how the indicator colors relate to the run status.

Indicator color	Run status
White	**End**
Green	**Run** or **Manual**
Yellow	**Hold**
Red	**Pause**

Error indication

When a warning or an alarm is issued from a system, the background of the system symbol starts flashing and the background color turns yellow.

Changing parameters

The separation system can be controlled with manual instructions issued from the Manual menu in System Control. These instructions can be used during the run to change system conditions in response to the results observed.

- When changing or loading a microplate during a run, the system must first be set to Pause before pressing the Eject button on the system control panel.

Problems during a run

In case of problems during the run, see **9.1 Troubleshooting** on page 282.

6.8 Procedures after a run

About this section

This section describes how to clean the system, tubing and columns after performed protein purification. It also includes how to perform metal ion stripping of the affinity columns.

In this section

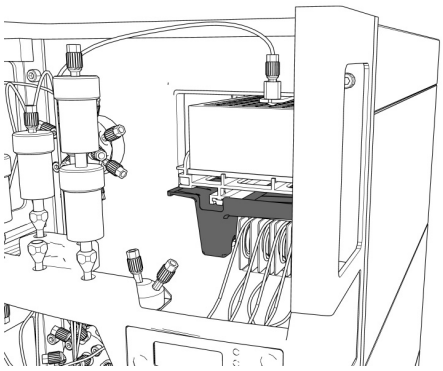
This section contains the following sub-sections

6.8.1 Emptying drip plate

Introduction

The drip plate, located below the microplate in the fraction collector, should be emptied when necessary.

The purpose of the drip plate is to collect any overflow from the microplate. Overflow might indicate an error, see **9.1 Troubleshooting** on page 282. If the drip plate becomes full, there is a risk of damage to the system.



Emptying drip plate

To empty the drip plate in the fraction collector follow the procedure below:

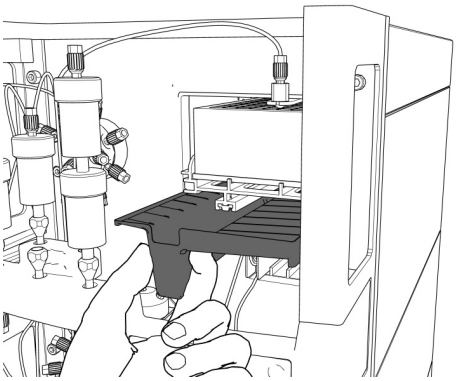
WARNING!

Do not put any body parts in front of or close to the fraction collector. The microplate can be ejected and retracted both manually and automatically by the system.

CAUTION!

Ensure that no parts like tubing or columns are positioned in front of the fraction collector, hindering the ejection of the microplate. The movement of the plate might destroy columns or tubing positioned in front of the fraction collector.

Step	Action
1	Check that the system is in **Idle** mode with no active run.
2	Press the **Eject** button to eject the collector plate out of the system, if necessary. Each time the button is pressed, the sled switches between inside and outside the system.

Step	Action
3	Carefully remove the drip plate by sliding it outwards. 
4	Empty any fluid into waste.
5	Slide the drip plate back into position.
6	Check the function by pressing the **Eject** button to load the collector plate.

6.8.2 Cleaning system

Introduction

When running different types of samples or purification methods after each other, the sample inlet tubing and the system flow path should be cleaned between the runs. This will prevent sample contamination, protein precipitation, and buffer mixing.

When leaving the system for the weekend or for a longer time, the cleaning procedure should be finished with 20% ethanol to prevent bacterial growth in the tubing.

Note: *Do not leave the system with salt buffer in the flow path. It might damage the pump.*

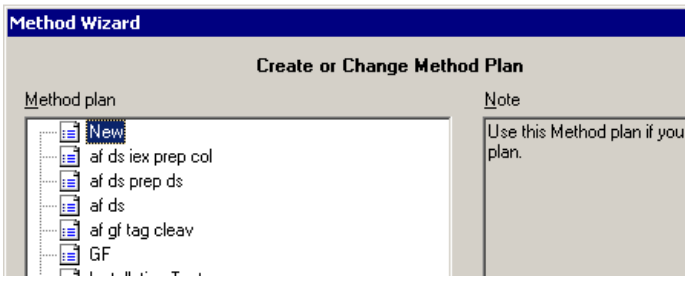
Prerequisites

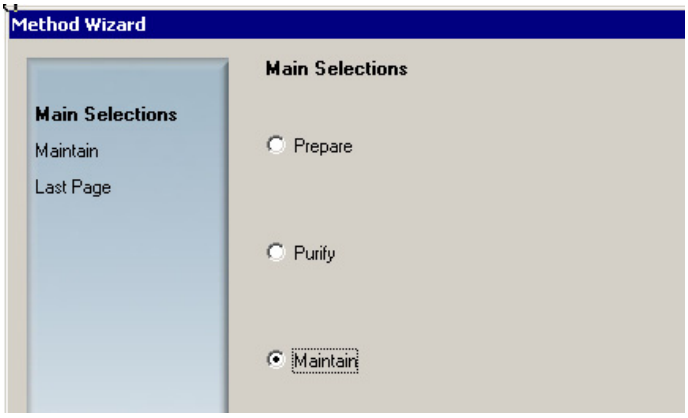
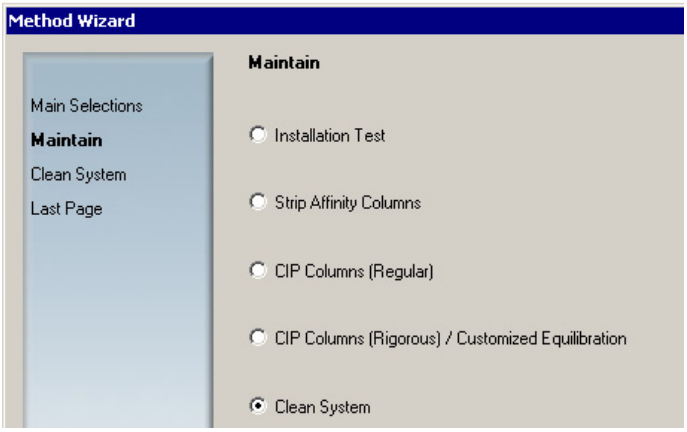
The following are required for automated system cleaning:

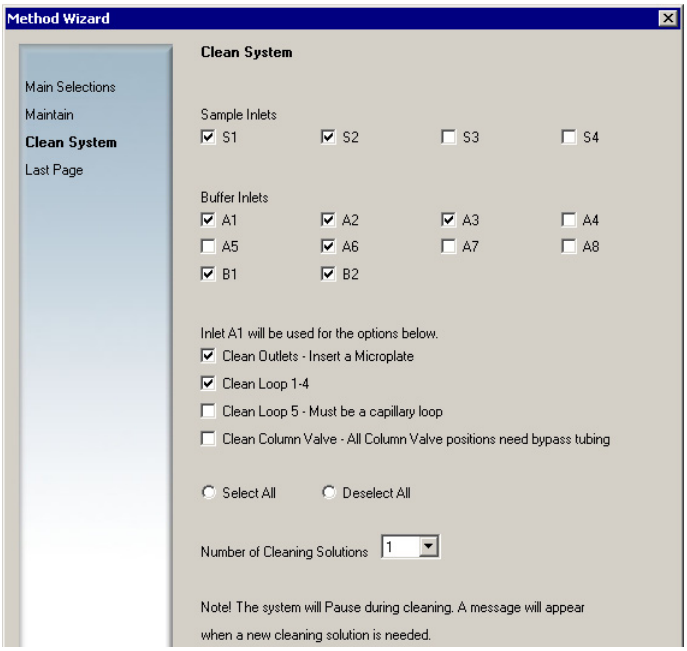
- Cleaning solutions, for example NaOH and deionized water. Up to five different cleaning solutions can be used in one cleaning run. The operator moves the inlet tubing from solution to solution.
- 20% Ethanol when the system will be unused for a longer time.
- A method plan for cleaning the system.

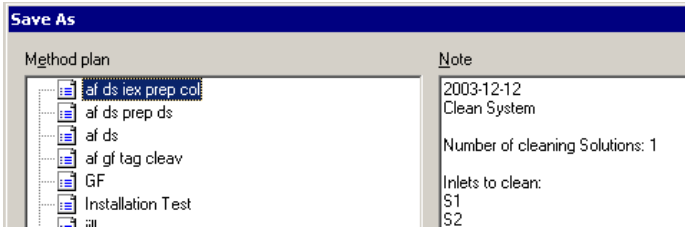
Creating a method plan for cleaning system

To create a method plan for cleaning the system:

Step	Action
1	In the **Method Editor** module in UNICORN, start the Method Wizard by for example selecting **File:Method Wizard**.
2	Click **Next** to create a new method plan. 

Step	Action
3	Select **Maintain** and click **Next**. 
4	Select **Clean System** and click **Next**. 

Step	Action
5	Select suitable cleaning options and click **Next**.  **Sample Inlets** The sample inlets to be cleaned. **Buffer Inlets** The buffer inlets to be cleaned. **Clean Outlets** The outlets will be cleaned from inlet A1 (a microplate must be inserted). **Clean Loop 1–4** The capillary loops to be cleaned from inlet A1. If this option is selected, the option to **Clean Loop 5** will be displayed. **Clean Column valve** The column inlet tubing will be cleaned from inlet A1. ***Note:*** *Check that all column valve positions have bypass tubing.* **Number of Cleaning Solutions** Up to five different cleaning solutions can be used. The tubing chosen in the method plan will be cleaned with one cleaning solution at the time. The system will pause and a message appear when the inlets shall be inserted into a new cleaning solution.
6	Click **Next** and **Finish**.

Step	Action
7	Enter a **Method plan name** and click **OK** to save the method plan.  *Result*: A method plan for cleaning system is now ready to be run on a single or on several ÄKTAxpress separation systems.

Cleaning the system

To run the method plan for cleaning the system:

Step	Action
1	Wash the outside of the inlet tubing with water and/or ethanol.
2	Immerse the tubing ends to be used in the container with cleaning solution 1 (see also the **Summary** page). If the system will not be used for a longer time, use a bacteriostatic solution, e.g. 20% ethanol, as the final wash solution.
3	If the column valve is to be cleaned, remove the columns and reconnect the tubing to the column block.
4	If the outlets are to be cleande, insert a microplate.
5	Run the cleaning method plan as any other method plan.

Flushing the tubing manually

The tubing can also be flushed using the following manual instructions (not column or outlet tubing):

- PumpWash
- SystemWash
- LoopWash

These instructions can be executed from the **Pump Instructions** dialog box by selecting **Manual:Pump** in **SystemControl**. They also appear in the method plans where they are automatically executed.

Note: *The system is always in Pause mode during PumpWash. Hence, it is only possible to click END or CONTINUE during PumpWash.*

For more information about the instructions, click **Help** in the dialog box.

6.8.3 Cleaning columns

Introduction

When running different types of purification methods and different samples after each other, the columns should be cleaned between the runs or according to the column instructions. This will remove unspecific bound proteins and prevent column clogging.

CIP Column is a Method Wizard option specifically made for cleaning columns. The method is adapted to the columns used when setting up the method plan in the Method Wizard, e.g. flow rate and pressure settings, column volume, etc.

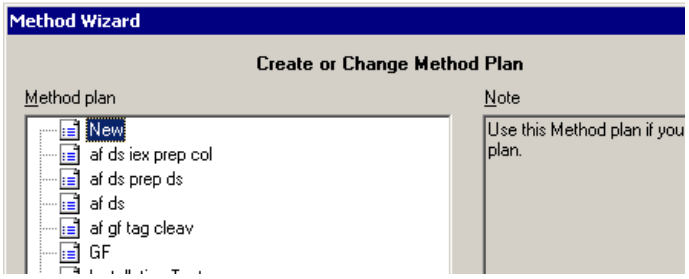
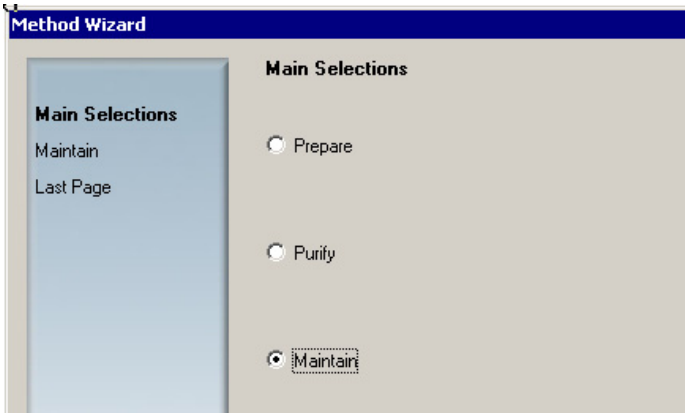
Prerequisites

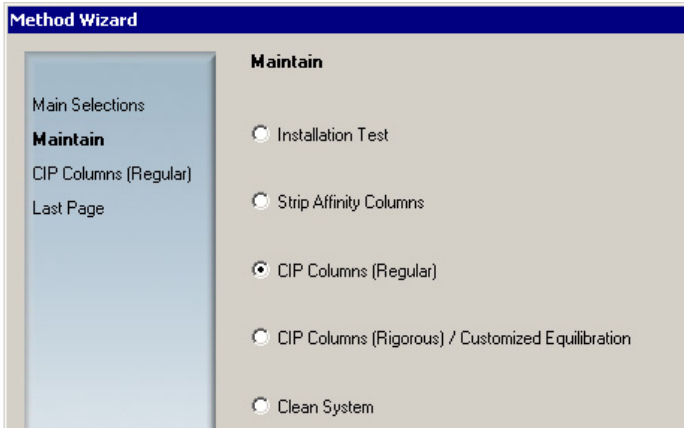
The following are required for cleaning the columns:

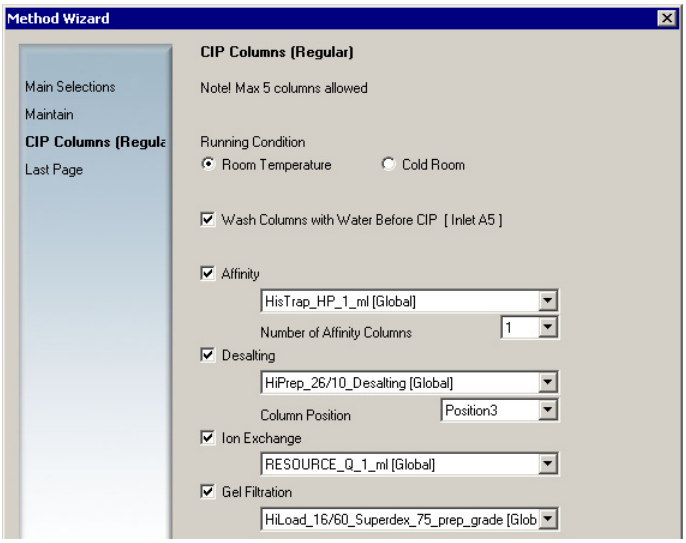
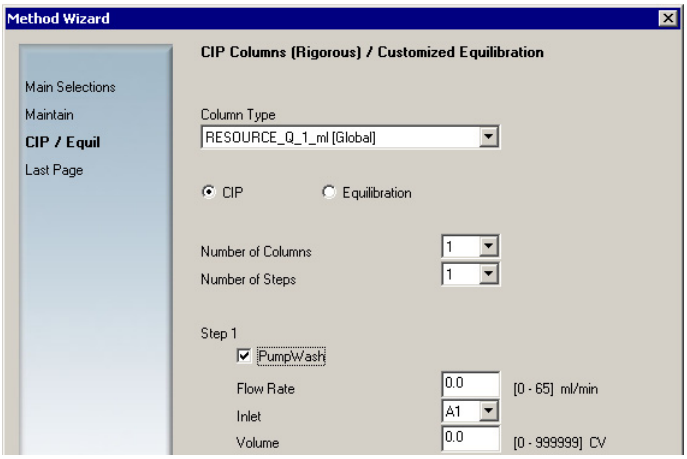
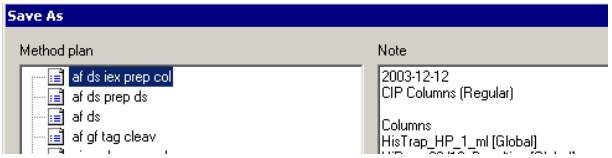
- Cleaning solutions, for example 0.5 M NaOH and deionized water. Up to nine cleaning solutions can be used in one cleaning run. See the column instructions.
- A method plan for cleaning the columns.

Creating a method plan for column cleaning in place

To create a method plan for column cleaning in place:

Step	Action
1	In the **Method Editor** module in UNICORN, start the Method Wizard by for example selecting **File:Method Wizard**.
2	Click **Next** to create a new method plan. 
3	Select **Maintain** and click **Next**. 

Step	Action
4	Select one of the two cleaning-in-place methods: • **Regular** The standard CIP procedure for the chosen columns two solutions per column. The solutions and inlets are specified on the Summary page in the wizard in System Control when starting the run. They are also described in the dialog Help. • **Rigorous / Customized Equilibration** A method plan that gives a range of options to design a protocol for cleaning or equilibration with up to nine cleaning solutions. Equilibration is recommended for removing ethanol from new columns. Click **Next**. 

Step	Action
5	Select suitable cleaning options and click **Next**. Also refer to the User Instruction supplied with each column for column cleaning procedures and column storage instructions. *Regular:*  *Rigorous / Customized Equilibration:* 
6	Click **Next** and **Finish**.
7	Enter a **Method plan name** and click **OK** to save the method plan.  *Result*: A method plan for cleaning columns is now ready to be run on a single or on several separation systems.

Cleaning the columns

To run the method plan for column cleaning in place:

Step	Action
1	Run the cleaning in place method plan as any other method plan.

6.8.4 Performing metal ion stripping of affinity columns

Introduction

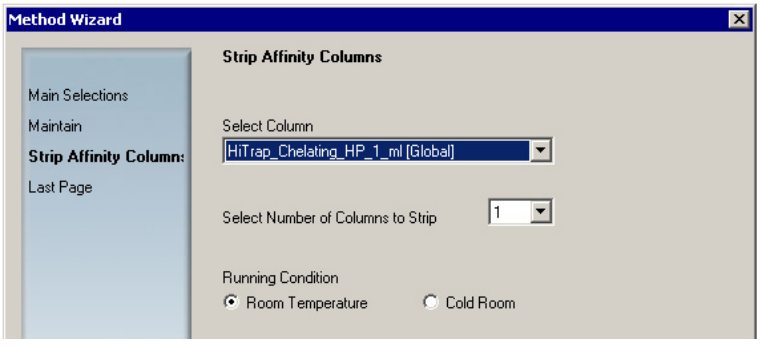
Before regenerating HiTrap Chelating and HisTrap affinity columns, metal ions must be removed from the columns.

Note: *Always remove metal ions before or directly after storing the columns in ethanol.*

During the procedure the column is flushed with 5 CV of EDTA solution and then washed with 10 CV of deionized water.

Creating a method plan for performing metal ion stripping

To create a method plan for performing metal ion stripping.

Step	Action
1	Click the **Method Wizard** icon in the **Method Editor**.  *Result*: The **Method Wizard** dialog box appears.
2	Select **New** method plan in the **Method plan** field and click **Next**.
3	Click **Maintain** on the **Main Selections** page. Click **Next**.
4	Click **Strip Affinity Columns** on the **Maintain** page. Click **Next**.
5	On the **Strip Affinity Columns** page: • Select column type. • Select the number of affinity columns • Select running conditions. Click **Next**. 
6	On **Last page** click **Finish** and save the metod plan.

- Run the method plan according to the standard procedure described in **6.6.1 Starting a run using a method plan** on page 186.

- Immerse the inlet tubing in flasks according to the check list on the Summary page.

Note: *The Metal Ion Stripping inlet tubing (A8) have to be filled before starting the stripping run. See* ***6.5.3 Purging the pump and inlet tubing*** *on page 156.*

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7 Evaluation

About this chapter

This chapter contains descriptions of how results from a run can be evaluated.

In this chapter

This chapter contains the following sections

7.1 Evaluation procedure overview

Introduction

This section is an overview of the normal procedure for evaluation of results after a run with ÄKTAxpress.

The normal evaluation procedure

Normally 5 steps are performed after a run:

Step	Action
1	Find and open results, see **7.2** on page 210
2	View results and adjust the viewing, see **7.3** on page 212
3	Adjust pooling, see **7.4** on page 213
4	Create and print pooling protocol, see **7.5** on page 216
5	Print report, see **7.6** on page 218

Optional evaluation actions

Optional actions can be performed in the evaluation part of UNICORN. For more information, see UNICORN Online Manual.

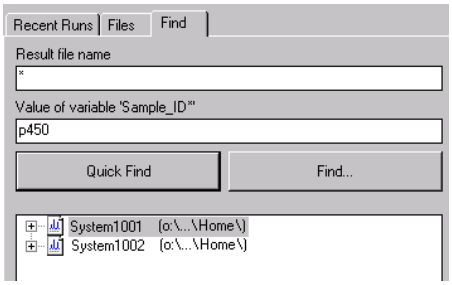
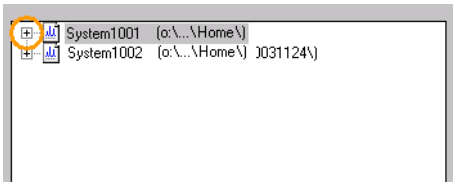
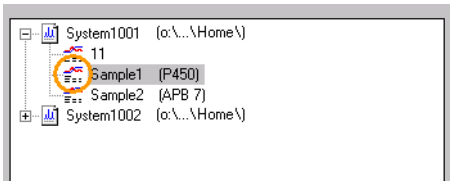
7.2 Finding and opening results

Introduction

This section describes how the result files can be found and opened in the UNICORN folders.

Recommended finding and opening of files

In UNICORN it is recommended to find and open result files in the **Evaluation** module the following way (to receive automatic pooling):

Step	Action
1	Use the **Recent Runs** tab, or the **Find** tab, in the **File Navigator** (on the left hand side of the **Evaluation** window) to locate the result file.  ***Note:*** *See How to use the File Navigator in the UNICORN Online Manual for detailed instructions on how to locate files and set up File Navigator preferences.*
2	Click the **+** button to expand the list for the result file. 
3	Double-click the sample file to open it.  *Result:* The chromatogram of the sample is displayed and the fractions are automatically pooled in the software. By default, the chromatograms in a run are shown as opened windows. The chromatogram window on top is the active window. There is also a minimized **Temporary** chromatogram window. See *Basic presentation of chromatograms* in the UNICORN Online Manual for further information about chromatograms.

Alternative finding and opening of files

See *How to open a result file* in the UNICORN Online Manual for alternative ways to open result files. Automatic pooling will not be performed if result files are opened in any of the alternative ways.

7.3 Viewing results

Introduction

This section describes how to view the results.

Zooming in the chromatogram

If the window is zoomed to show the peaks only, it is easier to evaluate the chromatogram.

In the active chromatogram window, zooming in on a designated area of the chromatogram is the easiest and quickest way to enlarge different parts of a curve. To do this:

Step	Action
1	Open a result file.
2	• Place the mouse pointer in any corner of the area to be magnified. • Press and hold the left mouse button. A magnifying glass icon will be added to the mouse pointer arrow on the screen. • Drag a box to cover the area to be magnified, and release the mouse button. *Result:* The selected region is now displayed in the entire chromatogram window, together with appropriate scales for the Y and X axes.
3	Use the arrow keys on the keyboard to move around in the chromatogram at the current zoom scale.
4	Possible undo-actions when zooming: • **Undo zoom** Right-click in the window and select **Undo zoom** to undo the last zoom step. • **Reset zoom** Right-click in the window and select **Reset zoom** to reset all zoom steps at once.

Further instructions

Further instructions on how to change the viewing of the chromatogram can be found in *How to view results* in the UNICORN Online Manual.

7.4 Pooling fractions and adjusting the pools

Introduction

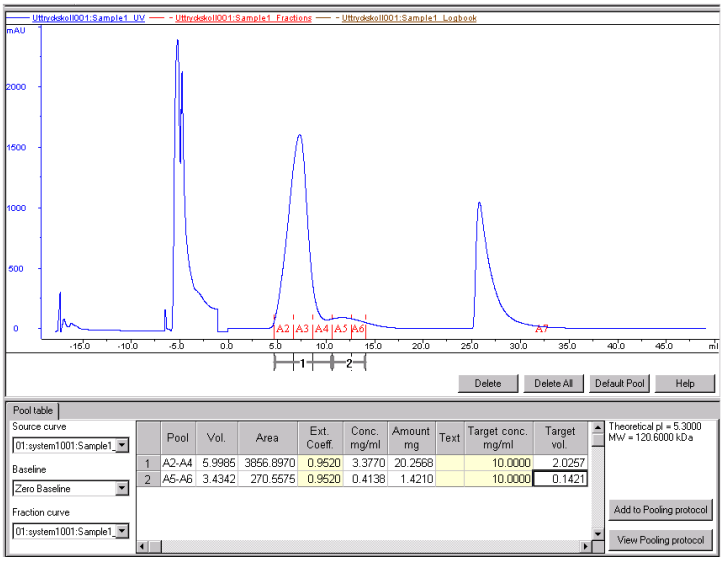
After the last purification step of each protocol a fractionation is performed. The resulting fractions are shown in the chromatogram. A pooling suggestion of the fractions can be provided in UNICORN to calculate protein concentration and amounts. This section describes the steps involved when creating the pooling suggestions and adjusting the pools.

Note: *When the fractionation of a peak is finished, it will in the chromatogram appear as the subsequent fraction volume is very large. This is because the position of the fraction collector is shown and only the delay volume for the next peak (if any) actually is collected in this well.*

Note: *The collection in the microplate is performed in a serpentine manner i.e. first in A1, A2, ..., A11, A12 and then in B12, B11, ..., B2, B1, and so on. To display all fractions used, select Show all fractions in the Pooling Protocol dialog.*

Pooling fractions and adjusting the pools

The table below describes how to view the suggested pool fractions and adjust the pools.

Step	Action
1	If the results were opened via Recent Runs or Find tab in the Evaluation module, UNICORN will automatically display a suggested pooling of the fractions. The pooled fractions are listed in a table below the chromatogram and the pooled peaks are numbered sequentially in the chromatogram. ***Note:*** *If the results were opened any alternative way, the pooling is not automatically performed. Choose* **Operations:Pool** *to pool the fractions before continuing below.*  ***Note:*** *Only adjacent fractions will be pooled. The fraction numbers for each pool are listed in the table as a range in retention order, e.g. A6–A7 etc.* ***Note:*** *If the extinction coefficient has been entered in the wizard in System Control, concentration and amount are automatically calculated. Otherwise: enter the extinction coefficient manually by marking a pool and then typing the value in the extinction coefficient field. The concentration is calculated in mg/ml or M and Amount is calculated in mg or mole)*

Step	Action
2	The pooled fractions can be adjusted manually: • **To include or exclude adjacent fractions in a pool** Click the numbered marker under the pool and drag the sideline. • **To add more pools** Click between the droplines under a fraction to create a new pool, and drag the sidelines to include more adjacent fractions. • **To delete pools** Click the numbered marker to select the pool and click the **Delete** button. Click the **Delete All** button to clear all pools. • **To restore the pools created by UNICORN** Click the **Default Pool** button.
3	To select another baseline curve, click the **Default Pool** button in the **Baseline** droplist.

Note: *To calculate concentration and amount for peaks that have not been fractionated, perform a simulated peak fractionation. See UNICORN Online Manual for more information.*

7.5 Creating pooling protocols

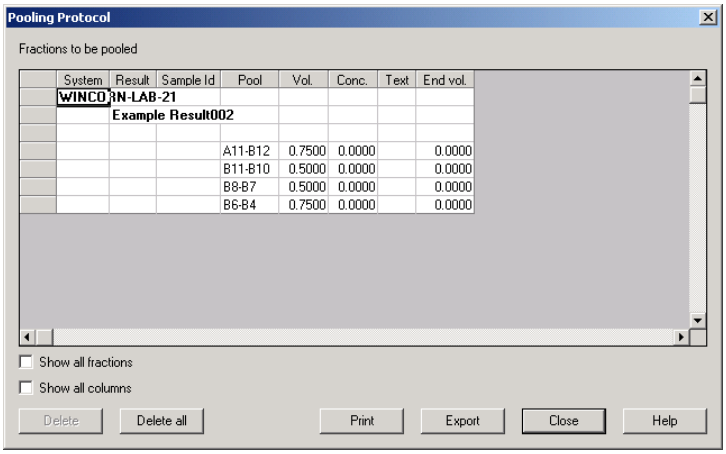
Introduction

Information about pooled fractions from different result files can be assembled in the pooling protocol. The pooling protocol can be printed for use when pooling the samples physically. This section describes how to add pools to the pooling protocol and how to print or export the pooling protocol.

Creating pooling protocol

Once the pools have been satisfactorily adjusted, they can be added to the pooling protocol.

The table below describes how to add pools to the **Pooling Protocol** and send the list to a printer or export the list to a file.

Step	Action
1	• Open a result file in the **Evaluation** module. • Make sure the pools are satisfactorily adjusted. See **7.4 Pooling and adjusting the pooling** on page 213. • Click the **Add to Pooling Protocol** button. *Result*: The pooled fractions from the active result file is added to the **Pooling Protocol**.
2	• Repeat step 1 to add pooled fractions from other result files.
3	• Click the **View Pooling Protocol** button. *Result*: The **Pooling Protocol** dialog box opens. 

Step	Action
4	• Click **Show all fractions** to display the individual fractions instead of fraction ranges for the pools. • Click **Show all columns** to display all the information columns from the **Pool table**. **Possible actions in the Pooling Protocol** ***Note:*** *The information in the Pooling Protocol is saved for the individual UNICORN user. The Pooling Protocol can only be cleared by clicking the* **Delete** *or* **Delete all** *button.* • To delete a single pool: select a pool and click the **Delete** button • To clear the whole protocol: click the **Delete all** button. • To print the protocol: click the **Print** button to print the protocol on the default Windows printer. • To save the protocol as a file: click the **Export** button to save the protocol in one of the following formats: - text (.txt) - Excel (.xls) - HTML (.htm) - XML (.xml)
5	• Click the **Close** button to close the **Pooling Protocol** dialog box. *Result*: If the protocol was exported or only edited, the dialog box will close. If the protocol was printed, a dialog box will open, asking if the list is to be deleted and a new one started.

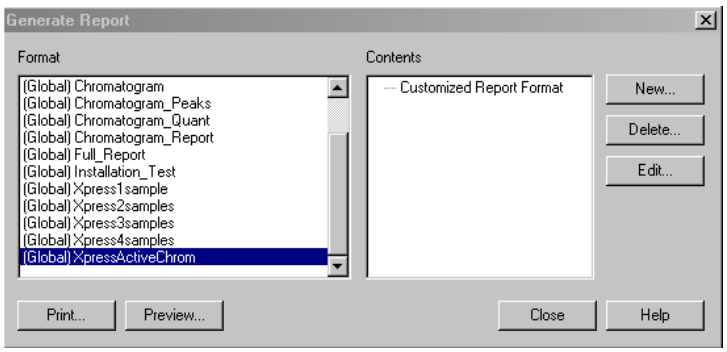
7.6 Printing report

Introduction

Reports can be printed after a completed run. To find information on how to create reports, see the UNICORN Online Manual. This section describes how to print reports from runs with ÄKTAxpress.

How to print a standard report

The table below describes how to print a **Standard** report format in the **Evaluation** module.

Step	Action
1	Open a result file.
2	• Select **File:Report**. *or* • Click the **Report** icon.  *Result*: The **Generate Report** dialog box opens. 
3	• Select **Xpress1sample, Xpress2samples, Xpress3samples** or **Xpress4samples** report format, depending on how many samples are included in the result file. The report format **XpressActive Chrom** can also be selected if only the active zoomed window should be included in the report. • Click the **Edit** button to modify the report format if needed.
4	• Click the **Print** button. *Result*: The **Print** dialog box opens. • Choose what pages and how many copies to print. • Click **OK**. *Note*: Printers are set up in the **File** menu of the **UNICORN Manager**.

Customized reports

For instructions on how to print customized reports, see UNICORN Online Manual.

8 Maintenance

About this chapter

This chapter describes the maintenance activities for ÄKTAxpress.

In this chapter

This chapter contains the following sections

8.1 Maintenance program

Introduction

This section specifies the preventive maintenance to be performed on ÄKTAxpress. The maintenance is divided into:

- Monthly maintenance
- Six monthly maintenance
- When required

For maintenance of columns see chapter **6.8.3 Cleaning columns** on page 202.

Periodic maintenance program

The following periodic maintenance should be made by the user of ÄKTAxpress.

Interval	Maintenance action
Monthly	See: **8.2.1 Checking flow restrictor** on page 223.
	See: **8.2.2 Changing pump rinsing solution** on page 225.
Six monthly	See: **8.3.1 Checking UV lamp run time** on page 227.
	See: **8.3.2 Cleaning UV cell** on page 228.
	See: **8.3.3 Cleaning conductivity cell** on page 231.
When required	See: **8.4.1 Externally cleaning the system** on page 233.
	See: **8.5.1 Calibration of zero pressure** on page 242.
	See: **8.5.2 Calibration of conductivity cell** on page 243.
	See: **8.6.1 Replacing capillary loops** on page 247.
	See: **8.6.2 Replacing tubing and connectors** on page 249.
	See: **8.6.3 Replacing flow restrictor** on page 262.
	See: **8.6.4 Replacing UV lamp** on page 264.
	See: **8.6.5 Replacing check valves** on page 269.
	See: **8.6.6 Replacing pump piston seal** on page 272.
	See: **8.6.7 Replacing a damaged pump piston** on page 280.
	See: **6.5.3 Purging the pump and inlet tubing** on page 156.

8.2 Monthly maintenance

About this section

This section describes the monthly maintenance.

In this section

This section contains the following sub-sections

Topic	See
Checking flow restrictor	8.2.1
Changing pump rinsing solution	8.2.2

8.2.1 Checking flow restrictor

Maintenance interval

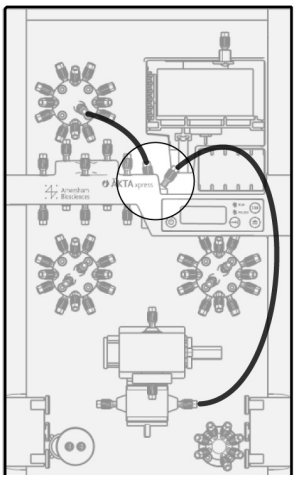
Every month.

Reason for maintenance

A flow restrictor opening at *too high pressure* will cause a high pressure alarm from the system. In exceptional cases this can result in column rupture.

A flow restrictor opening at *too low pressure* might cause air bubbles in the buffer/solution in the flow path.

The position of the flow restrictor:

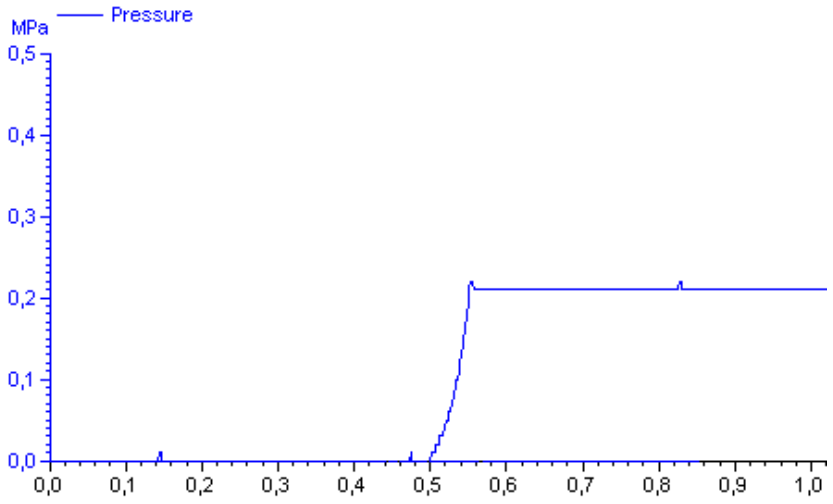


In addition, a flow restrictor is integrated in the mixer.

Checking flow restrictor

To check the flow restrictor:

Step	Action
1	Immerse inlet tubing A1 into deionized water.
2	Start a flow from A1 to *injection valve waste* by: • Start UNICORN and select **System Control: Manual: Flowpath**. • Select **Injection Valve** and **Waste**. Click **Execute**. • Select **System Control:Manual:Pump**. • Select a flow rate of 5 ml/min. Click **Execute**.
3	Start a flow from A1 to *outlet valve waste* by: • Select **System Control: Manual: Flowpath**. • Select **Injection Valve** and **Inject**. Click **Execute**.

Step	Action
4	Inspect the pressure curve in the UNICORN **System Control** window.  Check that the pressure difference is within the range 0.1 to 0.3 MPa. If this is not the case, the flow restrictor should be replaced, see **8.6.3 Replacing flow restrictor** on page 262.

8.2.2 Changing pump rinsing solution

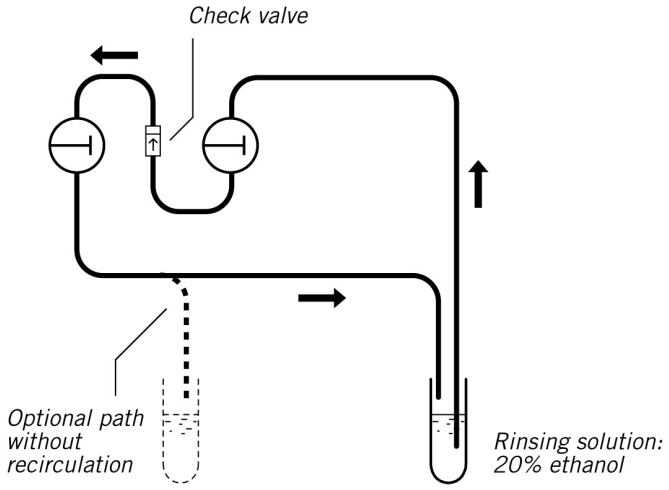
Maintenance interval

Every month.

Reason for maintenance

The pump rinsing solution should be changed to avoid bacteriological growth. The rinsing solution can be placed in a 50 ml test tube mounted in the right hand column holder.

The pump piston seal rinsing system:



Changing solution

To change the pump rinsing solution:

Step	Action
1	Empty the liquid container and fill it with 20% ethanol. Place the test tube for example in the right hand column holder
2	Repeat the procedure for all systems. If required, several systems can use the same liquid container. ***Note:*** *Do not connect rinsing system tubing from several systems in serial. The tubing from each system must be routed directly to the liquid container.*

8.3 Six monthly maintenance

About this section

This section describes the six monthly maintenance activities.

In this section

This section contains the following sub-sections

8.3.1 Checking UV lamp run time

Maintenance interval

Every 6 months.

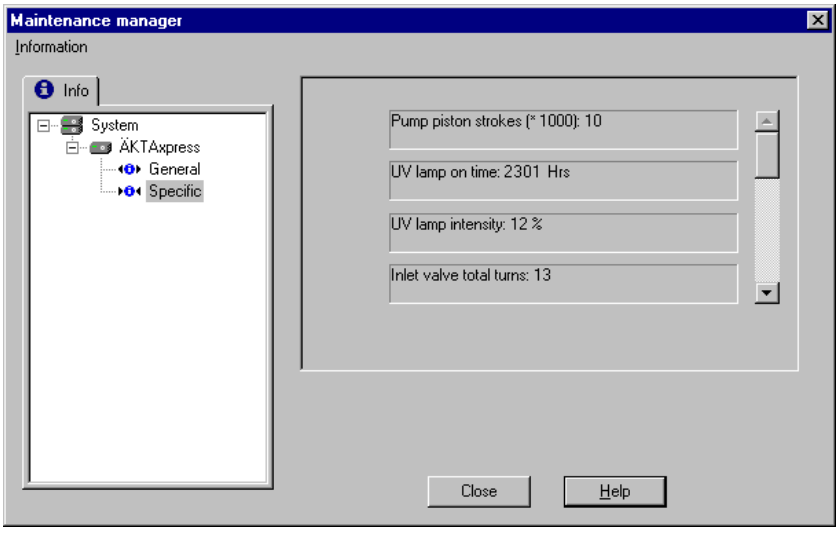
Reason for maintenance

The UV lamp has a life time of typically:

	Wavelength 254 nm	Wavelength 280 nm
In room temperature	7000 h	3500 h
In cold room	2000 h	2000 h

Checking UV lamp run time

To check the UV lamp run time:

Step	Action
1	From UNICORN **System Control** select **System:Maintenance**.
2	In the **Maintenance manager** open **ÄKTAxpress** and **Specific**. *Result*: The UV lamp on time is shown in hours together with other life time parameters. 
3	Read the run time. Take suitable action considering the lamp life time: • No action. • Check that a new UV lamp is available or order replacement lamp. • Replace the lamp, see **8.6.4 Replacing UV lamp** on page 264.

8.3.2 Cleaning UV cell

Maintenance interval

Every 6 months.

Reason for maintenance

A clean UV flow cell is essential for correct operation of the UV monitor.

> **CAUTION!**
> Do not allow solutions containing dissolved salts, proteins or other solid solutes to dry out in the UV cell. Do not allow particles to enter the cell. Damage to the UV cell might occur.

There are two procedures for cleaning the UV cell:

- **Inplace cleaning** Use this procedure for general cleaning in case of problems.
- **Offline cleaning** Use this procedure as preventive maintenance every 6 months.

Performing inplace cleaning

To make an inplace cleaning of the UV flow cell:

Step	Action
1	Pump a cleaning or sanitizing agent through the flow cell. **WARNING!** **CORROSIVE CHEMICALS**. NaOH is corrosive and therefore dangerous to health. Avoid spillage and wear protective glasses. The standard recommendation is to pump 1 M NaOH at a flow rate of 1 ml/min for 2 minutes.
2	Stop the flow and leave the cell filled with liquid for 30 minutes.
3	Rinse with buffer or deionized water.
4	If this does not correct the problem, follow the instructions for making offline cleaning.

Performing offline cleaning

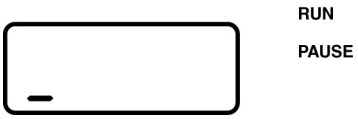
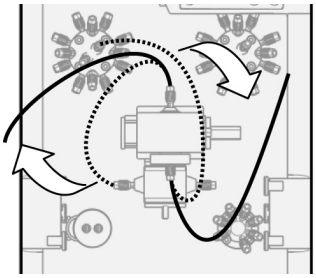
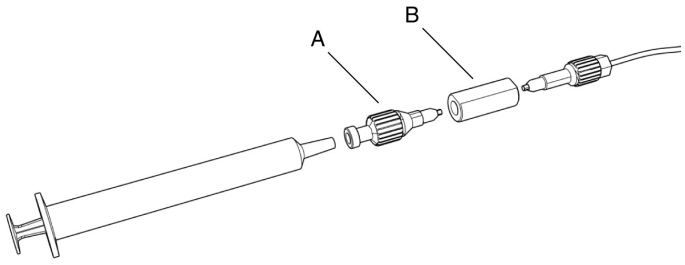
To perform offline cleaning of the UV flow cell:

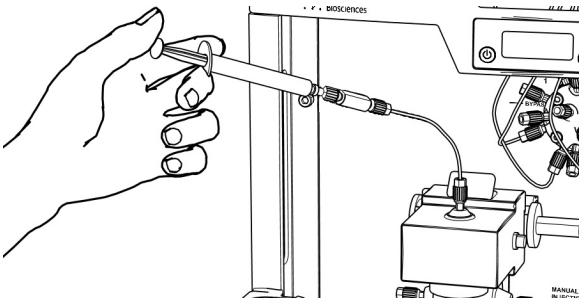
WARNING!

The UV monitor uses high intensity ultra-violet light. Do not remove the optical unit while the lamp is ON to prevent injury to eyes.

Required tools and materials:

- Syringe
- Surface active detergent solution like Decon 90, Deconex 11, RBS 25 or equivalent
- Deionized water

Step	Action
1	Set the system in *Standby* mode by pressing the **On/Standby** button. This will disconnect the system from UNICORN. 
2	Fill a syringe with deionized water.
3	Remove the UV monitor capillary tubing from the conductivity cell and the column valve. 
4	Connect a syringe to the upper capillary tubing using the connecting pieces A and B.  **A**: Luer female to 1/16" male: 18-1112-51. **B**: Union 1/16" to 1/16": 18-3855-01.

Step	Action
5	Inject water through the cell in small amounts while holding the lower capillary tubing in a flask. 
6	Fill the syringe with a 10% surface active detergent solution (like Decon 90, Deconex 11, RBS 25 or equivalent), and inject through the cell 5 times.
7	For the last injection, leave the detergent solution in the flow cell for at least 20 minutes.
8	Rinse the syringe and then flush the flow cell with water (10 ml).
9	Reconnect the capillary tubing.

8.3.3 Cleaning conductivity cell

Maintenance interval

Every 6 months.

Reason for maintenance

A clean flow cell is essential for ensuring correct measurements. If the conductivity measurements are not comparable to previous results, the electrodes in the flow cell may be contaminated and requires cleaning.

Performing in-place cleaning

To make an inplace cleaning of the conductivity flow cell:

Step	Action
1	Pump a cleaning or sanitizing agent through the flow cell. **WARNING!** **CORROSIVE CHEMICALS**. NaOH is corrosive and therefore dangerous to health. Avoid spillage and wear protective glasses. The standard recommendation is to pump 1 M NaOH at a flow rate of 1 ml/min for 2 minutes.
2	Stop the flow and leave the cell filled with liquid for 15 minutes.
3	Rinse with buffer or deionized water.
4	Make a calibration of the conductivity monitor, see **8.5.2 Calibration of conductivity cell** on page 243.

8.4 Maintenance when required

About this section

This section describes the maintenance activities to be made when required.

In this section

This section contains the following sub-sections

Topic	See
Externally cleaning the system	8.4.1
Cleaning check valves	8.4.2
Checking pump pressure	8.4.3
Checking tubing leakage	8.4.4
Running installation test	8.4.5

8.4.1 Externally cleaning the system

Maintenance interval

When required.

Reason for maintenance

The system should be kept dry and clean to ensure proper functioning of the system. Chemical stains and dust should be removed.

Actions

To clean the system follow the procedure below:

Step	Action
1	Check that no active run is in progress on the system. The run indicator should be *off*.
2	Disconnect the mains power cable.
3	To remove stains spray the system with 20% ethanol and wipe off the excess. ***Note:*** *Be careful not to bend any tubing.*

8.4.2 Cleaning check valves

Introduction

Faulty operation of the check valves is usually indicated by:

- irregular flow
- very low flow
- unstable pressure traces

Probable causes of this are air or dirt in a check valve preventing it from closing to seal and hold the pressure.

To solve the problem, perform:

- Inplace cleaning

If the problem remains, continue with:

- Offline cleaning

Performing in-place cleaning

To perform inplace cleaning of the check valves:

Note: *It is recommended to use degassed buffers and solutions*

Step	Action
1	Pump deionized water at a flow rate of 40 ml/min for 2 minutes.
2	Change solvent to 100% methanol and pump at a flow rate of 40 ml/min for 4 minutes.
3	Change solvent to deionized water and flush the system. If this does not correct the problem, follow the instructions for performing offline cleaning.

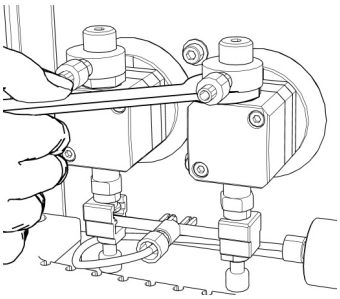
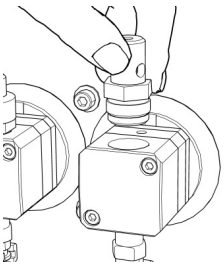
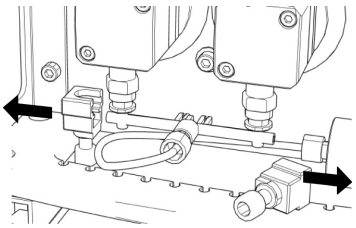
Performing offline cleaning

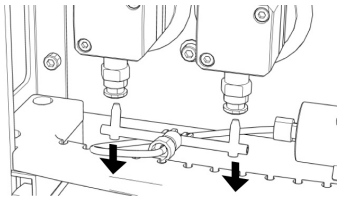
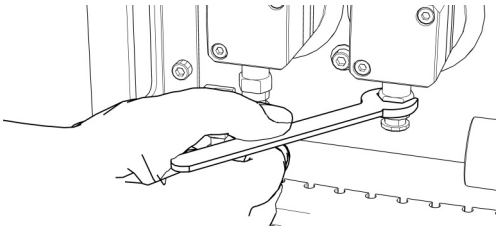
To perform offline cleaning of the check valves:

> **CAUTION!**
> Check valves have precision matched components and should only be disassembled by a trained person. If the problem cannot be corrected, the check valve should be replaced completely.

Required tools and material:

- 13 mm wrench
- 18 mm wrench
- Ultrasonic bath
- 100% methanol

Step	Action
1	Change solvent to deionized water and flush out all used buffers/solutions. Move all input buffers bottles below the level of the pump heads, to prevent siphoning.
2	Disconnect electrical power from the system.
3	Remove the tubing from the pump heads.
4	Loosen the upper valve from the pump head using the 18 mm wrench. 
5	Gently remove the check valve. **CAUTION!** Handle the check valves with care when they have been removed from the pump heads to prevent loss of any internal components. 
6	Unscrew the two white knurled screws under the pump to release the support bracket locking the inlet manifold into the inlet valve. 

Step	Action
7	Gently lower and remove the complete manifold. 
8	Loosen the lower valve from the pump head using the 13 mm wrench. **CAUTION!** Handle the check valves with care when they have been removed from the pump heads to prevent loss of any internal components.  Gently remove the check valve.
9	Immerse the complete valves in methanol and place them in an ultrasonic bath for some minutes. Then repeat the ultrasonic bath with deionized water.
10	Refit the check valves. The inlet check valve (with a lip for the manifold and a larger diameter opening) is fitted to the side marked IN of the pump head. Tighten the valves until fully finger-tight and then use the wrench to tighten a further 1/3rd (110°) of a turn. ***Note:*** *Do not overtighten the valves as damage to the internal components can occur.*
11	Refit the outlet tubing and the inlet manifold.
12	Purge the pump carefully and check that the pumping action has been corrected, see **6.5.3 Purging the pump and inlet tubing** on page 156.

8.4.3 Checking pump pressure

Introduction

Problems in the pump can cause abnormal pressure readings. Examples of pump problems:

- Air trapped in the pump heads
- Leaking connections
- Leaking pump piston seal
- Check valve malfunctioning
- Piston damage

Checking the pump function

To check the pump function:

Step	Action
1	Disconnect the **Waste** tubing from the injection valve, port 1 and connect a capillary tubing giving a counter pressure of about 1 MPa at 1 ml/min, to port 1.
2	Set the injection valve to position **Waste**.
3	Start a flow of 1 ml/min or less and observe the pressure curve over several minutes.
4	If the pressure pulsation seems abnormal: • Purge the pump heads, see **6.5.3 Purging the pump and inlet tubing** on page 156. • Check the tubing connectors for leakage. • Check the piston seals, check valves and pistons. • Check the number of piston strokes by selecting **System: Maintenance** in **System Control**. The life time is 4000 (x1000), i.e. 4 000 000 strokes.

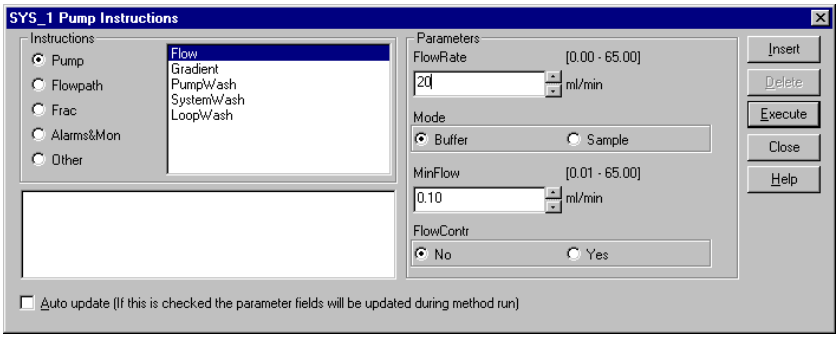
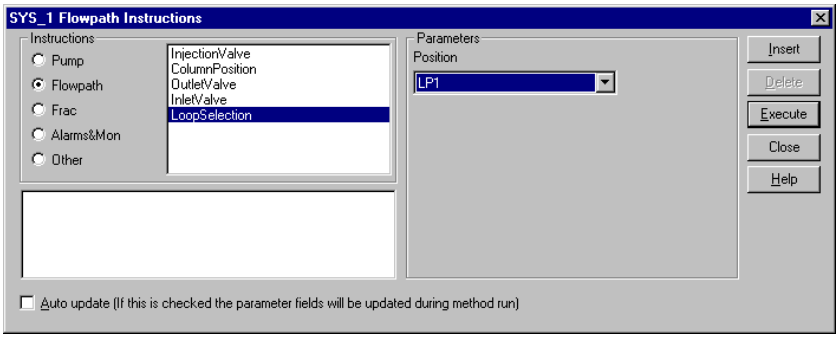
8.4.4 Checking tubing leakage

Introduction

Problems with leaking connectors can be found running a tubing leakage test.

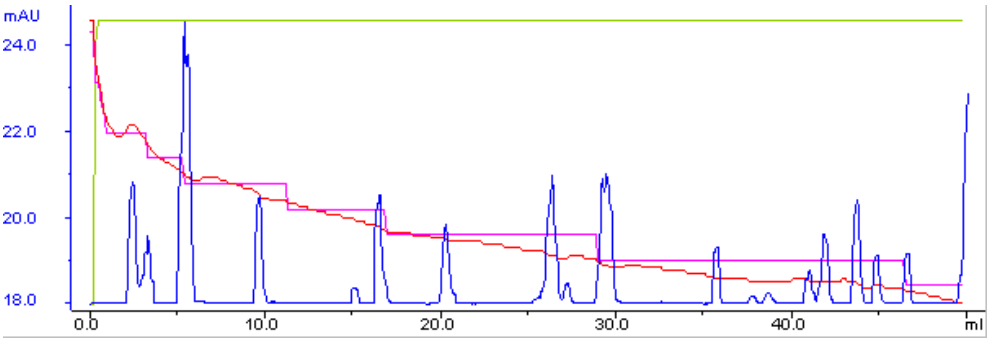
Checking the tubing leakage

To check for leakage in the system tubing:

Step	Action
1	Check that the inlet tubing to be tested is properly immersed in a flask with deionized water.
2	Start the pump: • Select **System Control:Manual:Pump**. • Select a flow rate of, for example 20 ml/min.  • Click **Execute** to start the flow.
3	Select a flow path matching the tubing to be checked, for example: • **InletValve** and **A1**. • **OutletValve** and **F12**. • **LoopSelection** and **LP1**.  • Click **Execute** to set the valves into position.
4	Inspect the UV curve for abnormal peaks, see below.

Abnormal pressure curves

Below is an examples of abnormal UV curve due to leaking connectors causing air bubbles in the tubing.



8.4.5 Running installation test

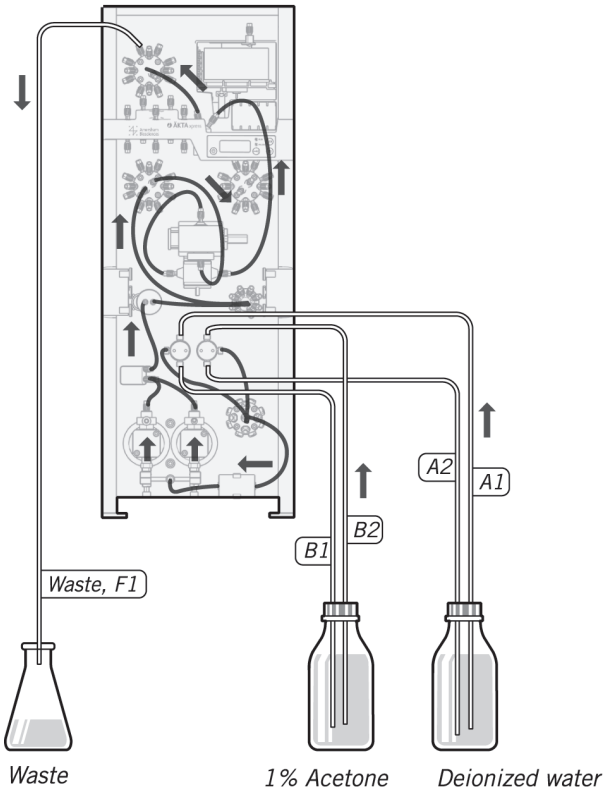
Introduction

To check the function of the separation system, an installation test can be performed on all separation systems e.g. after a prolonged stop. The test can be started to run simultaneously on all systems if required.

The installation test will check:

- The *liquid delivery,* by pumping liquid from buffer inlets to waste.
- The *gradient formation,* by producing a linear gradient and a series of step gradients of acetone.

The principle flow path of the installation test:



Procedure

To run the installation test follow the instructions in *ÄKTAxpress Installation Guide*.

8.5 Calibration procedures

About this section

This section describes some calibration procedures of ÄKTAxpress.

In this section

This section contains the following sub-sections

8.5.1 Calibration of zero pressure

Introduction

Problems in the pressure calibration can cause abnormal pressure readings in chromatograms. For specification of the technical data and signal drift of the pressure sensor, see **10 Reference information** on page 307.

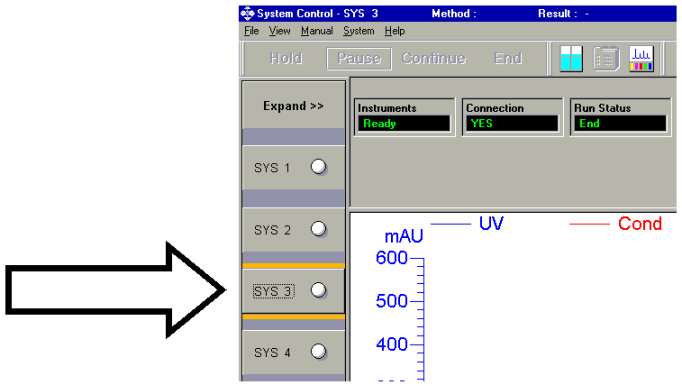
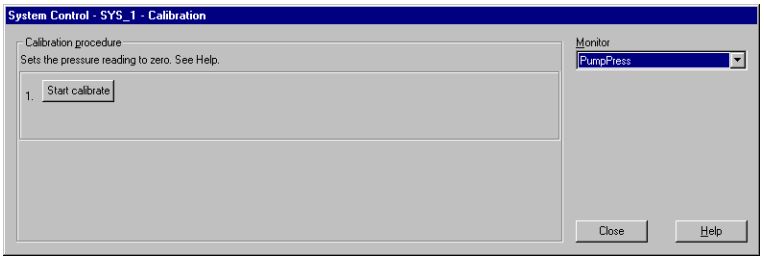
The zero pressure reading of the pressure sensor can be calibrated. The amplification is factory calibrated and cannot be changed.

Maintenance interval

When required and after replacing a pressure sensor.

Calibrate zero pressure

To calibrate the zero pressure:

Step	Action
1	In the UNICORN **System Control** module, select the system to be calibrated by clicking the system name. 
2	In the menu, select **System:Calibrate** and choose **PumpPress** under **Monitor**. 
3	Click **Start calibrate** and wait until it is enabled again (10–20 s).  *Result*: The injection valve will automatically be switched to Waste and the pressure reading set to zero.
4	Click **Close** to finish the operation.

8.5.2 Calibration of conductivity cell

Introduction

Problems in the conductivity cell can cause abnormal conductivity readings in chromatograms.

There are two different procedures:

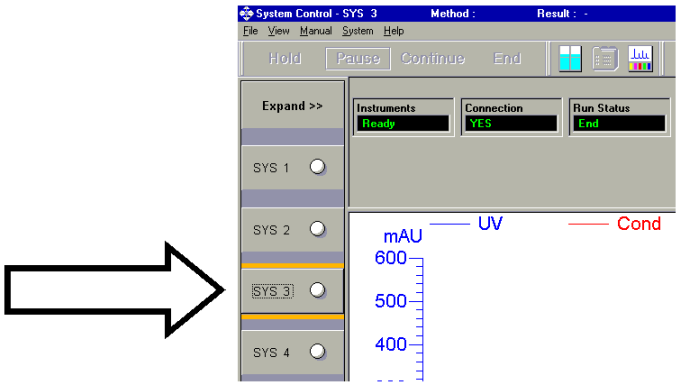
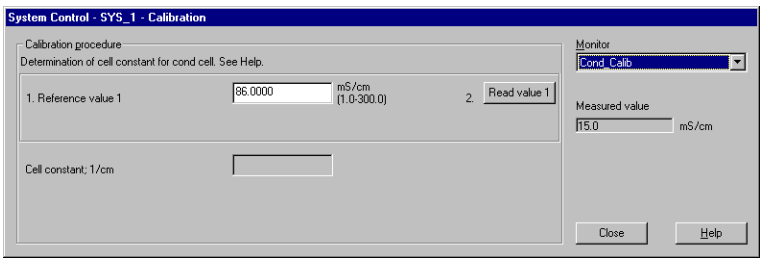
- Calibration of current conductivity cell
- Setting the conductivity cell constant, after replacement

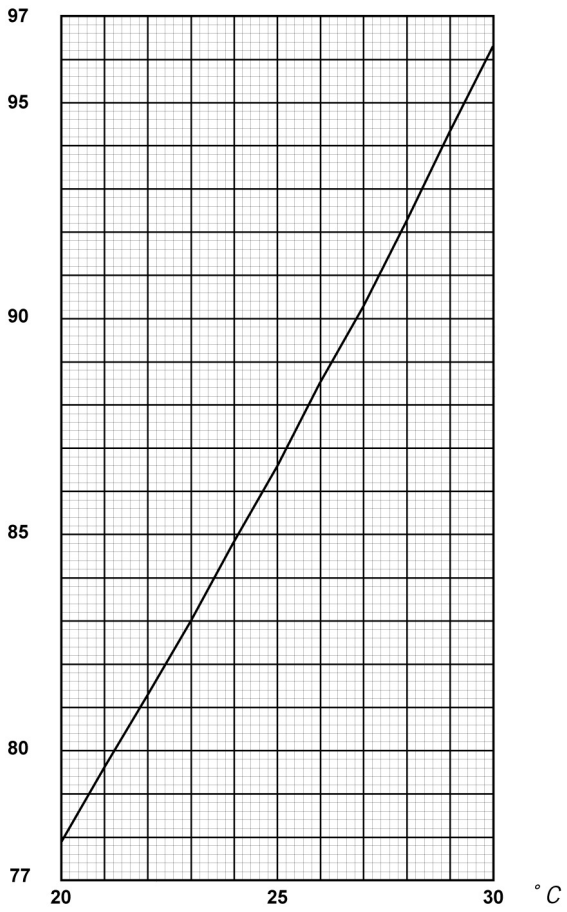
Calibrate current conductivity cell

To calibrate the current conductivity cell:

Note: *Calibration of the conductivity cell is not normally required. The cell constant is calibrated and set at the factory.*

Note: *Perform the test at room temperature, 20–30 °C.*

Step	Action
1	Prepare 20 ml of calibration solution 1.00 M NaCl, 58.44 g/l and immerse the **A1** tubing into the solution. ***Note:*** *Make sure that the temperature of the calibration solution is the same as the room temperature.*
2	In the UNICORN **System Control** module, select the system to be calibrated by clicking the system name. 
3	In the menu, select **System:Calibrate** and choose **Cond_Calib** under **Monitor**. 

Step	Action
4	Start a manual run at 10 ml/min to fill the conductivity cell with the calibration solution. Pump calibration solution through the cell until the conductivity signal is stable and then set the system in Pause.
5	Read the conductivity value displayed under **Measures value** and compare it with the theoretical value from the graph below. *mS/cm*  *If the displayed value corresponds with the theoretical value:* No further action is required. Proceed to step 8. *If the displayed value differs from the theoretical value:* Proceed to step 6.
6	Enter the theoretical conductivity value according to the graph, in the field **Reference value 1**.
7	Click **Read value 1**.  The new cell constant is saved in the system.

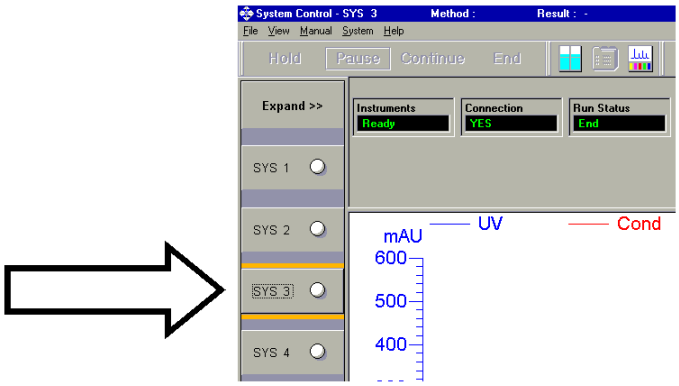
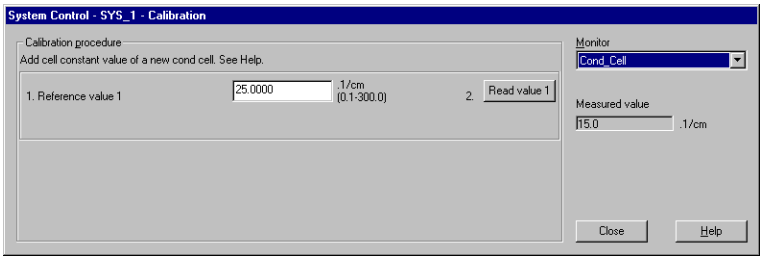
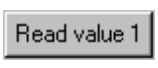
Step	Action
8	Click **Close** to finish the operation.

Setting the conductivity cell constant

If the conductivity cell has been replaced, a cell constant has to be set.

The cell constant is shown on the packaging of the new cell. If the packaging is missing, perform a normal calibration according to **8.5.2 Calibrate current conductivity cell** on page 245.

To enter the cell constant of a new conductivity cell:

Step	Action
1	Read the cell constant from the packaging of the new cell.
2	In the UNICORN **System Control** module, select the system with the new conductivity cell by clicking the system name. 
3	In the menu, select **System:Calibrate** and choose **Cond_Cell** under **Monitor**. 
4	Enter the cell constant in the **Reference value 1** field.
5	Click **Read value 1**.  After a few seconds, a new cell constant is calculated and saved in the system.
6	Click **Close** to finish the operation.

8.6 Replacement procedures

About this section

This section describes replacement procedures of ÄKTAxpress.

In this section

This section contains the following sub-sections

8.6.1 Replacing capillary loops

Maintenance interval

When required, for example when a capillary loop has clogged.

Reason for maintenance

Clogged tubings may contaminate or cause high back pressure, and hence preventing a successful purification run.

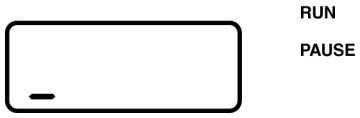
Required material

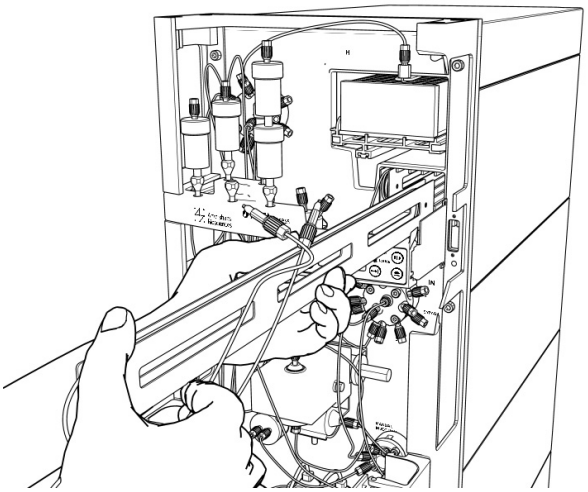
Capillary loop spare part: 11-0003-02

> **CAUTION!**
> Only spare parts approved or supplied by Amersham Biosciences may be used for maintaining and servicing the system.

Replacing capillary loops

To replace the capillary loops:

Step	Action
1	Press the **Eject** button to put the collector plate in *load* (inserted in system) position.
2	Set the system in *Standby* mode by pressing the **On** button. This will disconnect the system from UNICORN. 
3	On the loop valve, disconnect the two fingertight connectors of the capillary loop to be replaced.

Step	Action
4	Slide the loop out of the compartment. 
5	Insert the new loop and tighten the two connectors.
6	Press the **On** button to reconnect the system to UNICORN.
7	Verify the function by flushing the replaced loop and check the connectors for leakage. Set the injection valve to position **Reinject** and the loop valve to the position of the new capillary loop.

8.6.2 Replacing tubing and connectors

Maintenance interval

When required, for example when a tubing has clogged.

Reason for maintenance

Clogged tubing may contaminate or cause high back pressure, and hence preventing a successful purification run.

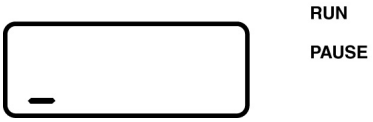
Required material

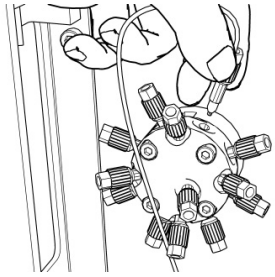
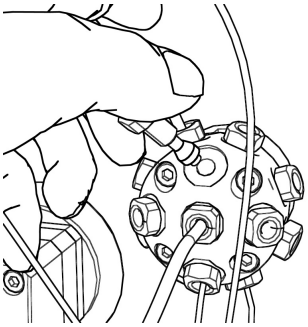
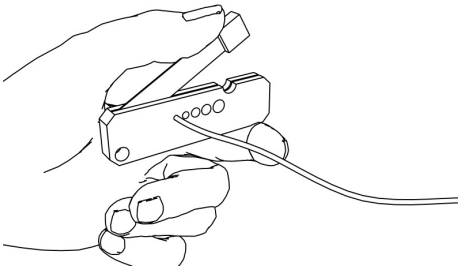
Extra tubing is included in the accessory kit:

> **CAUTION!**
> Only spare parts approved or supplied by Amersham Biosciences may be used for maintaining and servicing the system.

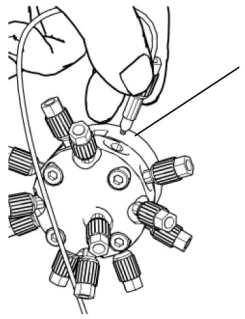
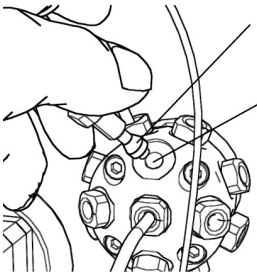
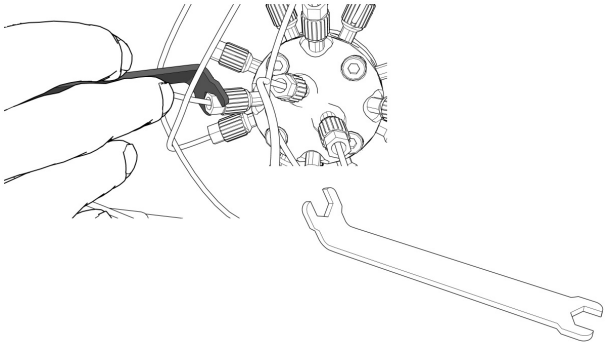
Replacing tubing

To replace the tubing:

Step	Action
1	Set the system in *Standby* mode by pressing the **On** button. This will disconnect the system from UNICORN. 

Step	Action
2	Loosen the connectors to remove the tubing. The system contains two types of tubing: • 1 mm capillary tubing with *fingertight* connectors:  • 1.6 mm capillary tubing with *M6* or *UNF 5/16"* (air sensor, pump inlet) connectors including ferrules: 
3	If the tubing has labels, remove the labels to be used with the new tubing later. Discard the tubing and connectors.
4	Cut the new tubing to the same length as the old tubing. Use the cutting tool to get a straight angle cut. ***Note:*** *When replacing system tubing, use the original length to ensure that the correct delay volumes are maintained. Inlet and outlet tubing may be shortened if required.* For a specification of the tubing, see **8.6.2 Specification of system tubing** on page 260.  The cutting tool is included in the accessory kit.
5	Put the old labels on the new tubing.

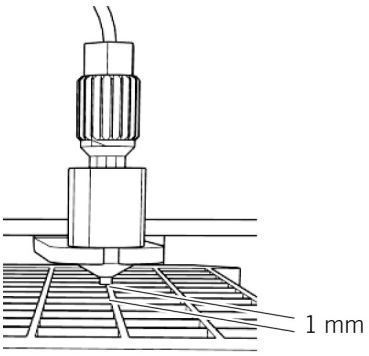
Step	Action
6	Mount the connectors on the tubing. **For fingertight connectors:** 1. Slide the nipple onto the tubing. **For M6 and UNF 5/16" connectors:** 1. Slide the nipple onto the tubing. 2. Slide the ferrule onto the tubing with the thick end towards the end of the tubing.

Step	Action
7	Attach the new tubing to the system: • Insert the tubing with nipple into the port. Make sure to insert the tubing *all the way into the bottom* of the port before tighten the connector.  ***NOTE:*** *Insert the tubing fully to the bottom of the port*  Ferrule ***NOTE:*** *Insert the tubing fully to the bottom of the port* • Tighten the connector properly. For areas difficult to access, use the fingertight-key, available in the accessory kit. - *Fingertight connectors* should be tighten by fingers only, but for areas difficult to access, use the special key available in the accessory kit. - *M6 and 5/16"* should be tighten using the special key for M6. 
8	Press the **On** button to reconnect the system to UNICORN.

Step	Action
9	Verify the new tubing by: • Running a flow through the replaced capillary tubing and check the connectors for leakage. • Running the tubing leakage test, see **8.4.4 Checking tubing leakage** on page 238.

Adjusting the fraction collector tubing

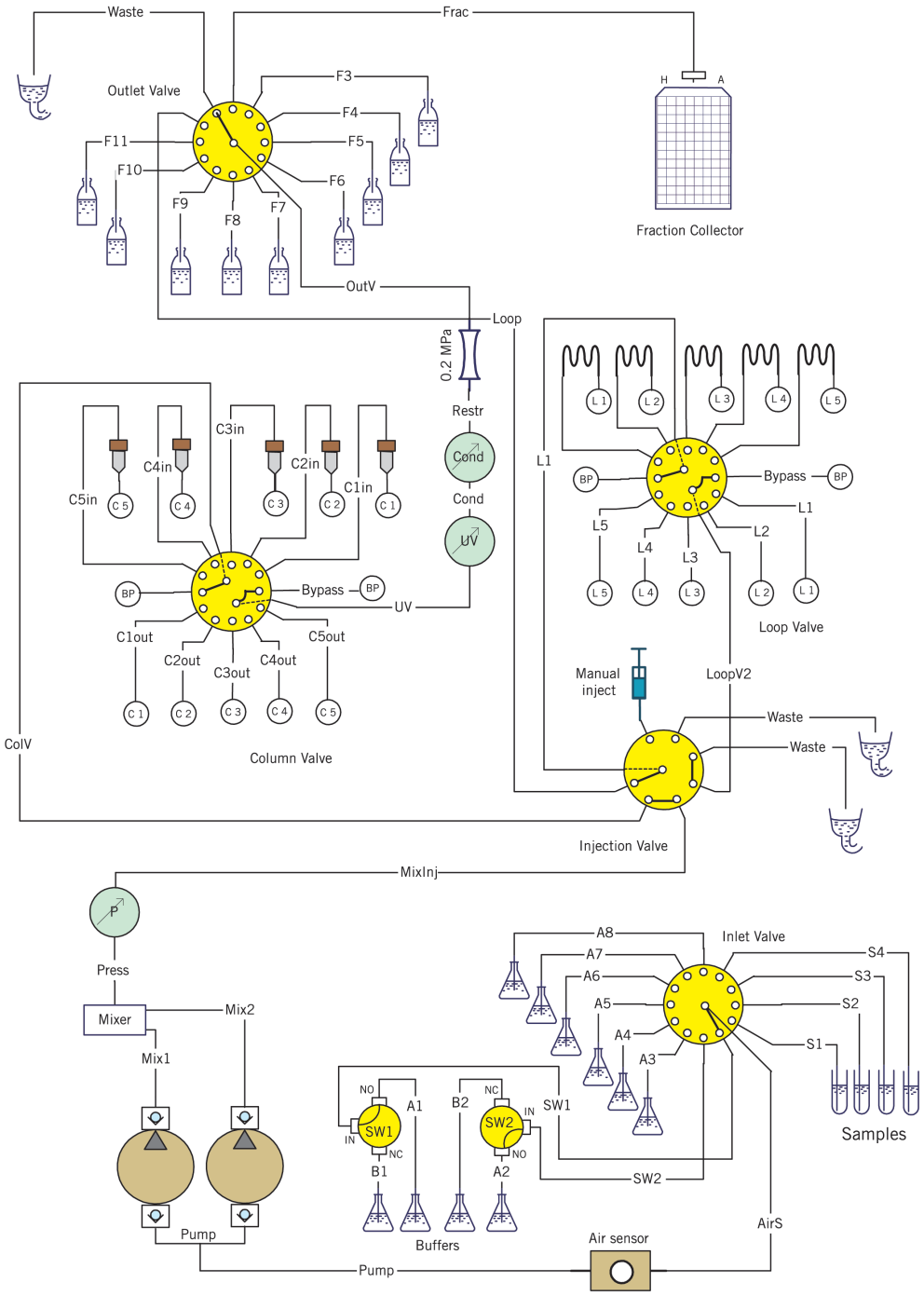
The fraction collector tubing should be adjusted in height when replaced.

Step	Action
1	Adjust the capillary tubing end to match the height of the microplate. The capillary end should be positioned about 1 mm above the microplate. 

Specification of system tubing

ÄKTAxpress has the following tubing connections for the system flow path.

For information about the piston rinsing tubing see **8.6.2 Specification of piston rinsing tubing** on page 261.



Label (bold) or designation	Description	Length, mm	Inner diameter, mm	Material
A1	Buffer inlet, switch valve 1 port NO	1500	1.6	Teflon
B1	Buffer inlet, switch valve 1 port NC	1500	1.6	Teflon
A2	Buffer inlet, switch valve 2 port NO	1500	1.6	Teflon
B2	Buffer inlet, switch valve 2 port NC	1500	1.6	Teflon
A3	Buffer inlet, inlet valve port 3	1500	1.6	Teflon
A4	Buffer inlet, inlet valve port 4	1500	1.6	Teflon
A5	Buffer inlet, inlet valve port 5	1500	1.6	Teflon
A6	Buffer inlet, inlet valve port 6	1500	1.6	Teflon
A7	Buffer inlet, inlet valve port 7	1500	1.6	Teflon
A8	Buffer inlet, inlet valve port 8	1500	1.6	Teflon
S1 red	Sample inlet, inlet valve port 12	500	1.6	Teflon

Label (bold) or designation	Description	Length, mm	Inner diameter, mm	Material
S2 yellow	Sample inlet, inlet valve port 11	500	1.6	Teflon
S3 blue	Sample inlet, inlet valve port 10	500	1.6	Teflon
S4 green	Sample inlet, inlet valve port 9	500	1.6	Teflon
SW1	Switch valve 1 port IN to inlet valve port 1	350	1.6	Teflon
SW2	Switch valve 2 port IN to inlet valve port 2	350	1.6	Teflon
AirS	Inlet valve port IN to air sensor	190	1.6	Teflon
Pump	Air sensor to pump manifold	230	1.6	Teflon
Mix1	Pump head 2 to mixer	120	1	Tefzel
Mix2	Pump head 1 to mixer	120	1	Tefzel
Press	Mixer to pressure sensor	160	1	Tefzel
MixInj	Pressure sensor to injection valve port 5	260	1	Tefzel

Label (bold) or designation	**Description**	**Length, mm**	**Inner diameter, mm**	**Material**
ColV	Injection valve port 6 to column valve IN	350	1	Tefzel
C1in	Column valve port 6 to column 1	350	1	Tefzel
C1out	Column block port 1 to column valve port 12	190	1	Tefzel
C2in	Column valve port 5 to column 2	350	1	Tefzel
C2out	Column block port 2 to column valve port 11	190	1	Tefzel
C3in	Column valve port 4 to column 3	350	1	Tefzel
C3out	Column block port 3 to column valve port 10	190	1	Tefzel
C4in	Column valve port 3 to column 4	350	1	Tefzel
C4out	Column block port 4 to column valve port 9	190	1	Tefzel
C5in	Column valve port 2 to column 5	350	1	Tefzel

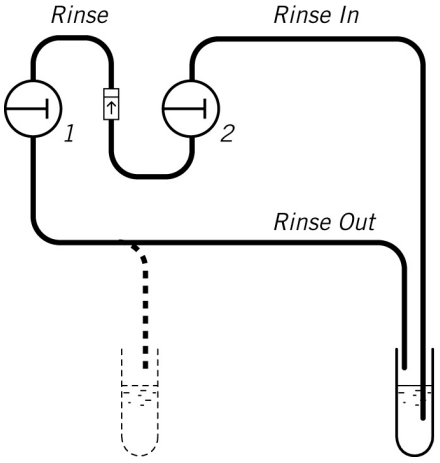
Label (bold) or designation	Description	Length, mm	Inner diameter, mm	Material
C5out	Column block port 5 to column valve port 8	190	1	Tefzel
Bypass	Column valve port 1 to port 7	160	1	Tefzel
UV	Column valve port OUT to UV cell	200	1	Tefzel
Cond	UV cell to conductivity cell	200	1	Tefzel
Restr	Conductivity cell to restrictor	350	1	Tefzel
OutV	Restrictor to outlet valve port IN	160	1	Tefzel
Loop	Outlet valve port 12 to injection valve port 7	500	1	Tefzel
LoopV1	Injection valve port center?? to loop valve port IN	260	1	Tefzel
Bypass	Loop valve port 1 to port 7	160	1	Tefzel
LoopV2	Loop valve port OUT to injection valve port 4	260	1	Tefzel

Label (bold) or designation	Description	Length, mm	Inner diameter, mm	Material
L1 capillary loop	Loop valve port 2 to 8	12800 (10 ml)	1	Tefzel
L2 capillary loop	Loop valve port 3 to 9	12800 (10 ml)	1	Tefzel
L3 capillary loop	Loop valve port 4 to 10	12800 (10 ml)	1	Tefzel
L4 capillary loop	Loop valve port 5 to 11	12800 (10 ml)	1	Tefzel
L5 capillary loop	Loop valve port 6 to 12	12800 (10 ml)	1	Tefzel
Frac	Outlet valve port 2 to fraction collector	260	1	PEEK
F3 red	Fraction outlet, outlet valve port 3	1500	1	Tefzel
F4 yellow	Fraction outlet, outlet valve port 4	1500	1	Tefzel
F5 blue	Fraction outlet, outlet valve port 5	1500	1	Tefzel
F6 green	Fraction outlet, outlet valve port 6	1500	1	Tefzel
F7 red	Fraction outlet, outlet valve port 7	1500	1	Tefzel
F8 yellow	Fraction outlet, outlet valve port 8	1500	1	Tefzel

Label (bold) or designation	Description	Length, mm	Inner diameter, mm	Material
F9 blue	Fraction outlet, outlet valve port 9	1500	1	Tefzel
F10 green	Fraction outlet, outlet valve port 10	1500	1	Tefzel
F11	Fraction outlet, outlet valve port 11	1500	1	Tefzel
Waste	Injection valve port 2	1500	1	Tefzel
Waste	Injection valve port 3	1500	1	Tefzel
Waste	Outlet valve port 1	1500	1	Tefzel

Specification of piston rinsing tubing

ÄKTAxpress has the following tubing connections for the piston rinsing tubing.



Designation	Description	Length, mm	Inner diameter, mm	Material
Rinse In	Rinsing solution inlet to pump head 2 lower port	1350	1.2	PTFE

Designation	Description	Length, mm	Inner diameter, mm	Material
Rinse	Pump head 2 upper port to pump head 1 lower port	200 (including check valve)	3	Silicone
Rinse Out	Pump head 1 upper port to rinsing solution outlet	1350	1.2	PTFE

8.6.3 Replacing flow restrictor

Maintenance interval

When required, for example when the restrictor opens at too high or too low pressure.

Reason for maintenance

A flow restrictor opening at *too low pressure* might cause air bubbles in the UV flow cell.

A flow restrictor opening at *too high pressure* will cause a high pressure alarm from the system. In exceptional it might cause column rupture.

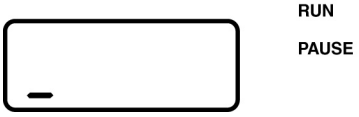
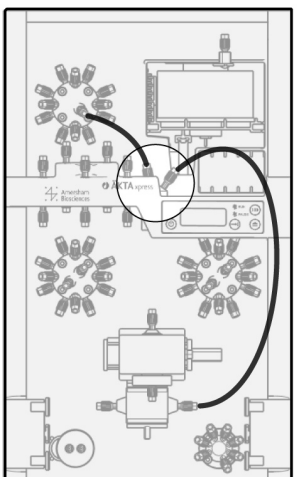
Required material

Flow restrictor FR-902: 18-1121-35

> **CAUTION!**
> Only spare parts approved or supplied by Amersham Biosciences may be used for maintaining and servicing the system.

Replacing flow restrictor

To replace the flow restrictor:

Step	Action
1	Set the system in *Standby* mode by pressing the **On** button. This will prevent runs from being started from UNICORN. 
2	Remove the two capillary tubing from the old restrictor. 
3	Lift off the restrictor from the holder.

Step	Action
4	Refit the capillary tubing to the new restrictor. Make sure that the tubing from the conducitivity cell is attached to the **IN** port on the flow restrictor.
5	Press the **On** button to reconnect the system to UNICORN.
6	Check the function, see **8.2.1 Checking flow restrictor** on page 223.

8.6.4 Replacing UV lamp

Maintenance interval

Replace the UV lamp when:

- The UV signal trace has excessive noise.
- The run time has exceeded the typical life time of the lamp

The UV lamp has a life time of typically:

	Wavelength 254 nm	**Wavelength 280 nm**
In room temperature	7000 h	3500 h
In cold room	2000 h	2000 h

The lamp run time can be checked from UNICORN, see **8.3.1 Checking UV lamp run time** on page 227.

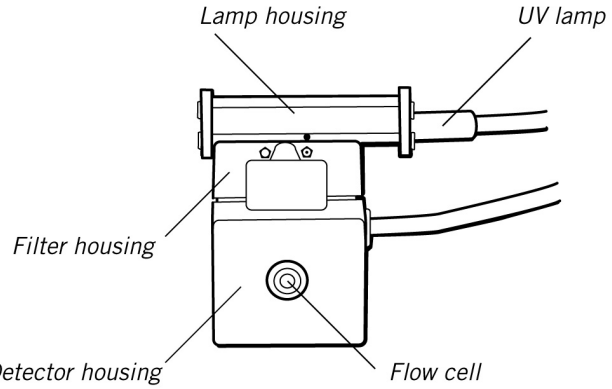
Required material

- Hg lamp in housing including cable: 18-1128-22
- Cross head screwdriver

> **CAUTION!**
> Only spare parts approved or supplied by Amersham Biosciences may be used for maintaining and servicing the system.

Replacing the UV lamp

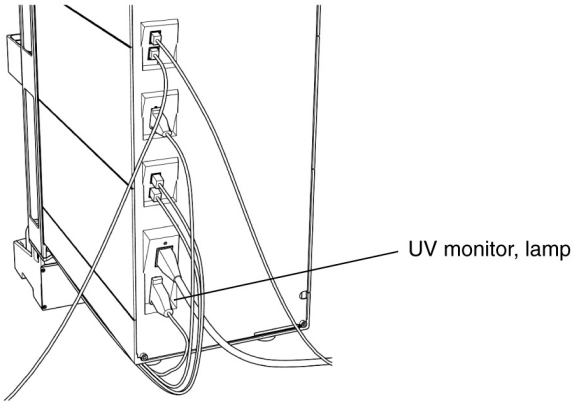
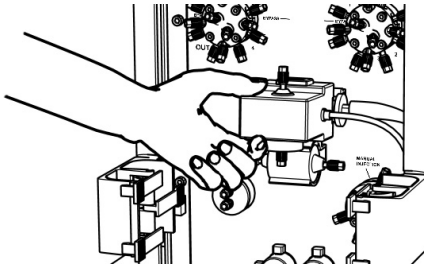
When replacing the lamp, the complete *lamp housing* including UV lamp should be replaced.

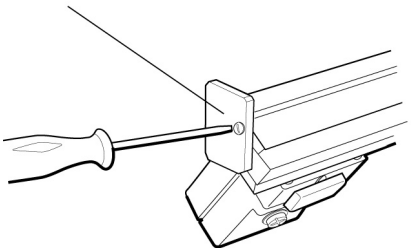


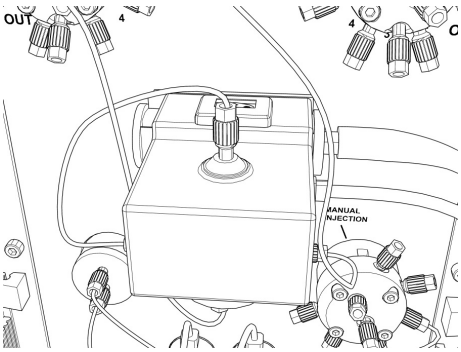
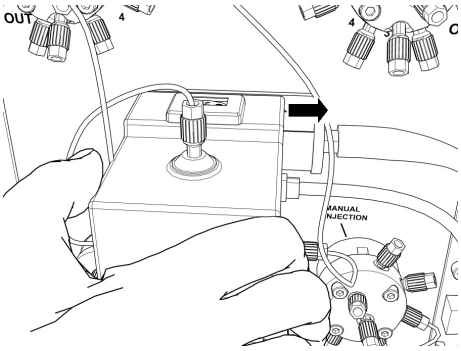
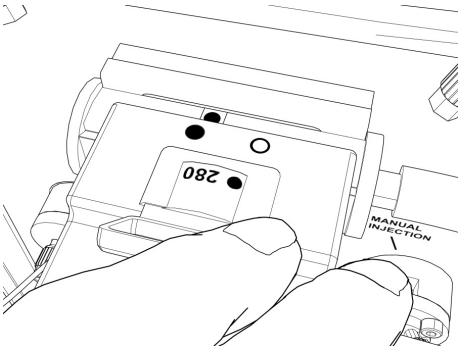
To change the UV lamp including housing:

Note: *It is recommended that an Amersham Biosciences service engineer changes the UV lamp. The user can change the lamp but cannot reset the lamp run time in UNICORN.*

WARNING!
The UV monitor uses high intensity ultra-violet light. To prevent injury to eyes do not remove the optical unit while the lamp is ON.

Step	Action
1	Disconnect the power to the system, for example by unplugging the mains power cable from the system. **CAUTION!** To prevent equipment damage, the mains power to the separation system must be disconnected before any cable is plugged or unplugged at the rear of the system.
2	Remove the connector **UV monitor, lamp** at the rear of the unit and loosen the cable from the cable chute under the system. 
3	Remove the UV monitor assembly from the system by moving it upwards until the unit is released from its holder. 

Step	Action
4	Use a cross head screwdriver to detach the end plate by removing one and loosening the other of the two holding screws on the lamp housing to be removed. *Lamp housing end plate* 
5	Slide the old lamp housing off the filter housing and discard it. **WARNING!** The UV monitor has a mercury (Hg) lamp that contains small amounts of mercury. The lamp must be handled with care and disposed of according to national and local environmental regulations.
6	On the new UV lamp housing, detach the end plate.
7	Slide the new lamp housing onto the filter housing making sure that the cable is on the right side. When sliding the lamp housing, depress the two pressure pads on the filter housing in turn to facilitate the installation.
8	Refit the lamp housing end plate.

Step	Action
9	Slide the lamp housing firmly into place and check that the detector housing is in its *right hand* position.  If this is not the case, slide the housing firmly to the right into place. A click will indicate that the housing is in position. 
10	Open the cap covering the filter wheel and check that the filter wheel is in the 280 nm position.  If this is not the case, turn the wheel into the correct position. A click will indicate that the wheel is in position.
11	Fix the new lamp cable in the cable duct and connect it at the back of the system.

Step	Action
12	Connect power to the system and set the unit in **Idle** mode. ***Note:*** *If a low intensity warning is given even after a change of UV lamp, the filter may need to be changed. Contact a local Amersham Biosciences representative.*
13	Reset the UV lamp run time counter in UNICORN. ***Note:*** *This step can only be made by an Amersham Biosciences service engineer.*

8.6.5 Replacing check valves

Introduction

A check valve should be replaced if dirt etc. cannot be removed by cleaning the check valve:

Spare part and tools required

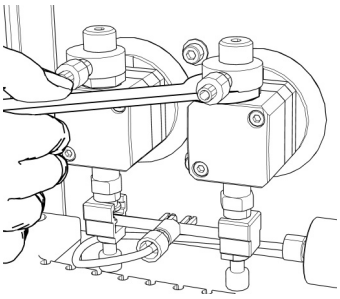
The following spare parts and tools are required:

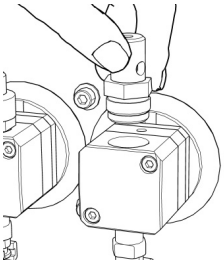
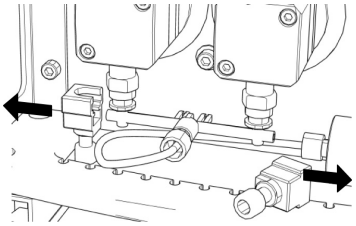
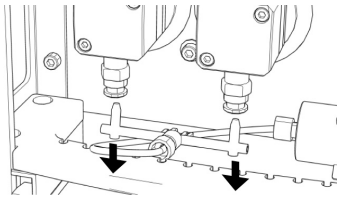
- Check valve kit 18-1128-66 containing one inlet check valve and one outlet check valve
- 13 mm wrench
- 18 mm wrench

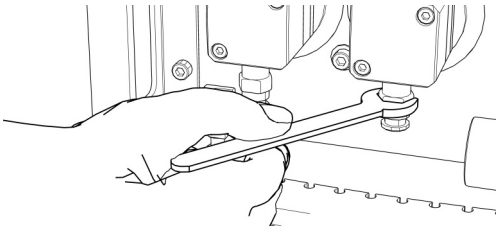
Replacing the check valves

> **CAUTION!**
> The check valves should only be disassembled by a trained person.

To replace the check valves:

Step	Action
1	Change solvent to deionized water and flush out all salt. Move all input buffers bottles below the level of the pump heads, to prevent siphoning.
2	Disconnect electrical power from the system.
3	Remove the tubing from the pump heads.
4	Loosen the upper valve from the pump head using the 18 mm wrench. 

Step	Action
5	Gently remove the check valve and replace it with a new one. **CAUTION!** Handle the check valves with care when they have been removed from the pump heads to prevent loss of any internal components. 
6	Unscrew the two white knurled screws under the pump to release the support bracket that locks the inlet manifold into the inlet valve. 
7	Gently lower and remove the complete manifold. 

Step	Action
8	Loosen the lower valve from the pump head using the 13 mm wrench. **CAUTION!** Handle the check valves with care when they have been removed from the pump heads to prevent loss of any internal components.  Gently remove the check valve.
10	Install the new check valves. The inlet check valve (with a lip for the manifold and a larger diameter opening) is fitted to the side marked IN of the pump head. Tighten the valves until fully finger-tight and then use the wrench to tighten a further 1/3rd (110°) of a turn. **CAUTION!** Do not over-tighten the valves since damage to the internal components can occur.
11	Refit the outlet tubing and the inlet manifold.
12	Reconnect the mains power cable.
13	Purge the pump carefully and check that the pumping action has been corrected, see **6.5.3 Purging the pump and inlet tubing** on page 156.

8.6.6 Replacing pump piston seal

Introduction

The pump piston seals should be replaced if:

- There are signs of liquid leakage between a pump head and the panel.
- The volume of the rinsing solution has increased or decreased.

> **CAUTION!**
> The pump head should only be disassembled by a trained person.

> **CAUTION!**
> Do not disassemble the pump head unless there is good reason to believe that there is an internal leakage. Always make sure that sufficient spare components are available before attempting to replace a spare part.

Note: *It is not possible to reinstall a used piston seal after removal.*

Note: *Always replace the piston seals on both pump heads at the same time.*

Note: *It is recommended to use degassed liquids.*

Spare part and tools required

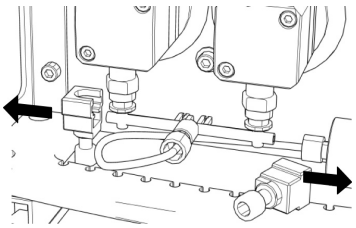
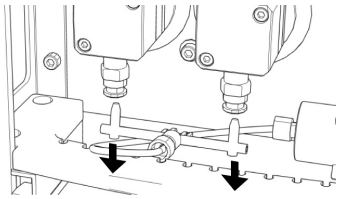
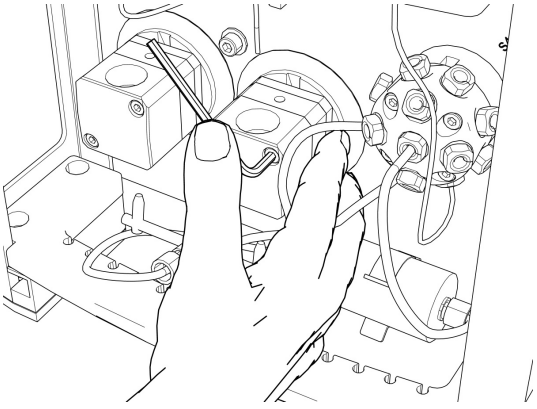
The following spare parts and tools are required:

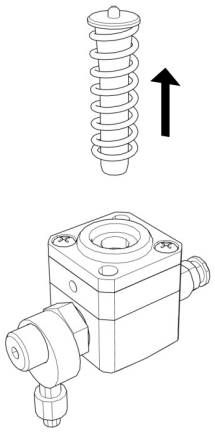
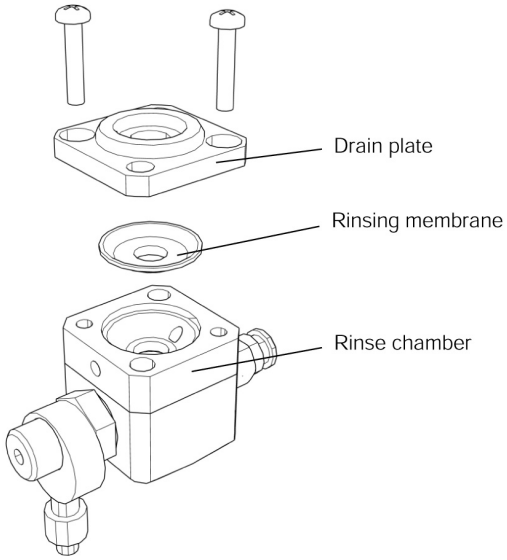
- Seal kit 18-1112-04 containing 2 piston seals and 2 rinse membranes
- 1/4 inch wrench
- 3 mm hex key
- Screwdriver
- 100% methanol for running in the new piston seal

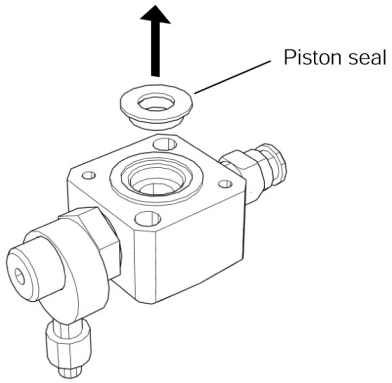
Replacing piston seal and membrane

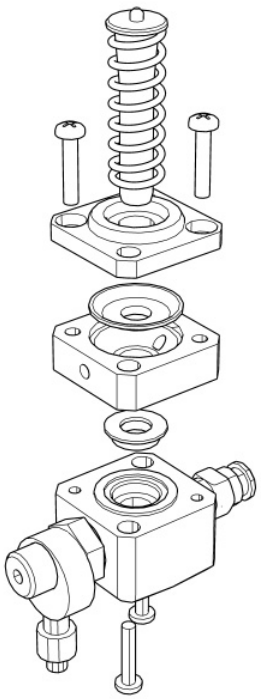
To replace the piston seal:

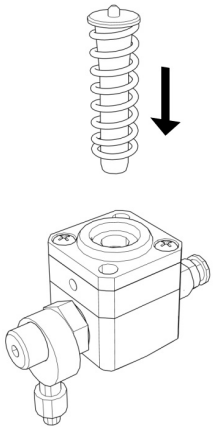
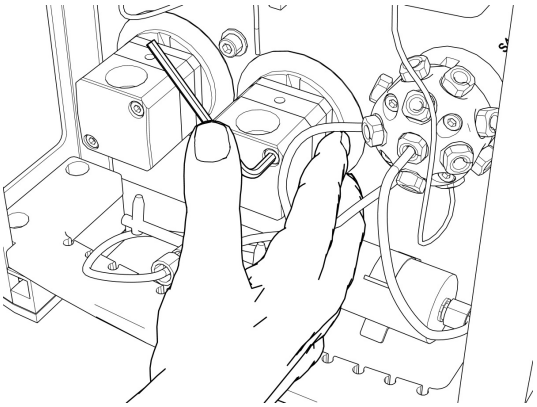
Step	Action
1	Change solvent to deionized water and flush out all salt. Move all input buffers bottles below the level of the pump heads, to prevent siphoning. **CAUTION!** **REPLACING SPARE PARTS!** Read the instructions carefully. Check the orientation of each part before continuing with the next instruction. Beware that for example, some individual parts of the pump head can be assembled incorrectly.

Step	Action
2	Disconnect the electrical power to the system to be maintained.
3	Remove the tubing: • Disconnect and remove the tubing from the pump head outlets (upper ports). • Remove the tubing of the piston seal rinsing system (plug-in fittings).
4	Unscrew the two white knurled screws under the pump to release the support bracket that locks the inlet manifold into the inlet valves. 
5	Gently lower and remove the complete manifold. 
6	• Using the hex key, unscrew and completely remove *one* of the two hex screws locking the pump head in position. • When unscrewing the second locking screw, push firmly on the front face of the pump head to compensate for the pressure of the piston return spring. Hold the pump head firmly to prevent it from twisting.  • Remove the second screw and, without allowing the pump head to twist sideways, carefully pull it out.

Step	Action
7	Place the pump head face down on the bench. Pull out the piston together with the return spring. 
8	Inspect the piston and return spring for sign of damage. If damaged, they should be replaced.
9	Wipe the piston with a clean cloth. If salt solutions have been used the piston may be slightly corroded. This corrosion can be removed with a rubber eraser. If it cannot be wiped or rubbed clean, scrape off any deposits with a scalpel or razor blade. Inspect the piston with a magnifying glass for scratches. Replace with a new piston if any scratches or cracks are found.
10	Remove the two screws securing the drain plate and the rinse chamber. Remove and discard the rinsing membrane. Remove the rinse chamber. 

Step	Action
11	Gently withdraw the piston seal. Discard the used seal. 
12	The pump head, rinse chamber and drain plate should be carefully rinsed or cleaned in an ultrasonic bath, if available. If dirt can be seen on any surfaces, the inlet and outlet check valves should be removed and cleaned separately, see **8.4.2 Cleaning check valves** on page 234.
13	Slightly wet the new seal with water. Place the seal in the hole on the pump head, and press it down into position with a hard flat object.

Step	Action
14	With the pump head still facing downwards on the bench, place the rinse chamber onto the head with the rinse ports in line with the inlet and outlet check valves. The conical depression in the rinse chamber should be facing upwards, ready to accept the new rinsing membrane. Fit the rinsing membrane with the conical face downwards. 
15	Place the drain plate on top of the assembly. Use the two screws to lock the complete assembly together. ***Note:*** *Align the drainage hole in the drainage plate with the inlet check valve (the opposite side of the pump head marked OUT/UP).*

Step	Action
16	Wipe clean the piston and remove all finger prints. Wet the piston with water and then insert it into the return spring. With the pump head facing downwards on the bench, insert the piston into the pump head by pushing it gently, but firmly, vertically downwards into the seal. ***Note:*** *Do not push the piston at an angle to the head and DO NOT twist the piston.* 
17	• Turn the head so that the inlet valve and drainage hole are facing downwards and the text UP/OUT on the pump head is facing upwards. Mount the complete pump head over the locating pins on the front panel. Locate the metal end of the piston and the spring towards the drive cam. • Hold the pump head firmly against the side panel of the housing with one hand. Do not allow the assembly to twist under pressure from the return spring. Using the hex key, fit and tighten *one* of the hex screws.  • Fit and tighten the remaining screw.

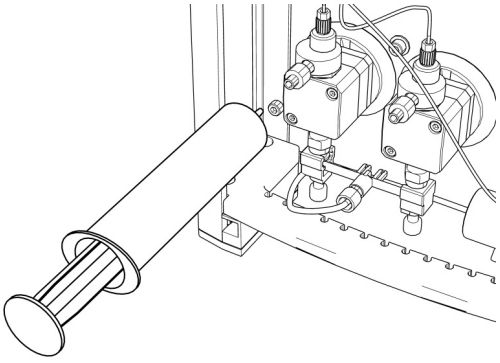
Step	Action
18	Refit the tubing: • Reconnect the outlet tubing to the outlet check valves. • Reconnect the inlet manifold. • Refit the tubing of the piston seal rinsing system.
19	Purge the pump and run in the new piston seal carefully following the instructions below.

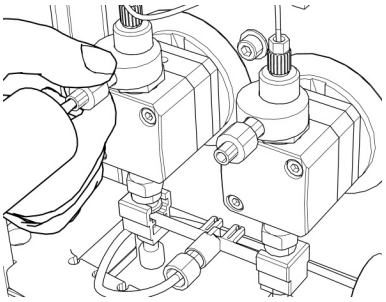
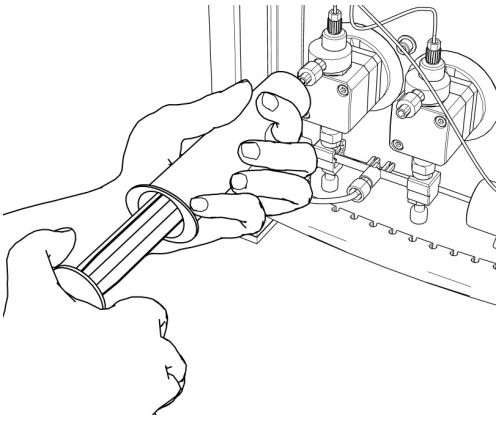
Running in the new piston seal

The piston seal should be run in using 100% methanol.

CAUTION!

Before the start of each run, ensure that there is an adequate supply of solution in the reservoirs. Never allow the pump to run dry, since this will affect the lifetime of the piston seals.

Step	Action
1	Fill a reservoir with 100% methanol and immerse the inlet tubing A1.
2	Connect a male Luer syringe, of at least 30 ml, to the left purge valve. Two syringes are included in the accessory kit supplied with the system. 

Step	Action
3	Turn the left purge valve counter clockwise half a turn to open it. 
4	Slowly draw buffer A1 with the syringe. When fluid starts entering the syringe, continue to draw a few milliliters before closing the purge valve. Check that there is no visible air left in the **A1** tubing. 
5	Repeat the steps 2, 3 and 4 for the right pump head.
6	Check that the outlet tubing is not blocked.
7	Connect a thin capillary or a column that will give sufficient back pressure (see below).
8	Reconnect the mains power cable to the system.
9	Run at a flow rate of 1 ml/min at a back-pressure < 0.5 MPa for 15 minutes.
10	Run at 20 ml/min at a back pressure of 2–3 MPa for 15 minutes.
11	Finally, perform system cleaning, see **6.8.2 Cleaning system** on page 198.

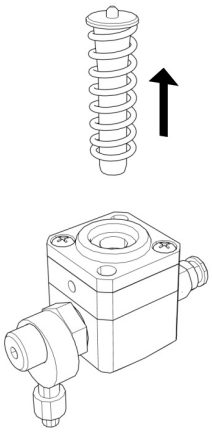
8.6.7 Replacing a damaged pump piston

Introduction

Typical symptoms of a damaged piston are observed as:

- excessive piston seal wear
- unstable pressure
- a reduction in the flow
- noise as the piston moves

The piston should be removed, examined for damage or salt precipitation and then replaced with a new piston if necessary.



If a damaged piston has been in operation, the piston seal will be destroyed and should also be replaced.

Spare parts and tools required

The following spare parts and tools are required.

- Piston kit, 18-1112-13 containing piston, spring, seal and rinse membrane
- 1/4 inch wrench
- 3 mm hex key
- Screwdriver
- 100% methanol for running in the new piston seal

Replacing a piston

To replace the piston and the seal follow the instructions in **8.6.6 Replacing pump piston seal** on page 272.

9 Troubleshooting and corrective actions

About this chapter

This chapter describes the troubleshooting and corrective actions of ÄKTAxpress.

In this chapter

This chapter contains the following sections

9.1 Introduction to troubleshooting

Introduction

This section describes the troubleshooting procedure and includes a general check list of common errors to be checked.

Troubleshooting procedure

To troubleshoot ÄKTAxpress:

Step	Action
1	Always start checking the *General check list* below
2	Find the fault and corrective actions in one of the different sections depending on the type of problem: • **Problems during a run** Errors related to failure to load samples, high back pressure and unsatisfactory elution from the columns, see **9.2 Problems during a run** on page 284. • **Monitoring problems** These are errors detected during a run or when evaluating the resulting chromatograms. For example: ghost peaks, noisy signal, signal drift, non-linear gradients and unstable readings, see **9.3 Monitoring problems** on page 290. • **Leakage problems** Problems related to physical problems with the flow path, see **9.4 Leakage problems** on page 297. • **Alarms and connection problems** Alarms in UNICORN and error codes on the separation systems, see **9.5 Alarms and connection problems** on page 298.
3	Make the recommended corrective actions.
4	If problem persists after corrective actions, contact a local Amersham Biosciences representative.

General check list

Check the following items before starting troubleshooting:

System checks:

- Is the correct system selected in UNICORN System Control, see **6.7 During a run** on page 193?
- Is the fan blowing at the back of the system?

Monitor checks:

- Is the UV monitor set to the correct wavelength, see **8.6.4 Replacing UV lamp** on page 264?
- Is the UV cell locking nut properly tightened to the stop position? For more details, see **8.6.4 Replacing UV lamp** on page 264.
- Is the air sensor sensitivity set to *low* in UNICORN System Control to avoid unnecessary stops due to minor air bubbles?

Flow path checks:

- Is all tubing connected correctly?
- Is there leakage at any of the connections?
- Is the inlet tubing immersed in correct buffer solutions?
- Is the gel filtration column located in the *left hand* column holder? If located in the right hand superloop holder it will hinder the movement of the fraction collector.
- Is any tubing interfering with the ejection and loading of the fraction collector?
- Is there a 2 ml, deep, 96-well microplate in the fraction collector (see **6.5.7 Preparing the fraction collector**) on page 173?

Purification checks:

- Does the positioning of the columns correspond to the selections made in the Method Wizard? Refer to the Summary page.
- Have all columns been cleaned and prepared according to the column recommendations?
- Have the samples been adjusted to binding buffer conditions?
- Have the samples been clarified by centrifugation and/or filtration prior to sample loading?
- Are the correct buffers used for the chosen columns and proteins?
- Are the chosen columns suitable for the chosen target proteins?
- The pH of some buffers changes with the temperature.

Note: *The mixer is designed for use with water based solutions only. If organic solvents are used, incorrect gradients or incomplete mixing may be the result.*

9.2 Problems during a run

Introduction

This section specifies troubleshooting for problems related to sample loading and elution.

Pressure problems

Find the possible cause and action for a specific problem in the table below.

Problem	Possible cause and action
High back pressure	• Make sure that the samples have been centrifuged and/or filtered through a 0.45 µm filter, and that no precipitation has occurred prior to sample loading. • Make sure that automatic flow control has not been disabled in the Advanced Zone. • A 5 ml affinity column (instead of a 1 ml column) is recommended for large sample volumes (more than 20–30 ml). • Clean or exchange columns. • Check the online filter, if used. It can become clogged if unfiltered buffers are used. • There might be dirt or residues in the flow path. Clean the system using a method plan for system cleaning, see **6.8.2 Cleaning system** on page 198. • If using a highly viscous sample, dilute it to ease sample loading. • Check the calibration of zero pressure, see **8.5.1 Calibration of zero pressure** on page 242.

Sample loading problems

Find the possible cause and action for a specific problem in the table below.

Problem	Possible cause and action
Sample loading failure	• Make sure that no air has entered the inlet tubing when moving the tubing from buffer solution to the sample. • Do not place the sample inlet tubing too close to the bottom of the sample tube. Air bubbles might otherwise be created, causing the sample loading to stop due to the air sensor. • Purge the pump to remove trapped air bubbles see **6.5.3 Purging the pump and filling the inlet tubing** on page 156. • Check the sample inlet tubing connectors. A ferrule could be distorted or a connector tightened too hard. Cut the tubing end using a tubing cutter and replace the ferrule. • If air bubbles keep appearing in the sample loading tubing, replace the tubing, see **8.6.2 Replacing tubing** on page 249. • If using a highly viscous sample, dilute it to ease sample loading. • Check that the flow restrictor generates a backpressure of 0.2 ± 0.05 MPa as follows: - Set the injection valve to one of the **Waste** tubings. - Run the pump manually at 10 ml/min with water. Note the backpressure in the Run Data window. - Connect the flow restrictor to the open end of the **Waste** tubing. Note the IN marking. - Run the pump manually at 10 ml/min with water. Note the backpressure. - Calculate the backpressure difference. If it is not within limit, replace the flow restrictor. See **8.6.3 Replacing flow restrictor** on page 262.

Elution problems

Find the possible cause and action for a specific problem in the table below.

No satisfactory elution from...	Possible cause and action
Affinity column	If the protein did not bind to the column, it will be found in the flowthrough that was collected in one of F3–F6. • Verify that the tagged target protein is present in the start sample. • Check that the sample has been applied onto the affinity column. • Check that appropriate wash and elution buffers were applied. • **HisTrap HP and HiTrap Chelating HP:** - If the target protein elutes during Wash 2, decrease the imidazole concentration of wash 2 solution. - If the absorbance curve does not return completely to the base line after peak elution, the quality of the imidazole might be poor. Change to imidazole with higher quality, or increase the Peak_Max factor to be able to detect peak end. • **GSTrap HP and FF:** - Many proteins bind better to GSTrap HP and FF at room temperature than in cold room whereas other protein shows the opposite. - Adding 1 mM DTT or other reducing agent to the buffer might increase the binding capacity. • **Tag cleavage:** - High glycerol concentrations in the buffer might decrease the activity of some proteases. - For operation and optimization hints see **4 Methodology overview** on page 53 and **6.5.8 Performing automatic affinity tag removal** on page 175.

No satisfactory elution from...	Possible cause and action
Desalting column	• Check that the eluted peak from the previous step was collected in a capillary loop by inspecting the peak start and peak end markers in the chromatogram. If not collected: - Open the used method plan and enter the *Advanced Zone*. - Change the watch parameters used in peak collection and run the sample again. • If the protein co-elutes with the salt peak, the recommendation is to add a small amount of salt, for example, 20 mM, to the desalting buffer. However, too high salt concentration might affect the binding capacity when using ion exchange in the following step. • For manual desalting protocols it is only possible to load up to: - 3 ml per sample loop if using two HiTrap Desalting columns in series. - 10 ml per sample loop if using a HiPrep 26/10 Desalting column.

No satisfactory elution from...	Possible cause and action
IEX column	• Check that the eluted peak from the previous step was collected in a capillary loop by inspecting the peak start and peak end markers in the chromatogram. If not collected: - Open the used method plan and enter the *Advanced Zone*. - Change the watch parameters used in peak collection and run the sample again. • Check that the appropriate size of desalting column was used in the previous step. • If the separation is not good enough: - Open the used method plan and enter the *Advanced Zone*. - Change the length and/or slope of the salt gradient, or - Decrease the flow rate. - If the problem persists, create a new method plan using a column that will give a higher resolution result, see **4 Methodology** on page 53. • If the protein did not bind to the column, it was directed to one of the fraction outlets F7–F10. - To protein binding, change the binding buffer or adjust buffer pH. - Check that the appropriate anion/cation exchange column is used. • To collect *all* IEX peaks, run the sample again using the AC-DS-IEX protocol. All peaks will then be collected in the microplate.

No satisfactory elution from...	Possible cause and action
Gel filtration column	• Check that the eluted peak from the previous step was collected in a capillary loop by inspecting the peak start and peak end markers in the chromatogram. If not collected: - Open the used method plan and enter the *Advanced Zone*. - Change the watch parameters used in peak collection and run the sample again. • If the separation is not good enough, change to a more suitable gel filtration column, or divide the start sample into aliquots. Perform repetitive or parallel purifications where less protein is applied in each run. • For manual gel filtration protocols it is only recommended to load up to 5 ml sample per samle loop.

9.3 Monitoring problems

Introduction

This section specifies troubleshooting for monitoring problems during a run.

The monitoring problems can be related to:

- UV curve
- Conductivity curve
- Pressure curve
- Air sensor

Faulty UV curve

Find the possible cause and action for a specific UV curve fault in the table below. If the problem remains, contact the local Amersham Biosciences representative.

Fault	Possible cause and action
Ghost peaks	• **Air in buffers** Check that there is no air in the buffers. Degas the buffers if necessary. It is recommened to use de-gassed buffers for best performance. • **Dirt or residues in the flow path from previous run** Clean the system using a method plan for System Cleaning, see **6.8.2 Cleaning system** on page 198. • **Residues in the columns from previous run** Replace or clean the columns using a method plan for Column cleaning, see **6.8.3 Cleaning columns** on page 202.

Fault	Possible cause and action
Noisy signal	• **Locking nut in optical unit not properly tightened.** Turn the locking nut to the stop position. See also **8.6.4 Replacing UV lamp** on page 264. • **Air in buffers** Check that there is no air in the buffers. Degas the buffers if necessary. • **The buffers might be impure** Make sure the buffers have been filtered. • **Air in the UV flow cell** There may be air in the flow cell. Check that the flow restrictor gives a back-pressure of 0.2 MPa, see **8.2.1 Checking flow restrictor** on page 223. • **Dirty UV cell** Clean the UV-cell, see **8.3.2 Cleaning UV cell** on page 228. • **Air might be trapped in the pump** Purge the pump according to the procedure in **6.5.3 Purging the pump and inlet tubing** on page 156.
Signal drift	See *Noisy signal* above.
Instability	See *Noisy signal* above.
Low sensitivity	• **Aging UV lamp** Check the lamp run time, see **8.3.1 Checking UV lamp run time** on page 227 and replace it if necessary, see **8.6.4 Replacing UV lamp** on page 264. • **UV lamp in wrong position** Check that the lamp position and the filter position both are set to the wavelength to be used; 280 nm or 254 nm, see **8.6.4 Replacing UV lamp** on page 264. • The theoretical extinction coefficient too low Calculate the theoretical extinction coefficient of the protein. If it is zero or very low at 280 nm, the protein cannot be detected.

Faulty conductivity curve

Find the possible cause and action for a specific conductivity curve fault in the table below. If the problem remains, contact the local Amersham Biosciences representative.

Problem	Possible cause and action
Baseline drift or noisy signal	• **Leaking tubing connections** Check for leaking tubing connections and correct, see **6.5.9 Checking the tubing** on page 180. • **Air in the conductivity flow cell** There may be air in the flow cell. Check that the flow restrictor gives a back-pressure of 0.2 MPa, see **8.2.1 Checking flow restrictor** on page 223. • **Column not equilibrated** Equilibrate the column. If necessary, clean the columns using a method plan for Column cleaning, see **6.8.3 Cleaning columns** on page 202. • **Air might be trapped in the pump** Purge the pump according to the procedure in **6.5.3 Purging the pump and inlet tubing** on page 156. • **Dirty conductivity cell** Clean the conductivity cell, see **8.3.3 Cleaning conductivity cell** on page 231.
Waves on the conductivity curve	• **Air might be trapped in the pump** Purge the pump according to the procedure in **6.5.3 Purging the pump and inlet tubing** on page 156.
Conductivity measurement with the same buffer appears to change over time	• **Dirty conductivity cell** Clean the conductivity cell, see **8.3.3 Cleaning conductivity cell** on page 231. • **The ambient temperature might have decreased or increased** The conductivity of the solution changes with temperature. Since there is no temperature compensation, the measured conductivity will be different at different temperatures. • **The buffer might loose its characteristics over time** Change buffer.

Problem	Possible cause and action
Ghost peaks in gradient profile	• **Air bubbles are passing through the flow cell** Check for leaking tubing connections and correct, see **6.5.9 Checking the tubing** on page 180. • **Air might be trapped in the pump** Purge the pump according to the procedure in **6.5.3 Purging the pump and inlet tubing** on page 156.
Non-linear gradients	• **Air might be trapped in the pump** Purge the pump according to the procedure in **6.5.3 Purging the pump and inlet tubing** on page 156. • **Dirt or residues in the flow path from previous run** Clean the system using a method plan for system cleaning, see **6.8.2 Cleaning system** on page 198. • **Organic solutions have been used** The mixer is designed for non-organic solutions only. • **Faulty switch valve** Flush through to clear any blockage by running e.g. System Wash.
Slow response to %B changes	See *Non-linear gradient* above. ***Note:*** *A delay of 3 ml plus the column volume is normal.*
Incorrect absolute conductivity value	• **Bad calibration** Calibrate the conductivity cell, see **8.5.2 Calibration of conductivity cell** on page 243. • **Incorrect calibration solution** Calibration solution, 1.00 M NaCl, not correctly prepared. Prepare a new calibration solution and recalibrate the conductivity cell, see **8.5.2 Calibration of conductivity cell** on page 243.

Problem	Possible cause and action
Incorrect or unstable reading	• **Cable not connected properly** Check that the conductivity flow cell cable is connected properly to the rear of the system, see the *ÄKTAxpress Installation Guide*. • **Air might be trapped in the pump** Purge the pump according to the procedure in **6.5.3 Purging the pump and inlet tubing** on page 156. • **Column not equilibrated** Equilibrate the column. If necessary, clean the columns using a method plan for Column cleaning, see **6.8.3 Cleaning columns** on page 202.

Faulty pressure curve

Find the possible cause and action for a specific pressure curve fault in the table below. If the problem remains, contact the local Amersham Biosciences representative.

Fault	Possible cause and action
Irregular flow	• **Air bubbles passing through or trapped in pump** Check that there is sufficient buffer in each liquid container. Check all connections for leakage. Use degassed solutions. Purge the pump according to the procedure in **6.5.3 Purging the pump and inlet tubing** on page 156. • **Check valves not functioning** There might be dirt in the check valves. Clean the valves, see **8.4.2 Cleaning check valves** on page 234. • **Blockage or partial blockage of flow path** Flush through to clear any blockage by running e.g. System Wash. If required, replace the tubing and connectors, see **8.6.2 Replacing tubing and connectors** on page 249. • **Pump piston assembly leaking** Replace the piston assembly in the pump head, see **8.6.7 Replacing a damaged pump piston** on page 280.
Noisy baseline signal	See *Irregular flow* above.
Irregular pressure trace	See *Irregular flow* above.

Faulty air sensor

Find the possible cause and action for a specific air sensor fault in the table below. If the problem remains, contact the local Amersham Biosciences representative.

Fault	Possible cause and action
Too high sensitivity	• **Wrong sensitivity setting** Change to low sensitivity in **System:Settings** in System Control. • **Air might be trapped in the pump** Purge the pump according to the procedure in **6.5.3 Purging the pump and inlet tubing** on page 156.
No response	• **The air sensor is not connected** Check the air sensor cable. • **Not functioning** Check the function of the air sensor. Replace if necessary. • **The air sensor is disabled** Check that the air sensor has not been disabled.

9.4 Leakage problems

Introduction

This section specifies troubleshooting for when there are leakage problems.

The problems can be related to:

- Pump leakage
- Other leakage in valves, tubing connectors, sensors, mixer and column block

Pump leakage

Find the possible cause and action for a specific problem below.

Problem	Possible cause and action
Low buffer flow and distrurbing noise	• **Bad piston spring** Disassemble the pump head and examine the piston spring. If the spring is corroded, check the piston seal and rinse membrane. Make sure that the rinsing system is always used when working with aqueous buffers with salt concentration, see **8.6.2 Specification of piston rinsing tubing** on page 261. If the piston is damaged, replace it, see **8.6.6 Replacing pump piston seal** on page 272.

Flow path leakage

Find the possible cause and action for a specific problem below. To perform a tubing leakage test see **8.4.4 Checking tubing leakage** on page 238.

Problem	Possible cause and action
Leakage around a pump head	• **Piston seal or rinsing membrane incorrectly fitted or worn** Replace the pump piston seals in the pump heads, see **8.6.6 Replacing pump piston seal** on page 272.
Leaking connection or crystallized material around tubing connectors	• **Tubing connector leaking** Unscrew the connector and check if it is worn or incorrectly fitted. If required, replace the faulty connectors, see **8.6.2 Replacing tubing** on page 249.
Internal leakage	• **Internal fault** The item must be replaced, contact a local Amersham Biosciences representative.

9.5 Alarms and connection problems

Introduction

This section specifies troubleshooting for when there are alarms and connection problems.

Alarms

Find the solution for a specific problem in the table below.

Problem	Solution
UNICORN error	See UNICORN help files and manuals.
Error code flashing on the separation system	Check the meaning of the error code in **9.6 Error code list** on page 300.

Connection problems

Find the solution for a specific problem below.

If the connection problem cannot be solved by the instructions below, check the log file: `c:\unicorn\bin\p4can_drvX.log`, where `X` is the system unit number. Contact a local Amersham Biosciences representative and be prepared to send the log file.

Problem	Solution
No text on the system's front display	• Check that the power cable is connected at the back of the separation system. • Check that the power socket has voltage present.
One segment on and system unit no. or "256" displayed	• If the "ÄKTAxpress program update" dialog does not appear in the UNICORN computer, the instrument update failed. Contact Service.
Two segments flashing and system unit no. steady	• Check that the UNICORN computer is turned on. • Start UNICORN and check the connection status as below.

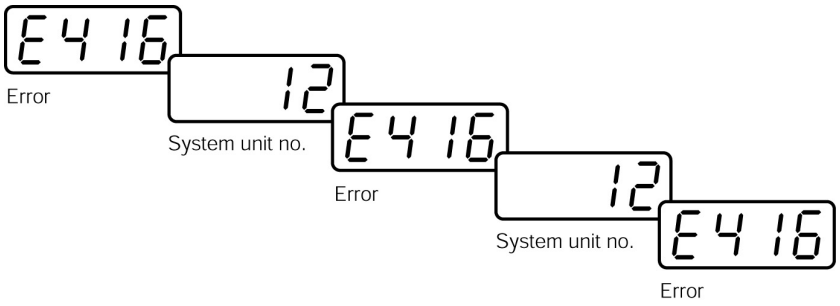
Problem	Solution
UNICORN Connection status: NO[1]	No contact with the systems. • Check the cables, see ÄKTAxpress Installation Guide. - Cables to the USB/CAN converter - UniNet cables between the systems and the USB/CAN converter • Check that the last system on the UniNet chain has the termination plug inserted, see ÄKTAxpress Installation Guide. • Remove and insert the USB cable to the USB/CAN converter. • If problem persists, restart the computer and reset the separation system.
UNICORN Connection status: NO[2]	No contact with the ÄKTAxpress driver. • Check the cables, see ÄKTAxpress Installation Guide. - Cables to the USB/CAN converter - UniNet cables between the systems and the USB/CAN converter • Check that the last system on the UniNet chain has the termination plug inserted, see ÄKTAxpress Installation Guide. • Remove and insert the USB cable to the USB/CAN converter. • Check the USB/CAN driver installation, see **9.7 Checking USB/CAN driver** on page 303. • If problem persists, restart the computer and reset the separation system.
UNICORN Connection status: NO[3]	Internal software error (OCI). • Check the system installation by using the UNICORN CD supplied. See *Adding systems in UNICORN* in *ÄKTAxpress Installation Guide*. • If trying to connect in *view mode* from a remote computer, check that the local UNICORN work-station is running.

9.6 Error code list

Introduction

This section contains a list of error codes and their meaning. The error codes are presented on the display of the separation system as a flashing code **Exxx**.

To enable identification of the system unit number, the display will alternate between flashing the error code and the system unit number.



Error codes

The following error codes can occur.

Code	Description
51–57	System error Internal error. Restart system. If problem remains, contact service.
60–61	System error Internal error. Restart system. If problem remains, contact service.
62	Illegal mode exchange It could be caused by a WATCH-instruction with action CONTINUE that occurred when the system was in RUN-state. Check the method. Otherwise, there could be an internal error. Restart system. If the problem remains, call service.
100–105	System error Internal error. Restart system. If problem remains, contact service.
106	Pressure calibration error Check that the system is at zero pressure.
107	Pump synchronization error By pressing END the pump will be synchronized. If this doesn't help, restart the system. If problem remains, contact service.

Code	Description
200–218	System error Internal error. Restart system. If problem remains, contact service.
219	Instruction ignored The method instruction or manual command could not be executed.
220	Set last tube error An attempt was made to set the last tube to a position already used. Set the last tube to a non-used position.
221–222	System error Internal error. Restart system. If problem remains, contact service.
223	Bad combination of valve positions The Injection valve is in position reinject and the Outlet valve is in position LoopFracF12.
224	CAN-bus error The communication with the instrument is broken. Restart system. If problem remains, contact service.
300–303	System error Internal error. Restart system. If problem remains, contact service.
400–401	System error Internal error. Restart system. If problem remains, contact service.
402	Cell constant out of range The calculated conductivity cell constant is outside the accepted range. Calibrate with a liquid of known conductivity in the range 0.1–300 mS/cm.
403	Cond sine table not calibrated The conductivity sine table has not been calibrated. Calibration of the conductivity sine table will start now and takes approximately 3 minutes to complete.
404	Conductivity cell error The cond cell is either not connected or broken.
405–408	System error Internal error. Restart system. If problem remains, contact service.
410–415	System error Internal error. Restart system. If problem remains, contact service.

Code	Description
416	The UV lamp is broken The UV lamp is broken. Replace the lamp.
417–418	System error Internal error. Restart system. If problem remains, contact service.
420–421	System error Internal error. Restart system. If problem remains, contact service.
422	Filter size error The filter size in the UV lamp is out of range. Check that the method is made for ÄKTAxpress.
423–427	System error Internal error. Restart system. If problem remains, contact service.
428	Measurement warning The UV lamp has been switched off, or the conductivity cell disconnected when measuring the absorbance or the conductivity. Try to switch on the UV lamp. If it does not help, restart the system. If the problem still remains, contact service.
429	Lamp intensity warning The intensity of the UV lamp is too low. Replace the lamp.
430	Dark current warning The dark current in the UV lamp is higher than the allowed limit. Check if the seal is broken.
431	UV auto zero warning The UV value was out of the allowed range (–0.2 to 2 AU) when trying to set the relative absorbance signal to zero.
480	No peak found No peak was found by the Peak_Select instruction.
481	No peak selected No peak has been selected. Use the Peak_Select instruction to select a peak.
500–501	System error Internal error. Restart system. If problem remains, contact service.

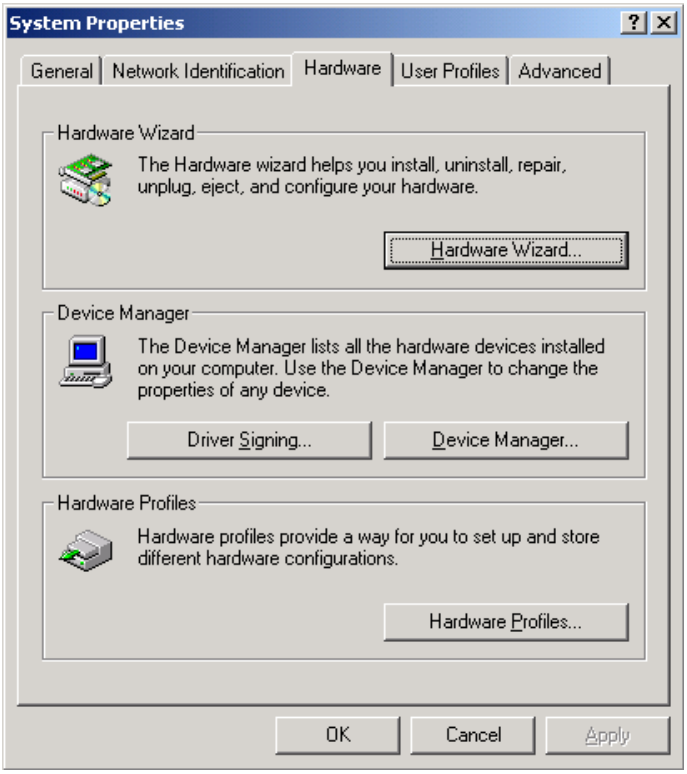
9.7 Checking USB/CAN driver

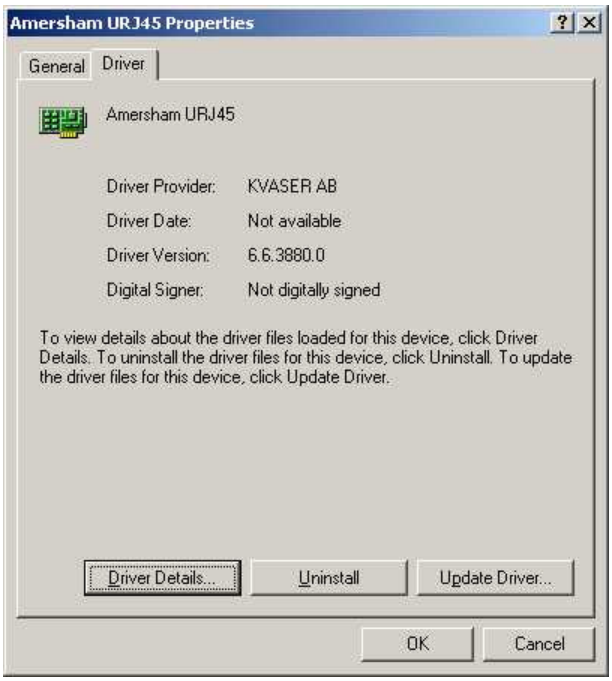
Introduction

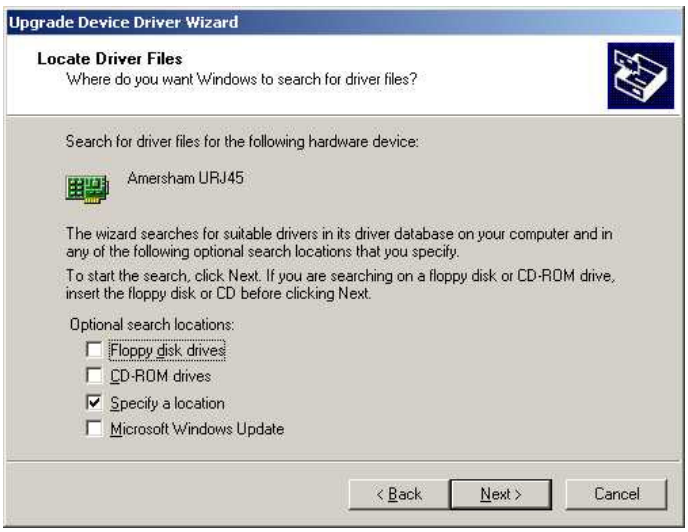
If there are connection problems between the computer and the separation systems, the USB/CAN driver installation can be checked:

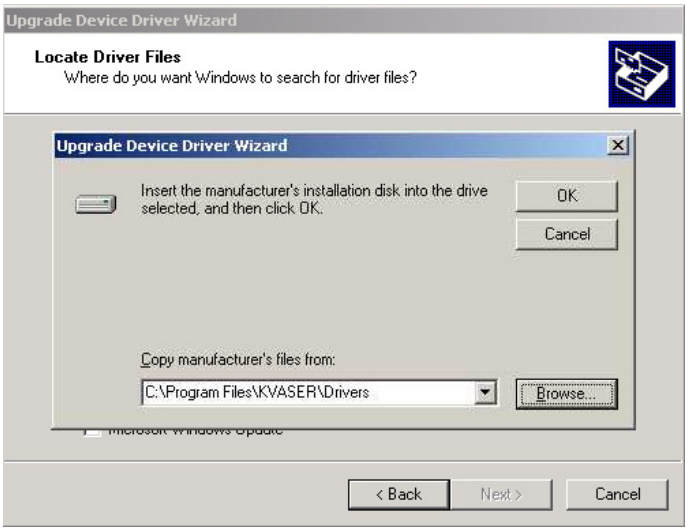
Checking the driver installation

To verify that the CAN drivers have been correctly installed:

Step	Action
1	Open the **System Properties** dialog box: • Open the **Control Panel** and choose **System** *or* • press down the Windows key and the **PauseBreak** key simultaneously.
2	In the **System Properties** dialog box, click the **Hardware** tab:  • Click the **Device Manager** button. *Result:* The **Device Manager** is opened.

Step	Action
3	In the Device Manager, click the plus sign to expand the **CAN Hardware (KVASER)** item:  • If the **Amersham URJ45** icon looks like the image above the installation was successful. If so, go to step 10. • If the **Amersham URJ45** icon has an exclamation or warning sign, you must reinstall the driver. Continue to step 4.
4	• Right-click the **Amersham URJ45** icon and choose **Properties**. *Result:* The **Amersham URJ45 Properties** dialog is opened. • Click the **Driver** tab: 
5	• Click the **Update Driver** button. *Result:* The **Upgrade Device Driver Wizard** is opened. • Click **Next**.

Step	Action
6	• Select the option **Search for a suitable driver....**  • Click **Next.**
7	• Select the option **Specify a location:**  • Click **Next.**

Step	Action
8	• Browse to the folder `C:\Program Files\KVASER\Drivers` and click **OK**:  • Click the **Next** button.
9	The wizard should find the correct driver:  • Click **Next** and then **Finish**.
10	Close the **Device Manager** and the **System Properties** dialog.

10 Reference information

About this chapter

This chapter includes reference information for ÄKTAxpress.

In this chapter

This chapter contains the following sections

10.1 System description

About this section

This section describes the components of ÄKTAxpress.

For a system overview, see **3.2 Separation system overview** on page 27.

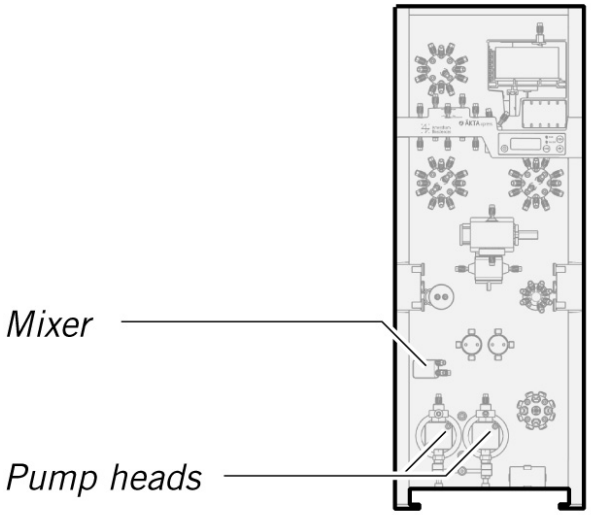
In this section

This section contains the following sub-sections

10.1.1 Pump and related components

Introduction

This section describes the pump and mixer in ÄKTAxpress.

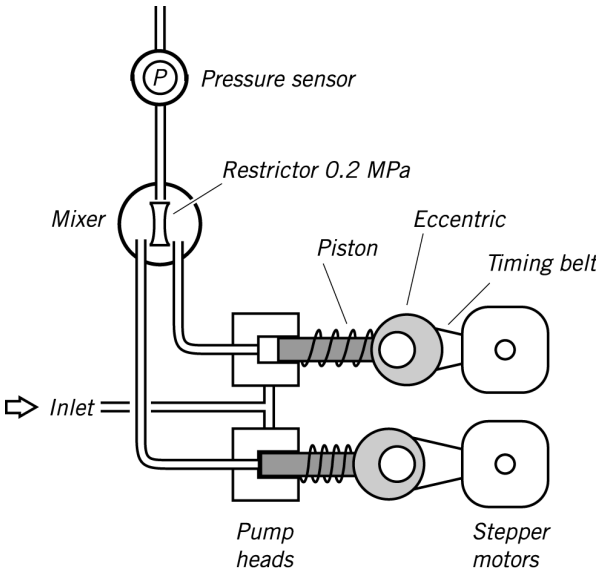


Pump

General

The ÄKTAxpress pump is a high performance laboratory pump for applications where accurately controlled liquid flow is required. The pump consists of two parallel pump heads with a cylinder/piston. Each pump head has two check valves and one purge valve.

The liquid is drawn into the inlet manifold by the action of the pump. Twin reciprocating pump heads work in unison to deliver a low-pulsation flow from the pump outlet.



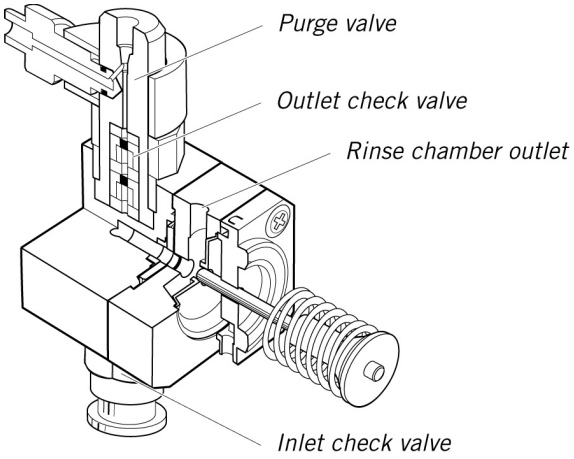
Each piston is driven by a simple robust cam (eccentric). These cams are driven by stepper motors via timing belts. The motor speed is varied to achieve linear movement. This produces the particular motor sound. This system guarantees an accurate, low pulsation flow over the entire flow rate range, independent of the back pressure. When an increase in flow rate is programmed, the motor speed accelerates gradually, giving a soft start and building up speed to the flow rate required.

When a decrease in flow rate is programmed, the motor speed reduces rapidly to the lower flow rate.

Pump heads

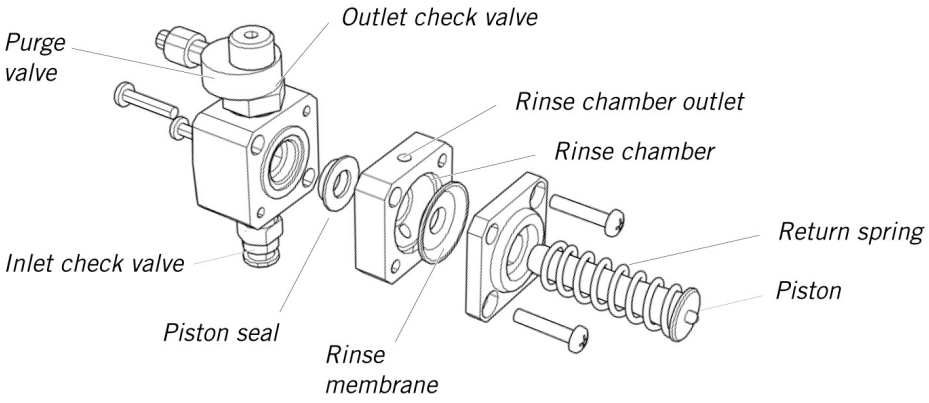
The individual heads are identical but are actuated in opposite phase to each other by individual stepper motors controlled by a microprocessor.

Each outlet check valve houses a purge valve and a fingertight connector. It is used for draining any unwanted liquid or to remove air from the system. The purge valve is opened by turning it counterclockwise half a turn.



The inlet to each pump head is fed from a common manifold. Liquid is drawn up into the pump head through a non-return check valve by the action of the piston being withdrawn from the pump chamber.

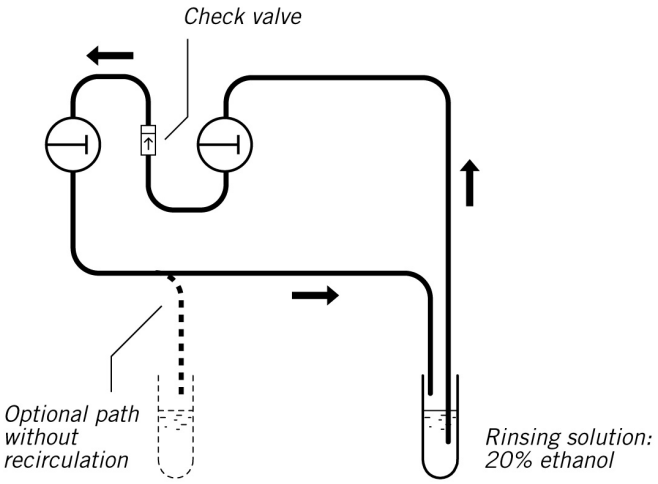
On the delivery stroke of the piston, the inlet valve is sealed by the pressure developed and eluent is forced out through a similar check valve at the outlet.



The pistons are actuated by cams (eccentrics) driven by the motors. Force for the retraction of the pistons is provided by coil springs. The length of stroke of the pistons is fixed and changes in the flow rate are made by varying the speed of the drive motor.

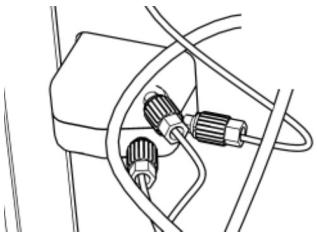
Piston seal rinsing system

Leakage between the pump chamber and the drive mechanism is prevented by a piston seal. The pistons and seals are continuously lubricated by the presence of liquid. To prevent any deposition of salts from aqueous eluents on the piston and to prolong the life of the seals, the low pressure chamber behind the pump head can be flushed continuously with a low flow of rinsing solution. The flexible rinsing membrane sweeps the piston and seals the rinsing system.



Mixer

The mixer is a static mixer with a volume of 0.25 ml.



Note: *Do not use organic solvents in buffers. The mixer is designed for use with water based solutions only. If organic solvents are used, incorrect gradients or incomplete mixing may be the result.*

Flow restrictor

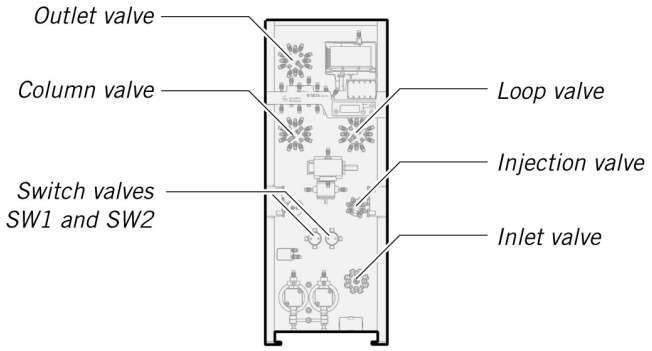
The flow restrictor is positioned directly after the UV and conductivity monitors. The restrictor generates a steady backpressure of 0.2 MPa to prevent air bubbles being formed in the monitors.

The flow restrictor is closed for pressures below 0.2 MPa.

10.1.2 Valves

Introduction

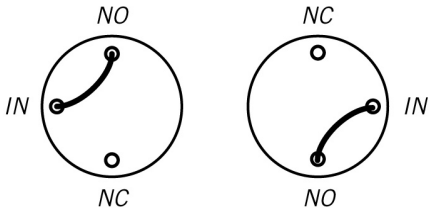
This section describes the valves in ÄKTAxpress:



Switch valves

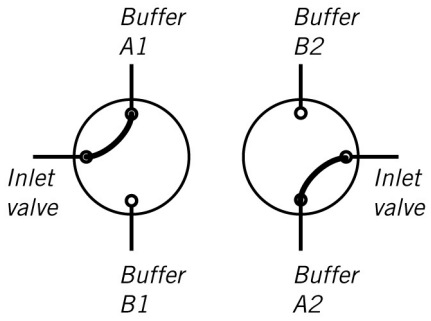
The switch valves SW1 and SW2 are 2-way 3-port valves used for buffer switching and gradient formation. The valves have one port marked IN and two ports marked NC (normally closed) and NO (normally open).

The valves have UNF 5/16" connectors. The valves have no user replaceable parts.

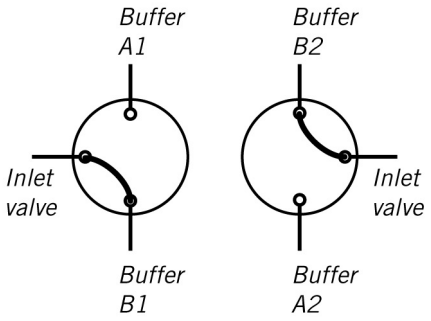


The two positions of the valve gives the following flow paths:

***A1/A2** position*



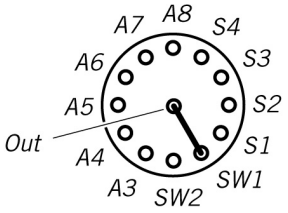
***B1/B2** position*



Inlet valve

The inlet valve is a 12-way 13 port valve. For each 12 positions an inlet port is connected to the central outlet port.

The valve has M6 connectors. The valve has no user replaceable parts.



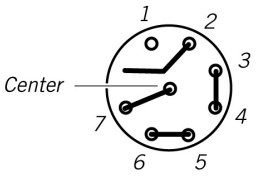
Default position, at startup

The valve is used for buffer selection and sample loading. For flow path details see **3.2.2 Liquid flow path** on page 34.

Injection valve

The injection valve is a 4-way 8-port valve used for main flow direction in the system. The valve is also used for manually injection of samples and filling superloop via a syringe.

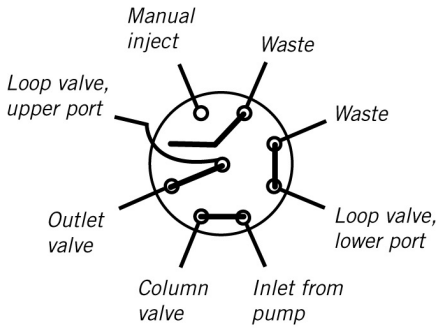
The valve has fingertight connectors. The valve has no user replaceable parts.



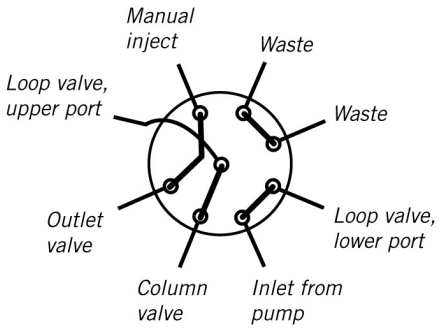
Default position, at startup (inject)

The four positions of the valve gives the following flow paths:

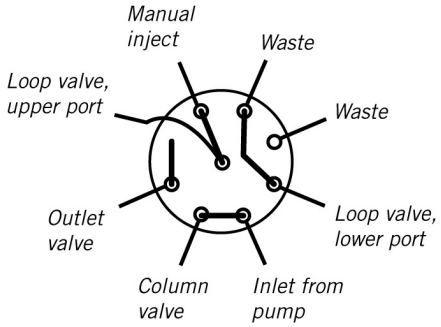
Inject *position*



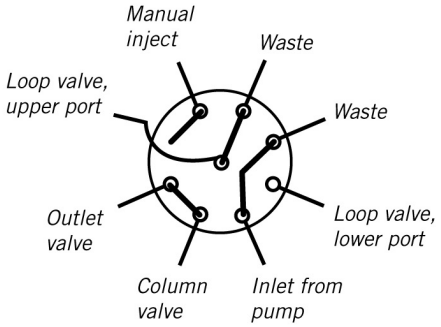
Reinject *position*



Load *position*



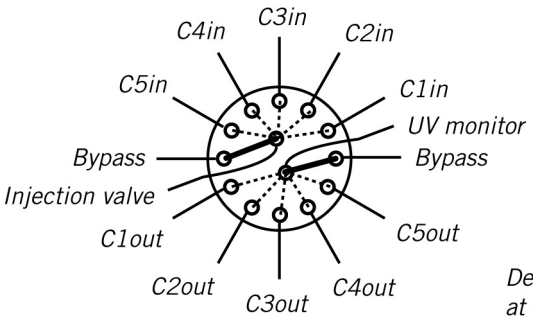
Waste *position*



Column valve

The column valve is a 2×6-way 14-port valve used for directing a flow to any of up to five columns and one bypass route.

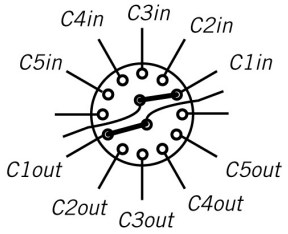
The valve has fingertight connectors. The valve has no user replaceable parts.



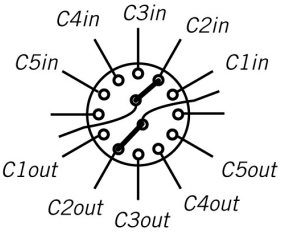
Default position, at startup

The six positions gives the following flow paths:

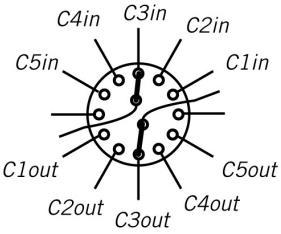
***Column 1** position*



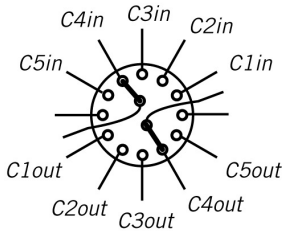
***Column 2** position*



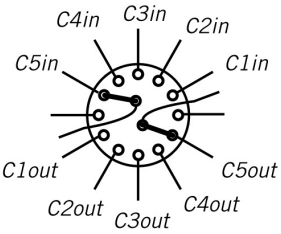
***Column 3** position*



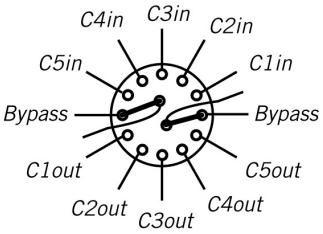
***Column 4** position*



***Column 5** position*



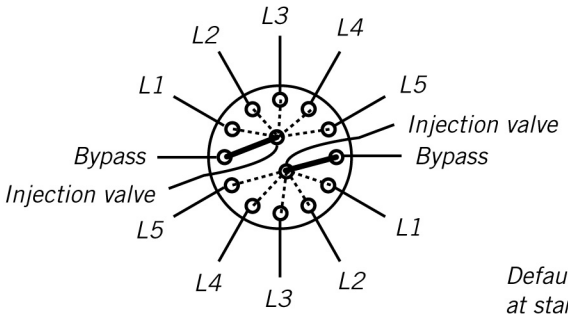
***Bypass** position (default)*



Loop valve

The loop valve is a 2×6-way 14-port valve used for directing a flow to any of up to five sample capillary loops and one bypass route. The loop valve is identical to the column valve.

The valve has fingertight connectors. The valve has no user replaceable parts.

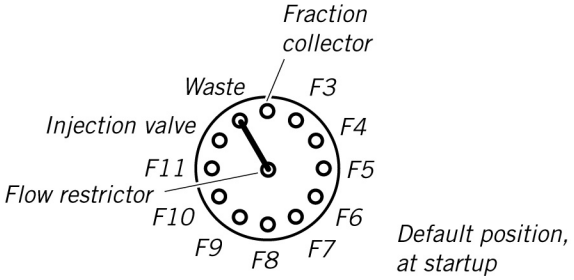


For flow path see **10.1.2 Column valve** on page 316.

Outlet valve

The outlet valve is a 12-way 13 port valve. For each 12 positions an outlet port is connected to the central inlet port.

The valve has fingertight connectors. The valve has no user replaceable parts.

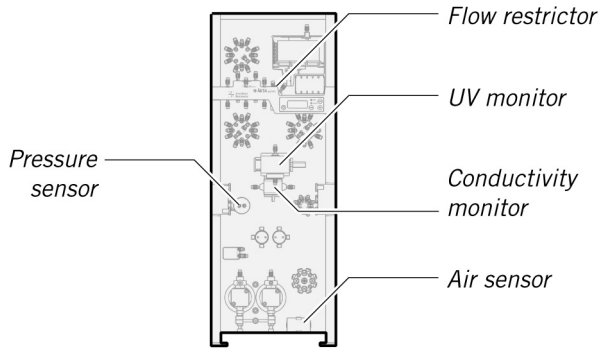


The valve is used for redirecting samples from a column to a capillary loop, passing samples to the fraction collector and passing liquids to flowthrough containers and waste. For flow path details see **3.2.2 Liquid flow path** on page 34.

10.1.3 Monitors

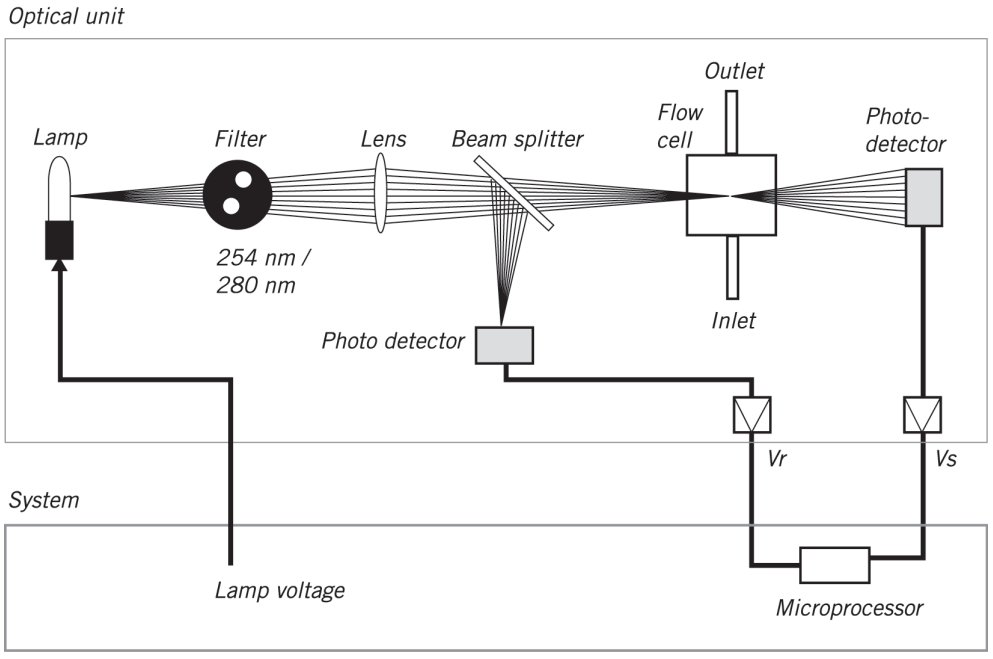
Introduction

This section describes the monitors and flow restrictor in ÄKTAxpress.



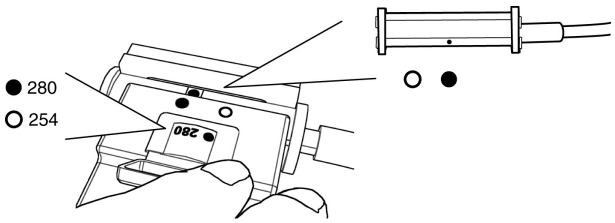
UV monitor

The UV optical unit houses the Hg lamp, the wavelength filter and the UV flow cell. The light beam is directed through a double conical or straight flowthrough cuvette of 2 µl illuminated volume to a photodetector. The photodetector current is fed to the signal processing circuitry in the system.



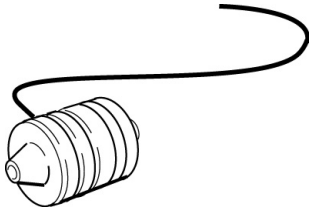
The reference signal comes from the same point in the lamp as the signal measuring the sample, thus assuring a stable baseline by eliminating the effects of variations in lamp intensity.

The Hg lamp emits light only at certain wavelengths. It does not emit light at 280 nm, so for this wavelength, the light is converted at a fluorescent surface before it passes the filter. On the lamp housing, there is a special exit for 280 nm light, which means that the lamp position needs to be changed when working with this wavelength.



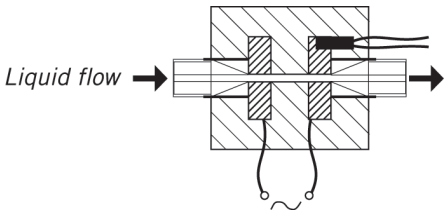
Conductivity monitor

The conductivity monitor flow cell is positioned below the UV housing.



The flow cell has two cylindrical titanium electrodes positioned in the flow path of the cell. An alternating voltage is applied between the electrodes and the resulting current is measured and used to calculate the conductivity of the eluent. The monitor controls the AC frequency and increases it with increasing conductivity between 50 Hz and 50 kHz giving maximum linearity and true conductivity values.

The conductivity is automatically calculated by multiplying the measured conductance by the flow cell's cell constant. The cell constant is precalibrated on delivery but can be measured with a separate calibration procedure, see **8.5.2 Calibration of conductivity cell** on page 243.



Pressure sensor

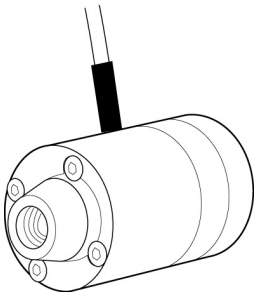
The pressure in the flow path is continuously monitored by a pressure sensor.

The liquid chamber in the pressure sensor housing is equipped with a titanium membrane. A strain gauge is attached to the rear side of the membrane. When the liquid pressure increases, the membrane bulges, which is detected by the strain gauge.

The pressure sensor housing is made of PEEK, other wetted parts are made of titanium and FFKM (perfluororubber).

Air sensor

The air sensor is a high precision monitor designed for continuous monitoring of air bubbles in the inlet flow path. When air is detected, the system is either paused, or performs an action that is set in the method plan.



The flow path has i.d. 1.5 mm. The air sensor is made of PEEK.

10.1.4 Fraction collector

Introduction

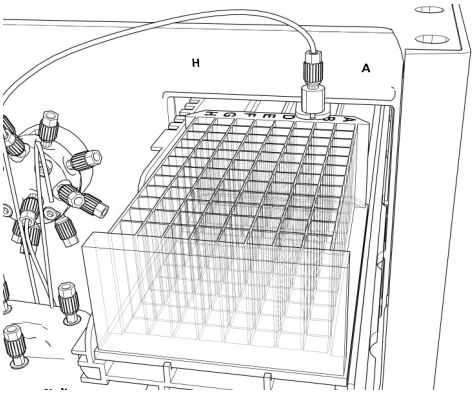
This section describes the fraction collector in ÄKTAxpress.

The fraction collector is used for:

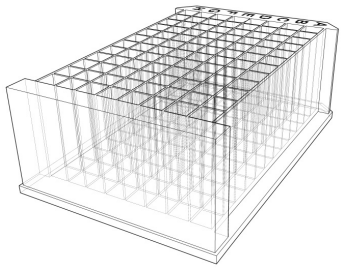
- Peak fractionation
- Elution fractionation

Fraction collector design

The fraction collector is an X-Y collector with a free arm. The design gives a compact unit with efficient operation.



The fraction collector holds 96 deep well microplates.



10.2 Technical specifications

About this section

This section contains specifications of ÄKTAxpress and its components.

In this section

This section contains the following sub-sections

Topic	See
System specifications	10.2.1
Components specifications	10.2.2
Wetted materials	10.2.3

10.2.1 System specifications

Introduction

This section specifies the general operating data of ÄKTAxpress. For components data see **10.2.2 Components specifications** on page 325.

Performance data

General performance data of ÄKTAxpress:

Parameter	Data
Flow rate range	0.1–65 ml/min
Pressure range	0–3 MPa (30 bar, 435 psi)

Gradient formation

Gradient formation data of ÄKTAxpress:

Linear gradients

Conditions:

- Water based buffers with different concentrations of salts

Parameter	Data
Linearity	±2%B within 20–80%B

Step gradients (quasi-isocratic mixing)

Conditions:

- Water based buffers with different concentrations of salts
- Mix proportions 5–95%

Parameter	Data
Concentration accuracy	±2%B

Physical data

Physical data of ÄKTAxpress:

Parameter	Data
Supply voltage	100–240 V AC, 50–60 Hz
Power consumption	Max. 120 VA
Degree of protection	EN 61010-1
Dimensions	w250 × d490 × h660 mm

Parameter	Data
Weight	30 kg
Ambient temperature – operation – storage	Temperature: +4 to +40° C –25 to +60° C
Relative humidity	20–95%, non condensing
Wetted material	See **10.2.3 Wetted materials** on page 328.

Standards

Compliance of ÄKTAxpress:

Parameter	Data
EMC	EN 61326 and EN 55011/CISPR 11 (Group I, Class A)
LVD	EN/IEC 61010-1, UL 61010A-1: 2003 and CAN/CSA C22.2 No 10101.1-92.

Column holders

Column holder data of ÄKTAxpress:

Parameter	Data
Column block capacity	Max. 5 small columns
Column holders	2 left hand side and 2 right hand side, designed for 30 mm waist columns

Microplate specifications

The microplates used in ÄKTAxpress must comply with the data:

Parameter	Data
Type	Deep well plate, 96 wells, height 45 mm
Well shape	Square wells

10.2.2 Components specifications

Introduction

This section specifies the operating data of the components in ÄKTAxpress. For general data of the system see **10.2.1 System specifications** on page 323.

Pump data

Pump data:

Parameter	Data
Flow rate range	0.1–65 ml/min
Flow rate accuracy	±2% or ±0.02 ml/min whichever is greater
Flow reproducibility	RSD < 0.5%
Maintenance interval	Sealings: 2000 hours

Mixer data

Mixer data:

Parameter	Data
Internal volume	0.37 ml

Flow restrictor data

Flow restrictor data:

Parameter	Data
Back pressure	0.2 MPa nominal

UV monitor data

UV monitor data:

Parameter	Data
Wavelengths	280 and 254 nm set by lamp position and filter
Optical path length	2 mm
Optical cell volume	2 µl
Detector cell volume	30 µl
Absorbance range	0.01 to 5 AU

Parameter	Data
Autozero range	-0.2 to 2.0 AU
Linearity, deviation	< 3% up to 2 AU
Static noise, short and long term	< 40 µAU
Static drift	±0.1 mAU/h
Hg lamp lifetime at 254 nm, – in room temperature – in cold room	Lifetime: 7000 hours 2000 hours
Hg lamp lifetime at 280 nm, – in room temperature – in cold room	Lifetime: 3500 hours 2000 hours

Conductivity monitor data

Conductivity monitor data:

Parameter	Data
Conductivity range	0.01–300.00 mS/cm
Accuracy	Max. ±0.4 mS/cm, typically < 0.1 mS/cm
Reproducibility, short and long term	Max. ±3% or ±15 µS/cm whichever is greater
Noise	Max. ±0.5% of full scale calibrated range, typically ±0.1%
Response time	< 3 s (0–95% of step)
Internal volume	24 µl

Pressure sensor data

Pressure sensor data:

Parameter	Data
Pressure range	0–3 MPa

Valve data

Valve data:

Inlet valve:

Parameter	Data
Internal volume, in–out	< 33 µl
Maintenance interval	10 000 positions, 1 year

Injection valve:

Parameter	Data
Internal volume, in–out	< 17 µl
Maintenance interval	10 000 positions, 1 year

Loop valve and column valve:

Parameter	Data
Internal volume, in–out	< 16 µl
Maintenance interval	10 000 positions, 1 year

Outlet valve:

Parameter	Data
Internal volume, in–out	< 15 µl
Maintenance interval	10 000 positions, 1 year

Capillary loops

Capillary loop data:

Parameter	Data
Volume	10 ml

Fraction collector

Fraction collector data:

Parameter	Data
Collector type	X–Y collector for 96 deep well microplates

10.2.3 Wetted materials

Introduction

This section specifies the wetted materials of ÄKTAxpress.

Wetted materials

The following wetted materials are used in ÄKTAxpress:

- **PEEK, polyetheretherketone**
  - Pump
  - UV monitor
  - Valves
  - Mixer
  - Flow restrictor
  - Online filter
  - Tubing
  - Unions, connectors
- **PTFE, polytetrafluoroethylene**
  - Pump
  - UV monitor
  - Mixer
  - Flow restrictor
- **FEP, fluorinatedethylenepropylene**
  - Tubing
- **ETFE, ethylenetetrafluoroethylene**
  - Flow restrictor
  - Unions, connectors
- **PCTFE, polychlorotrifluoroethylene**
  - Pump
  - Conductivity cell
- **PP, polypropylene**
  - Online filter
  - Inlet filter
- **PVDF, polyvinylidenefluoride**
  - Pump
- **PE, polyethylene**
  - Pump
- **PFR, fluororubber**
  - Flow restrictor

- **Titanium alloy**
  - Pump
  - UV monitor
  - Inlet filter
  - Conductivity cell
- **Quartz glass**
  - UV monitor
- **Aluminium oxide**
  - Pump
- **Stainless steel, Elgiloy, cobalt-chromium-nickel alloy**
  - Pump
- **Ruby, sapphire**
  - Pump
  - Online filter

10.3 Import file format

Introduction

This section describes how to create import files and how they are used.

Import file content

One or several import files can be used for storing information on one or several protein samples. When starting a run using the wizard in System Control, the import file can automatically be called upon, and the information collected and inserted in the wizard.

The import file may contain the following parameters for each sample ID:

- Sample volume.
- Isoelectric point.
- Extinction coefficient.
- Molecular weight.
- Free text.

Example

This illustration is an example of an import file, containing information on three different samples.

```
GFP-His Volume  10
GFP-His pI      6.19
GFP-His ExtCoeff        0.746
GFP-His MW      28
GFP-His FreeText        Green Fluorescent Protein
APB 7   Volume  10
APB 7   pI      5.95
APB 7   ExtCoeff        0.4
APB 7   MW      28
APB 7   FreeText        Unstable at room temperature
APB 13  Volume  10
APB 13  pI      5.56
APB 13  ExtCoeff        1.175
APB 13  MW      70
APB 13  FreeText        Avoid pH below 5.5
```

File specification

The import file can be created in a text editor, for example Notepad.. Follow the format description below:

- The file name is optional but the extension must be `txt`.
- Column 1: Sample ID; Column 2: Parameters; Column 3: Parameter values.
- The parameter names must be written according to the example below.
- Separate the columns by pressing "Tab" once.
- Press Enter to create a new line.
- The storage location of the import file is by default `C:\UNICORN\Server\MethodWizardImport\`. The path can be changed in each method plan, see **5.5.9 Advanced Zone for Purify - General advanced settings** on page 136.

10.4 Chemical resistance guide

Introduction

This section specifies the chemical resistance of ÄKTAxpress to some of the most commonly used chemicals in liquid chromatography.

Note: *ÄKTAxpress is intended to be used with water based solutions only. Organic solvents are not recommended due to the mixer design.*

Assumptions made

The ratings are based on the following assumptions:

- The synergistic effects of chemical mixtures have not been taken into account.
- Room temperature and limited overpressure is assumed.

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

List of chemicals

List of chemicals and their compatibility to ÄKTAxpress:

Chemical	Exposure <1 day	Exposure up to 2 months	Remarks
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	

Chemical	Exposure <1 day	Exposure up to 2 months	Remarks
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	Remarks
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	

Chemical	Exposure <1 day	Exposure up to 2 months	Remarks
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	OK	PP and PE are affected by long term use

10.5 Ordering information

Introduction

This section lists the systems, accessories and *user replaceable* spare parts available for ÄKTAxpress.

If not stated otherwise, the code number contains a pack of one (1) unit.

Systems

The following systems are available:

Item	Code no.
ÄKTAxpress system for system extension	18-6645-01
ÄKTAxpress, set of 4 systems	18-6645-04
ÄKTAxpress, set of 4 systems with computer	18-6645-05

Holders

The following holders are available for the system:

Item	Code no.
Tube holder	18-1177-80
Tubing holder	18-1177-81
Flask holder (optional)	18-1177-79
Large column holder clip	11-0002-92

Pump parts

The following spare parts are available for the pump:

Item	Code no.
Pump piston seal kit, including 2 piston seals and 2 rinse membranes	18-1112-04
Check valve kit, including 1 inlet and 1 outlet check valve	18-1128-66
Piston kit, including piston, spring, seal and rinse membrane	18-1112-13

Monitor parts

The following spare parts are available for the monitors:

UV monitor:

Item	Code no.
Hg optics with 254, 280 nm filters, excl. flow cell	18-1128-20
Hg lamp & housing complete	18-1128-22
UV flow cell 2 mm	18-1128-25
Filter 254 nm	18-0620-01
Filter 280 nm	18-0621-01
UV test kit, 2 mm flow cell, 280 nm	18-1129-63

Conductivity monitor:

Item	Code no.
Conductivity flow cell complete	18-1111-05

Air sensor:

Item	Code no.
Air sensor complete, 915N	11-0003-08

Flow restrictor

The following spare part is available for the flow restrictor:

Item	Code no.
Flow restrictor FR-902	18-1121-35

Cables

The following cables are available:

Item	Code no.
UniNet, 0.7 m	18-1109-74
UniNet, 1.5 m	18-1117-75
UniNet, 3.0 m	18-1109-75
UniNet, 15 m	18-1117-74

Item	Code no.
UniNet, Inline coupler	11-0003-79
Mains cable EU, 240 V	19-2448-01
Mains cable US, 120 V	19-2447-01

Fraction collector

The following spare part is available for the fraction collector:

Item	Code no.
Drip box	11-0002-95

Capillary loops

The following capillary loops are available:

Item	Code no.
Capillary loop	11-0003-02

Filters

The following filters are available:

Item	Code no.
Inlet filter set	11-0004-07
Online filter	18-1112-44
Screw lid GL45 incl. cap membrane	11-0004-10

Connectors and unions

The following connectors and unions are available:

Item	Code no.
Fingertight connectors 1/16"	18-1172-63
Fingertight stop plug	11-0003-55
M6/3.3 connectors	18-1172-64
Ferrule, yellow for M6 connector	18-1121-18
Nut 5/16 male 1/8 for pump inlet	18-1121-17
M6 key	19-7481-01

Item	Code no.
Fingertight key	11-0003-56
Tubing cutter	18-1112-46
Union M6 male to 1/16" female	18-1112-57
Union M6 female to 1/16" male	18-1112-58
Union M6 female to 1/16" female (PEEK)	18-1123-94
Union 1/16" male to 1/16" male	18-1120-93
Union 1/16" female to 1/16" female	18-3855-01
Union Luer female to 1/16" male	18-1112-51

Tubing

The following tubing is available:

Item	Code no.
Tefzel tubing i.d. 1 mm o.d. 1/16" (3 m)	18-1142-38
Teflon tubing i.d. 1.6 mm o.d. 1/8" (3 m)	18-1121-16
Desalt tubing Tefzel tubing i.d. 1.6 mm o.d. 1/16" (0.35 m)	11-0004-04

Columns

The following columns are available:

Affinity chromatography columns

Item	Code no.
HisTrap HP, 5 × 1 ml	17-5247-01
HisTrap HP, 100 × 1 ml	17-5247-05
HisTrap HP, 5 × 5 ml	17-5248-02
HisTrap HP, 100 × 5 ml	17-5248-05
HiTrap Chelating HP, 5 × 1 ml	17-0408-01
HiTrap Chelating HP, 1 × 5 ml	17-0409-01

Item	Code no.
GSTrap HP, 5 × 1 ml	17-5281-01
GSTrap HP, 100 × 1 ml	17-5281-05
GSTrap HP, 5 × 5 ml	17-5282-02
GSTrap HP, 100 × 5 ml	17-5281-05
GSTrap FF, 5 × 1 ml	17-5130-01
GSTrap FF, 2 × 1 ml	17-5130-02
GSTrap FF, 1 × 5 ml	17-5131-01

Desalting columns

Item	Code no.
HiPrep 26/10 Desalting, 1 × 53 ml	17-5087-01
HiPrep 26/10 Desalting, 4 × 53 ml	17-5087-02
HiTrap Desalting, 5 × 5 ml	17-1408-01

Ion exchange columns – anion-IEX

Item	Code no.
RESOURCE Q, 1 × 1 ml	17-1177-01
RESOURCE Q, 1 × 6 ml	17-1179-01
HiTrap Q HP, 5 × 1 ml	17-1153-01
Mono Q 5/50 GL, 1 × 1 ml	17-5166-01

Ion exchange columns – cation-IEX

Item	Code no.
RESOURCE S, 1 × 1 ml	17-1178-01
RESOURCE S, 1 × 6 ml	17-1180-01
HiTrap SP HP, 5 × 1 ml	17-1151-01
Mono S 5/50 GL, 1 × 1 ml	17-5168-01

Gel filtration

Item	Code no.
HiLoad 16/60 Superdex 75 prep grade, 1 × 120 ml	17-1068-01
HiLoad 16/60 Superdex 200 prep grade, 1 × 120 ml	17-1069-01

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