

# ÄKTA™ pilot 600

## User Manual



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# 1 Introduction

## About this chapter

This chapter contains important user information, descriptions of safety notices, intended use of the ÄKTA pilot 600 system, and lists of associated documentation.

---

## In this chapter

This chapter contains the following sections:

| Section                        | See page |
|--------------------------------|----------|
| 1.1 About this manual          | 5        |
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---

## 1.1 About this manual

### Purpose of this manual

The *User Manual* provides you with in-depth instructions and information for using the ÄKTA pilot 600 system. Basic instructions including important safety information are given in the *ÄKTA pilot 600 Operating Instructions*.

---

### Scope of this manual

The User Manual covers the ÄKTA pilot 600S (Standard) and ÄKTA pilot 600R (Regulatory) instruments. The instrument is controlled by a PC running UNICORN system control software version 7.3 or higher. For detailed information about the system control software, see the UNICORN user documentation. For information about chromatography columns, see the respective user manuals or instructions.



### Typographical conventions

Software items are identified in the text by ***bold italic*** text. A colon separates menu levels, thus ***File:Open*** refers to the ***Open*** command in the ***File*** menu.

Hardware items are identified in the text by **bold** text (for example, **Power**).

---

## 1.2 Important user information

### Read the *Operating Instructions* before using the product



**All users must read the entire *ÄKTA pilot 600 Operating Instructions* before installing, operating or maintaining the product.**

Always keep the *Operating Instructions* at hand when operating the product.

Do not operate the product in any other way than described in the user documentation. If you do, you may be exposed to hazards that can lead to personal injury and you may cause damage to the equipment.

---

### Intended use of the product

The ÄKTA pilot 600 system is a low-pressure automated liquid chromatography system providing:

- precision transportation of fluids to and from chromatography columns of varying sizes,
- detection and monitoring of UV absorbance, conductivity, and pH,
- fractionation of column eluate.

The system is intended for process development, scale up and scale down of processes, as well as sanitary production of material for pre-clinical and clinical phases of applicable scale. The working flow rate range is 0.1 to 600 mL/min, (or 600 to 1200 mL/min using dual pump flow), at pressures up to 2 MPa (20 bar).

Process engineers, process operators and other trained laboratory personnel are the intended users of the ÄKTA pilot 600 system.

The ÄKTA pilot 600 system shall not be used in any clinical procedures, or for diagnostic purposes.

The ÄKTA pilot 600 system shall not be used in a potentially explosive atmosphere or for handling flammable liquids.

---

## Prerequisites

In order to follow this manual and use the system in the manner it is intended:

- The user should have a general understanding of how the computer and Microsoft® Windows® work.
  - The user must understand the concepts of liquid chromatography.
  - The user must have read and understood the Safety instructions chapter in the *ÄKTA pilot 600 Operating Instructions*.
  - The ÄKTA pilot 600 system must have been installed according to the instructions in the *ÄKTA pilot 600 Operating Instructions*.
  - A user account must have been created according to the *UNICORN™ Administration and Technical Manual*.
- 

## Safety notices

This user documentation contains safety notices (WARNING, CAUTION, and NOTICE) concerning the safe use of the product. See definitions below.



### WARNING

**WARNING** indicates a hazardous situation which, if not avoided, could result in death or serious injury. It is important not to proceed until all stated conditions are met and clearly understood.



### CAUTION

**CAUTION** indicates a hazardous situation which, if not avoided, could result in minor or moderate injury. It is important not to proceed until all stated conditions are met and clearly understood.



### NOTICE

**NOTICE** indicates instructions that must be followed to avoid damage to the product or other equipment.

## 1 Introduction

### 1.2 Important user information

## Notes and tips

**Note:** *A note is used to indicate information that is important for trouble-free and optimal use of the product.*

**Tip:** *A tip contains useful information that can improve or optimize your procedures.*

---

## 1.3 Associated documentation

### Introduction

This section describes the user documentation that is delivered with the product, and how to find related literature that can be downloaded or ordered from GE.

### User documentation for ÄKTA pilot 600

The user documentation listed in the table below is available in printed or on the web PDF format. Translations of the *Operating Instructions* are provided on the User Documentation CD, together with the *Product Documentation* and *Unpacking Instructions*.

| Documentation  | Main contents  |
|--|--|
| <i>ÄKTA pilot 600 Operating Instructions</i>         | Instructions needed to prepare and operate the ÄKTA pilot 600 system in a correct and safe way.<br>System overview, site requirements, and instructions for moving the system within the same building.<br>Instructions for basic maintenance and troubleshooting. |
| <i>ÄKTA pilot 600 User Manual</i><br>(this document) | Additional information in order to get the optimal performance from the system.<br>Functional description of modules.<br>Instructions for maintenance and troubleshooting activities.  |
| <i>ÄKTA pilot 600 Site Preparation Guide</i>         | Information needed to prepare the site for installation and use of the ÄKTA pilot 600 system.  |
| <i>ÄKTA pilot 600 Unpacking Instructions</i>         | Instructions for handling the delivery package and unpacking the ÄKTA pilot 600 system.  |
| <i>ÄKTA pilot 600S Product Documentation</i>         | Specifications and material conformity.  |

## Product documentation binders (Regulatory version only)

In addition to the user documentation, the documentation package supplied with ÄKTA pilot 600R (Regulatory systems) also includes product documentation binders containing detailed specifications and traceability documents, specific to the individual system.

For more information about the ÄKTA pilot 600R instrument, see [Standard and Regulatory versions of the instrument, on page 16](#).

The following table provides some examples of documents that can be found in the documentation package delivered with ÄKTA pilot 600R.

| Document                           | Abbreviation | Purpose/Contents   |
|------------------------------------|--------------|--|
| Piping and Instrumentation Diagram | P&ID         | Schematic overview of the process flow, components and instruments and the control system. |
| General Specification              | GS           | Technical data for the system.   |
| Assembly Drawing                   | AD           | Physical layout. Provides dimensional data.  |
| Bill of Material                   | BOM          | Description of process-related components, including wetted materials and specifications.  |
| Declaration of Conformity          | DoC          | Declaration of Conformity for EU and/or other regions.                                     |
| Spare Part List                    | SPL          | List of spare parts available from GE.   |

## UNICORN documentation

The UNICORN documentation is listed in the following table. The documents are available in the **UNICORN Online Help and Documentation** section of the on-line help (see [Online help, on page 11](#)).

| Documentation   | Main contents  |
|---|--|
| Getting started with Evaluation (accessed through the online help in the UNICORN Evaluation module) | <ul style="list-style-type: none"> <li>• Video clips showing common workflows in the Evaluation module.</li> <li>• Overview of features of the Evaluation module.</li> </ul> |

| Documentation  | Main contents   |
|--|---|
| UNICORN Method Manual <sup>1</sup>                       | <ul style="list-style-type: none"> <li>• Overview and detailed descriptions of the method creation features in UNICORN.</li> <li>• Workflow descriptions for common operations.</li> </ul>  |
| UNICORN Administration and Technical Manual <sup>1</sup> | <ul style="list-style-type: none"> <li>• Overview and detailed description of network setup and complete software installation.</li> <li>• Administration of UNICORN and the UNICORN database.</li> </ul>                         |
| UNICORN Evaluation Manual <sup>1</sup>                   | <ul style="list-style-type: none"> <li>• Overview and detailed descriptions of the Evaluation Classic module in UNICORN.</li> <li>• Description of the evaluation algorithms used in UNICORN.</li> </ul>                          |
| UNICORN System Control Manual <sup>1</sup>               | <ul style="list-style-type: none"> <li>• Overview and detailed description of the system control features in UNICORN.</li> <li>• Includes general operation, system settings and instructions on how to perform a run.</li> </ul> |
| UNICORN OPC User Manual                                  | Instructions for setting up and using the UNICORN OPC server.   |

<sup>1</sup> Current UNICORN version is added to the title of the manual.

## Online help

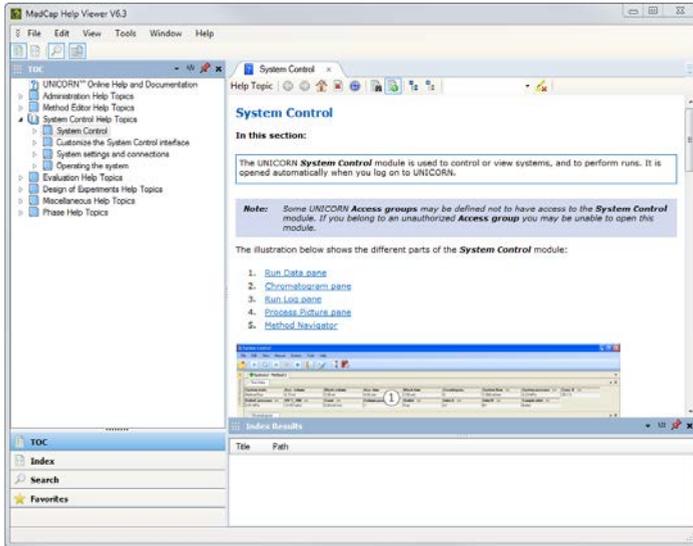
Online help in UNICORN software may be accessed in three ways:

- Use the **Help** menu to access help on the current module and contextual help (help on the currently context).
- Press the **F1** key on the keyboard to open the contextual help for the current context.
- Click on the Help symbol  if one is displayed. In general, Help symbols are shown in dialog boxes.

All of these approaches open the online help in a help browser (illustrated below) that supports access to UNICORN documentation and navigation among help topics using browse, search and index functions.

# 1 Introduction

## 1.3 Associated documentation



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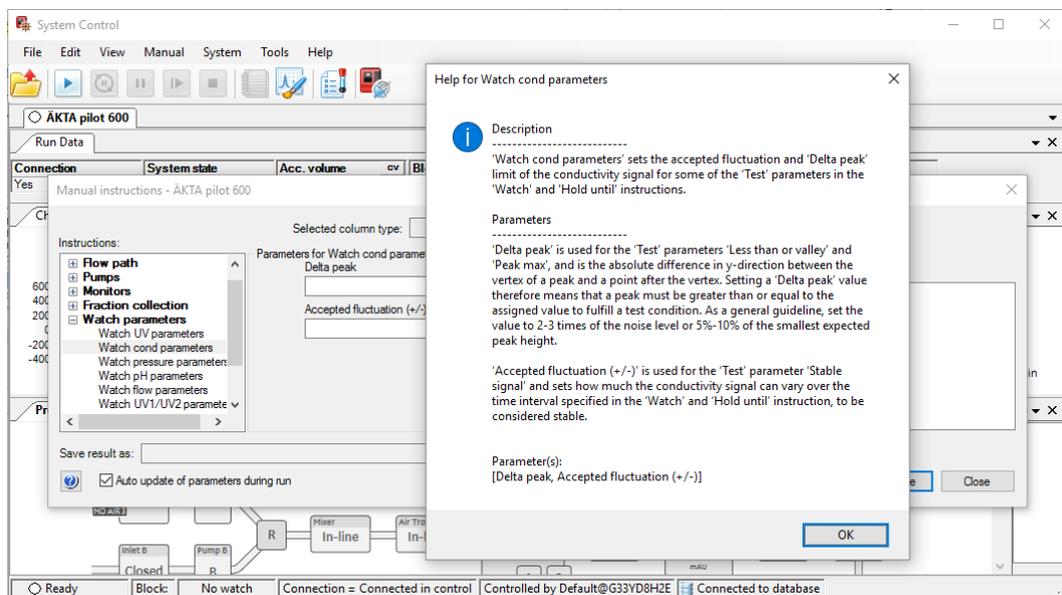
## Important information

In some contexts, important and supplementary information about settings is presented in tooltips, marked by the information symbol . Hover over the symbol to show the tooltip.

---

## Help for specific instructions

To access help for specific instructions in **System settings** and **Manual instructions** and for text instructions in the **Method Editor** module, select the instruction and press **F1**. Navigation among help topics is not supported from help displayed in this way.



## User documentation and other literature on the web

User documentation and other literature related to ÄKTA pilot 600 system may be downloaded from the web. Follow the steps below to access the documentation.

- | Step | Action   |
|------|--|
| 1    | Go to <a href="http://www.gelifesciences.com/aktapilot">www.gelifesciences.com/aktapilot</a> . |
| 2    | Navigate to <b>RELATED DOCUMENTS</b> .   |
| 3    | Select the type of document and download the chosen literature.                                |

## Access documentation from mobile units

Scan the code using your mobile phone or tablet computer to access the product page for ÄKTA pilot 600. Select documents to download under **RELATED DOCUMENTS**.



# 2 System description

## About this chapter

This chapter gives an overview of the ÄKTA pilot 600 instrument, available accessories, and the Instrument Configuration software.

---

## In this chapter

This chapter contains the following sections:

| Section  | See page |
|--|----------|
| 2.1 Description of the ÄKTA pilot 600 instrument | 16       |
| 2.2 Flow path                                    | 19       |
| 2.3 Accessories                                  | 21       |
| 2.4 Instrument Configuration software            | 27       |

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## 2 System description

### 2.1 Description of the ÄKTA pilot 600 instrument

## 2.1 Description of the ÄKTA pilot 600 instrument

### Introduction

This section gives an overview of the instrument and the available modules.

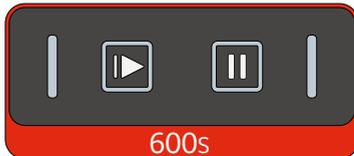
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### Standard and Regulatory versions of the instrument

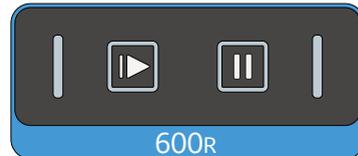
The ÄKTA pilot 600 instrument is available in two versions:

- ÄKTA pilot 600S, Standard instrument, with the possibility to install optional modules.
- ÄKTA pilot 600R, Regulatory instrument. The ÄKTA pilot 600R system is supplied fully configured according to the purchase specifications, and is tested at the factory. ÄKTA pilot 600R is delivered with the additional instrument specific, detailed documentation. Installing, removing or moving modules will make the documentation invalid.

The instrument version is indicated on the front panel as shown in the illustration below:



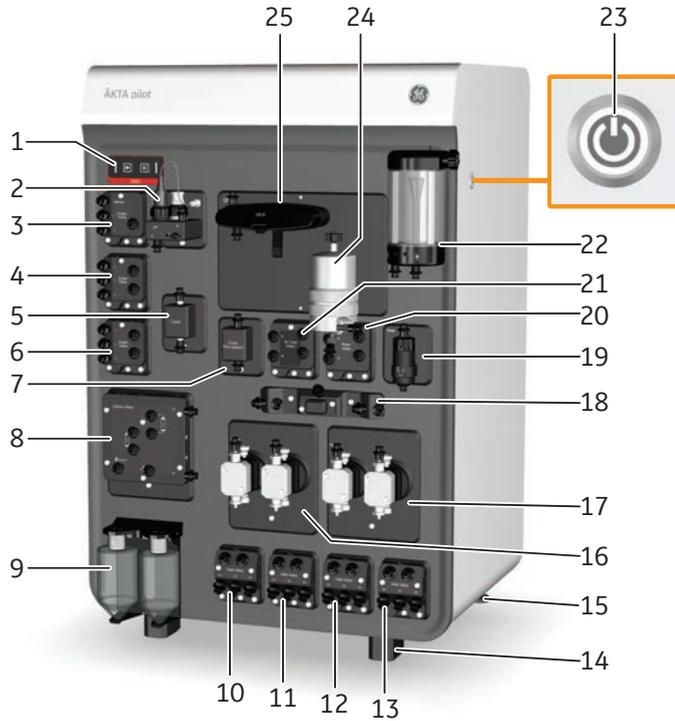
Standard instrument



Regulatory instrument

### ÄKTA pilot 600 instrument front panel

The following illustration shows an example of the ÄKTA pilot 600 instrument, with all types of modules installed. The location of modules can vary, depending on customer requirements. Optional modules are indicated in the table.



| Part | Description   | Label                           | Optional (*) |
|------|---|---------------------------------|--------------|
| 1    | Control panel. The instrument version is indicated below the control panel: <b>600S</b> (red) or <b>600R</b> (blue) | -                               |              |
| 2    | pH monitor  | <b>pH</b>                       | *            |
| 3    | Outlet valve 1-3, port 1 can be used for waste  | <b>Outlet Valve<br/>W/1 2 3</b> |              |
| 4    | Outlet valve 4-6  | <b>Outlet Valve<br/>4 5 6</b>   | *            |
| 5    | Conductivity monitor  | <b>Cond</b>                     |              |
| 6    | Outlet valve 7-9  | <b>Outlet Valve<br/>7 8 9</b>   | *            |
| 7    | Pre-column Conductivity monitor   | <b>Cond Pre-col-<br/>umn</b>    | *            |

## 2 System description

### 2.1 Description of the ÄKTA pilot 600 instrument

| Part | Description  | Label                           | Optional (*) |
|------|--|---------------------------------|--------------|
| 8    | Column valve, including pre- and post-column pressure sensor | <b>Column Valve</b>             |              |
| 9    | Bottles for pump rinsing solution                            | -                               |              |
| 10   | Inlet valve A1-A3  | <b>Inlet Valve<br/>A1 A2 A3</b> |              |
| 11   | Inlet valve A4-A6  | <b>Inlet Valve<br/>A4 A5 A6</b> | *            |
| 12   | Inlet valve B1-B3  | <b>Inlet Valve<br/>B1 B2 B3</b> |              |
| 13   | Inlet valve B4-B6  | <b>Inlet Valve<br/>B4 B5 B6</b> | *            |
| 14   | Fixed plastic feet   | -                               |              |
| 15   | Adjustable feet  | -                               |              |
| 16   | System pump A  | <b>Pump A</b>                   |              |
| 17   | System pump B  | <b>Pump B</b>                   |              |
| 18   | Flow restrictor, including system pressure sensor            | -                               |              |
| 19   | Mixer module   | <b>Mixer</b>                    | *            |
| 20   | Mixer valve  | <b>Mixer Valve</b>              | *            |
| 21   | Air trap valve, including air sensor                         | <b>Air Trap Valve</b>           |              |
| 22   | Air trap   | -                               |              |
| 23   | <b>ON/OFF</b> button   | -                               |              |
| 24   | In-line filter (stainless steel filter housing shown)        | -                               | *            |
| 25   | UV monitor   | <b>UV</b>                       |              |

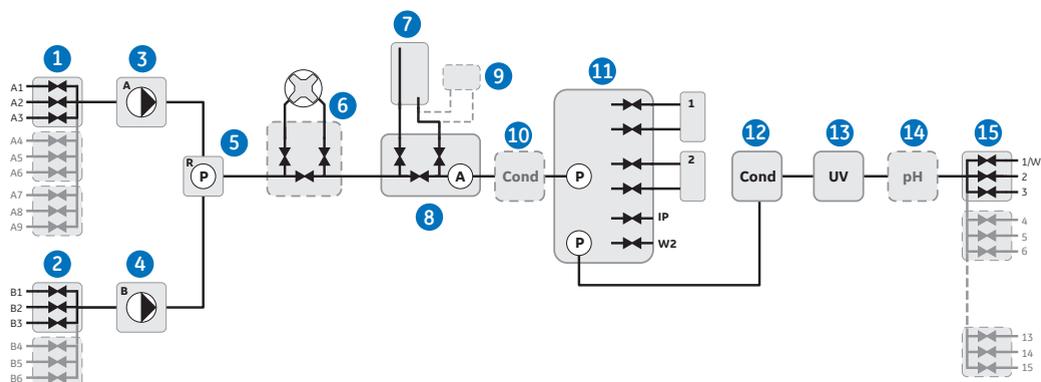
## 2.2 Flow path

### Introduction

This section gives a generalized overview of the flow path in the ÄKTA pilot 600 instrument. The flow path in a specific instrument is determined by the modules that are installed and by the current settings in UNICORN software. Several functions such as the mixer, air trap and columns can be switched in or out of the flow path as required by setting to *In-line* or *By-pass* in the software.

### Flow diagram

The following illustration shows a schematic diagram of the flow path. Optional components are marked with dashed outlines.



| Part | Function   |
|------|--|
| 1    | Inlet valve A with 3 ports. Can be expanded to 9 ports with optional inlet valves. |
| 2    | Inlet valve B with 3 ports. Can be expanded to 6 ports with optional inlet valves. |
| 3    | System pump A  |
| 4    | System pump B  |
| 5    | Flow restrictor and system pressure sensor   |
| 6    | Mixer and mixer valve (optional)   |
| 7    | Air trap   |

## 2 System description

### 2.2 Flow path

| Part | Function  |
|------|---|
| 8    | Air trap valve including air sensor   |
| 9    | In-line filter (optional)   |
| 10   | Pre-column Conductivity monitor with temperature sensor (optional)                  |
| 11   | Column valve including pre-column and post-column pressure sensors                  |
| 12   | Conductivity monitor with temperature sensor  |
| 13   | UV monitor  |
| 14   | pH module (optional)  |
| 15   | Outlet valve with 3 ports. Can be expanded to 15 ports with optional outlet valves. |

## 2.3 Accessories

### Introduction

This section gives an overview of the accessories supplied with the system and required for regular maintenance.

### Accessories included with the ÄKTA pilot 600 instrument

Up-to-date information on accessories supplied with the ÄKTA pilot 600 instrument may be obtained from the ÄKTA pilot 600 product pages on [www.gelifesciences.com/akta](http://www.gelifesciences.com/akta). The table below lists some examples.

| Accessory   | Illustration   |
|---|--|
| Socket screw wrench 7 mm                          |    |
| Tubing cutter                                     |    |
| SNAP connectors, 3.2 mm and 4.8 mm                |   |
| Connectors TC25-SNAP connector, 3.2 mm and 4.8 mm |    |
| SNAP connector-Stop plug, 3.2 mm                  |    |
| Union 5/16" male-SNAP connector 3.2 mm            |  |
| Reference capillaries 1 and 2                     |    |
| Tube bender                                       |    |

## 2 System description

### 2.3 Accessories

#### Using the Tube bender

The Tube bender, provided as an accessory with the instrument, helps in bending tubing without introducing kinks. Short lengths of tubing, especially the i.d. 4.8 mm tubing on the inlet side of the pumps, are particularly susceptible to kinking.

Use the Tube bender as described in the steps below.

| Step | Action   |
|------|--|
| 1    | Mount the Tube bender on mounting pins on the front of the instrument, for example, the mounting pins for the in-line filter holder.<br>The Tube bender can also be used while held in the hand. |
| 2    | Pull the tubing around the groove in the Tube bender until a suitable bend is obtained. Use the wider groove for i.d. 4.8 mm tubing and the narrower for i.d. 3.2 mm tubing.                     |

#### Optional accessories

The following optional accessories are available:

- Extension box for optional modules
- Extension stand for columns and accessories
- I/O-box to connect external equipment to the ÄKTA pilot 600 instrument
- Module front panel with rails

#### Extension box for optional modules

Optional modules can be installed in an Extension box that can be placed on the bench beside the instrument, or attached to the Extension stand.

The following illustration shows an outlet module installed in an Extension box.

**Note:** *Modules installed in an Extension Box will have protection class IP21, regardless of the instrument classification.*



| Part | Description   |
|------|---|
| 1    | Retaining screw for the module  |
| 2    | UniNet-9 cable: connect to a free <b>UniNet-9</b> connector at the back of the instrument |
| 3    | Bracket for hanging the Extension box on the Extension stand                              |

**Note:** *If the Extension box is used with ÄKTA pilot 600R, the documentation for the instrument will be invalid.*

## I/O-box

The external I/O-box can be used to connect other equipment in order to measure parameters such as refractive index, light scattering and fluorescence. The I/O-box can communicate with external equipment by digital or analog signals.

The I/O-box can be attached to the Extension stand or placed on the bench, using the supplied feet.

**Note:** *The I/O Box has protection class IP20 when placed on the bench, regardless of the instrument classification.*

## 2 System description

### 2.3 Accessories



### Extension stand for columns and accessories

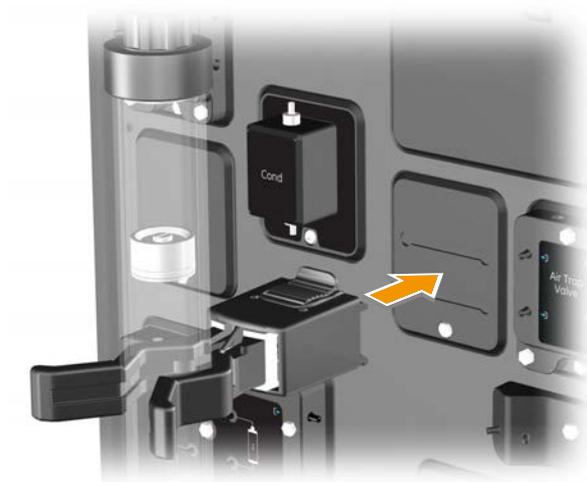
The Extension stand can be used to mount columns, the Extension box, and the I/O-box. The stand includes holders to organize the tubing.

The following illustration shows the Extension stand with a HiScale column attached.



## Module front panel with rails

A module front panel with rails is available for attaching ÄKTA accessory holders to the instrument front, for holding accessories such as Superloop™ 150 SNAP. The front panel with rails occupies one module position.



## Luer-SNAP 3.2 connectors

Some maintenance procedures require injection of liquid into a module from a syringe. Follow the instructions below to construct a secure connection from a Luer syringe to a module inlet.

**Note:** *It is important that the connection is secure, to avoid leakage of potentially hazardous liquids injected from the syringe.*

| Step | Action   |
|------|--|
| 1    | Prepare a length (5 to 10 cm) of 3.2 mm i.d. tubing with SNAP connectors at each end. The length of the tubing should be sufficient to allow access to the module inlet without interference from other modules on the instrument. |



## 2 System description

### 2.3 Accessories

| Step | Action |
|------|--------|
|------|--------|

- |   |   |
|---|---|
| 2 | Connect one end of the tubing to a 5/16" Male - SNAP 3.2 mm union (part no. 29274572, included with accessories). |
|---|---|



- |   |  |
|---|--|
| 3 | Connect the 5/16" Male union to a Luer - 5/16" Female union (part no. 11002707, ordered separately). |
|---|--|



## 2.4 Instrument Configuration software

### Introduction

UNICORN is a common software platform that is used for a range of different products from GE. The platform functionality is adapted to specific systems by installation of the Instrument Configuration software provided with the system.

This section gives a brief overview of the function and management of the Instrument Configuration software. More details are given in the UNICORN Administration and Technical Manual.

---

### Description

The Instrument Configuration adapts UNICORN functionality to the specific features and characteristics of the instrument in three areas, as listed in the table below.

| Area                   | Description   |
|------------------------|---|
| <b>Strategy</b>        | The software instruction set which controls the instrument.   |
| <b>Process Picture</b> | The graphic elements which are used to build the <b>Process Pictures</b> in the <b>Method Editor</b> and <b>System Control</b> modules. |
| <b>Phase Library</b>   | Predefined phases and methods adapted to the available options for the specific Instrument Configuration.                               |

### Installing the Instrument Configuration

The Instrument Configuration is installed as part of the procedure to define a system during UNICORN installation (see the *UNICORN Administration and Technical Manual* for detailed instructions).

---

### Updating the Instrument Configuration

Updated versions of the Instrument Configuration may be released by GE to provide improved system functionality. Detailed instructions for downloading and importing updated Instrument Configuration files are given in the *UNICORN Administration and Technical Manual*.

## 2 System description

### 2.4 Instrument Configuration software



#### **NOTICE**

It is important that the correct Instrument Configuration for the system is downloaded and installed. The system will be unusable if an incorrect Instrument Configuration is installed.

# 3 Description of modules

## About this chapter

This chapter gives additional information about the modules that are presented in *ÄKTA pilot 600 Operating Instructions*. Technical details can be found in *ÄKTA pilot 600S Product Documentation*, information about documentation for the R system can be found in *ÄKTA pilot 600 Operating Instructions*, and installation instructions can be found in respective module installation instruction.

---

## In this chapter

This chapter contains the following sections.

| Section                              | See page |
|--------------------------------------|----------|
| 3.1 External air sensors (optional)  | 30       |
| 3.2 Inlet valves                     | 33       |
| 3.3 Pumps                            | 35       |
| 3.4 Flow restrictor                  | 37       |
| 3.5 Mixer valve and mixer (optional) | 39       |
| 3.6 Air trap valve and air trap      | 41       |
| 3.7 In-line filter (optional)        | 45       |
| 3.8 Column valve                     | 47       |
| 3.9 Conductivity monitor             | 51       |
| 3.10 UV monitor                      | 53       |
| 3.11 pH monitor (optional)           | 55       |
| 3.12 Outlet valves                   | 57       |

---

## 3 Description of modules

### 3.1 External air sensors (optional)

## 3.1 External air sensors (optional)

### Introduction

This section describes the external air sensors that can be installed on the inlet tubing to detect incoming air if, for example, a buffer or sample container has run out of liquid.

### Illustration

The illustration below shows an external air sensor.



| Part | Description    |
|------|----------------|
| 1    | UniNet-9 cable |
| 2    | Inlet/outlet   |
| 3    | Inlet/outlet   |

### Function

The sensors are ultrasonic sensors that detect small or large gas bubbles depending on the sensitivity level.

When the alarm of an external air sensor is enabled in UNICORN and air is detected, the pumps pause and the text **AIR** is shown in red color in the **Process Picture**.

When the alarm of the external air sensor is disabled in UNICORN and air is detected, the pumps continue but the text **AIR** is still shown in red in the **Process Picture**.

Four external air sensors can be connected to the system. Each sensor has a predefined identity, 1, 2, 3 or 4, and must be connected in numerical order, see the separate installation instructions. Position the external air sensors depending on your goal, for example:

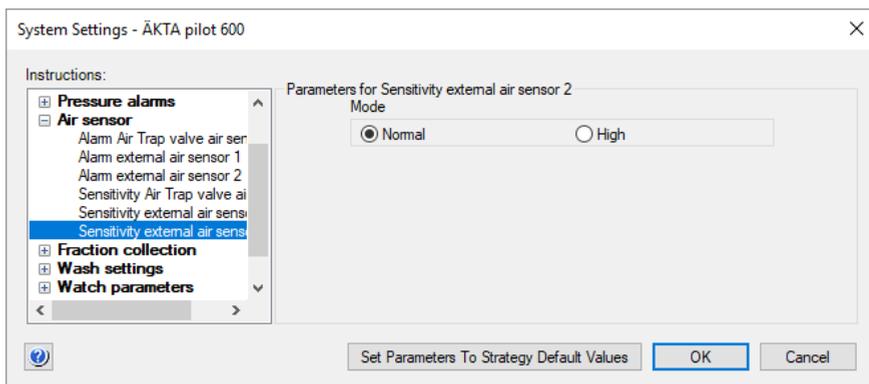
- To apply all sample feed onto the column, use a short piece of tubing between the external air sensor and the inlet valve. Remember to have the air trap in-line while applying sample using short tubing and high flow rates, because air might pass beyond the inlet valve.
- To prevent air entering the system flow path, place the external air sensor close to the liquid container. When running high flow rates, use a long (>70 cm) piece of tubing between the external air sensor and the inlet valve to make sure no air reaches the inlet valve.

In a predefined method, in the **Sample application** phase, the **External air sensor 1** can be used to facilitate sample application, see [Section 4.1 Sample application, on page 60](#).

## Air sensor sensitivity

The air sensor has two sensitivity levels, **High** that detects down to approximately 30  $\mu\text{L}$  bubbles, and **Normal** that detects down to approximately 1 mL bubbles. The level **Normal** is default for external air sensors.

To change the sensitivity, select **System:Settings** in the **System Control** module to open the **System Settings** dialog box. Select the external air sensor in the **Air sensor** list, and set the sensitivity as required, see illustration below.



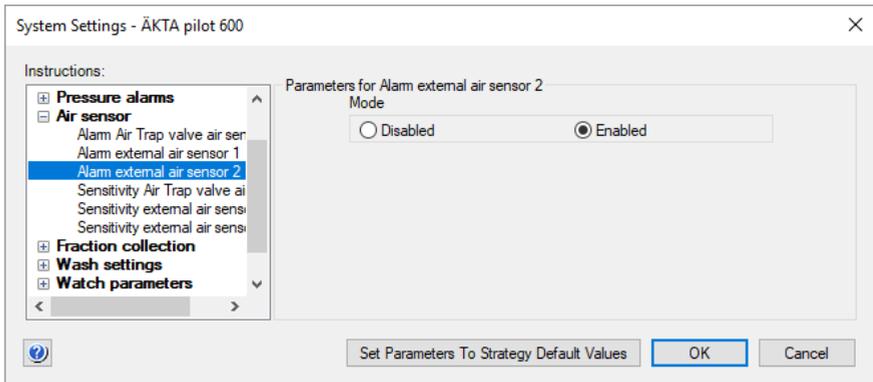
## Air sensor alarm

The air sensor alarms are disabled by default. If an alarm is enabled, detected air pauses the pumps.

To enable the alarm, select **System:Settings** in the **System Control** module to open the **System Settings** dialog box. Select external air sensor in the **Air sensor** list, select **Enabled** and click **OK**, see illustration below.

### 3 Description of modules

#### 3.1 External air sensors (optional)



Any selections in a method run, for example in the method settings, overrides the selections made in system settings.

---

## 3.2 Inlet valves

### Introduction

This section describes the inlet valves that are used to select samples and buffers, for pump A and pump B respectively, during the chromatography run.

### Illustration

The illustration below shows the front of the inlet valve module for pump A.



| Part | Description                                     |
|------|---|
| 1    | <b>A1</b> inlet                                 |
| 2    | <b>A2</b> inlet                                 |
| 3    | <b>A3</b> inlet                                 |
| 4    | Outlet, to the right pump head of system pump A |
| 5    | Outlet, to the left pump head of system pump A  |

## 3 Description of modules

### 3.2 Inlet valves

#### Function

The inlet valve modules consist of a number of membrane valves. The first A inlet port, **A1**, is used for buffer solution. **A2** and **A3** are used for sample or buffer solutions. If more A inlets are required, up to three inlet valve modules can be installed which results in nine A inlets.

The B inlets are used for buffer and wash solutions. If more B inlets are required, another B inlet module can be installed which results in six B inlets.

**Note:** *When running isocratic dual pump flow, make sure that the same buffer solution is pumped in pump A and pump B. For example, place the A and B inlets in the same buffer container. See [Section 4.4 Dual pump flow, on page 81](#).*

---

#### Inlet tubing

Tubing with inner diameter 4.8 mm and SNAP 4.8 connectors is used on the valve inlets. An adapter can be used between the SNAP 4.8 connector and the larger TC25 clamp to use tubing with larger diameter. Tubing with larger diameter is needed in the following cases:

- High viscosity solutions
  - Long inlet tubing
  - Tank or pipe with TC25 clamp
-

## 3.3 Pumps

### Introduction

This section describes the two high precision system pumps, A and B, that pump buffers and samples through the flow path.

### Illustration

The illustration below shows the front of pump A.



| Part | Description  |
|------|--|
| 1    | Rinsing solution inlet, from the rinsing solution bottle |
| 2    | Inlet, from <b>Inlet Valve</b> module                    |
| 3    | Rinsing solution inlet, from left pump head              |
| 4    | Inlet, from <b>Inlet Valve</b> module                    |
| 5    | Rinsing solution outlet, to right pump head              |
| 6    | Outlet, to the flow restrictor                           |

## 3 Description of modules

### 3.3 Pumps

| Part | Description   |
|------|---|
| 7    | Rinsing solution outlet, to the rinsing solution bottle |
| 8    | Outlet, to the flow restrictor                          |

## Function

Each system pump module consists of two pump heads. The individual pump heads are identical but actuated in opposite phase to each other. The two pistons and pump heads work alternately to give a continuous, low pulsation, liquid delivery. The two system pumps can be used individually or in combination, to generate isocratic or gradient elution in purification methods for flow rates up to 600 mL/min. The pumps can also be used in combination to generate dual flow in the flow range 600-1200 mL/min, for isocratic runs and limited gradient runs.

---

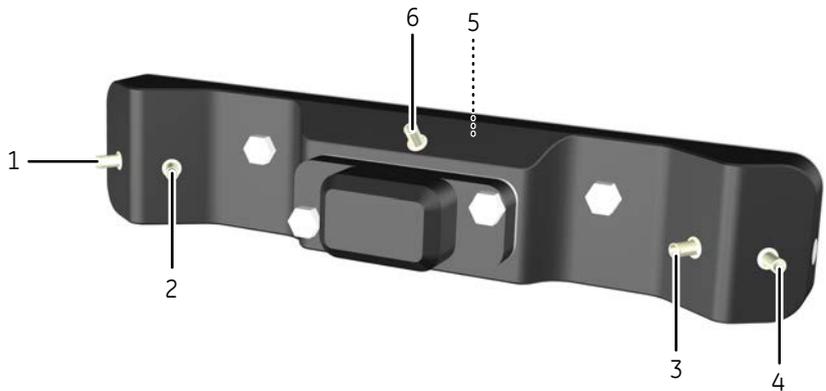
## 3.4 Flow restrictor

### Introduction

This section describes the flow restrictor, including the system pressure sensor. The flow restrictor prevents siphoning in the system, improves the flow rate and gradient accuracy at low flow rate, and monitors the overall system pressure.

### Illustration

The illustration below shows the front of the flow restrictor module.



| Part | Description                               |
|------|---|
| 1    | Inlet from system pump A, left pump head  |
| 2    | Inlet from system pump A, right pump head |
| 3    | Inlet from system pump B, left pump head  |
| 4    | Inlet from system pump B, right pump head |
| 5    | Position of the system pressure sensor    |
| 6    | Outlet, to the next module                |

## 3 Description of modules

### 3.4 Flow restrictor

#### Function

The module has two separate restrictors for A and B pumps respectively. The flow path inside this module is designed to use inlets from pump A for sample application.

The system pressure sensor is located inside the flow restrictor module. This sensor measures the pressure near the outlet, and UNICORN compares the value with the highest allowed and the lowest allowed system pressure.

---

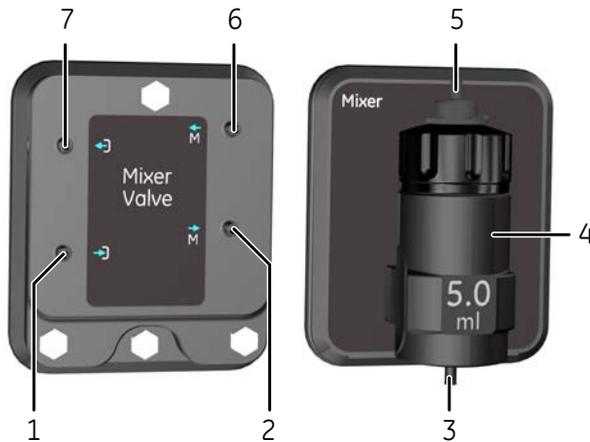
## 3.5 Mixer valve and mixer (optional)

### Introduction

This section describes the mixer valve and the dynamic mixer that control the buffer mixing from the two system pumps.

### Illustration

The illustration below shows the front of the mixer valve module (left) and the front of the mixer module (right).



| Part | Description                     |
|------|---------------------------------|
| 1    | Inlet, from the previous module |
| 2    | Outlet, to the mixer            |
| 3    | Mixer inlet                     |
| 4    | Mixer chamber, 5 mL             |
| 5    | Mixer outlet                    |
| 6    | Inlet, from the mixer           |
| 7    | Outlet, to the next module      |

## 3 Description of modules

### 3.5 Mixer valve and mixer (optional)

#### Function

The mixer valve module consists of a number of membrane valves. The mixer valve directs the flow through the mixer, or by-passes the mixer, for example during sample application. The mixer mixes the A and B buffers in a 5 mL mixer chamber to a homogeneous buffer composition.

If the mixer is in-line, the mixer stir magnet starts and stops automatically at start and end of a method. If it is by-passed, it does not start.

---

#### When to use

Some examples with recommendations to use the mixer or not are shown in the table below.

| Example                        | Recommended | Comment   |
|--------------------------------|-------------|---|
| HIC with flow rate < 30 mL/min | YES         | Strongly recommended at high salt concentrations.                                   |
| Flow rate > 100 mL/min         | N/A         | At high flow rates, the buffers are sufficiently mixed without the need of a mixer. |
| Sensitive samples              | NO          | Set the mixer to by-pass during sample application.                                 |

## 3.6 Air trap valve and air trap

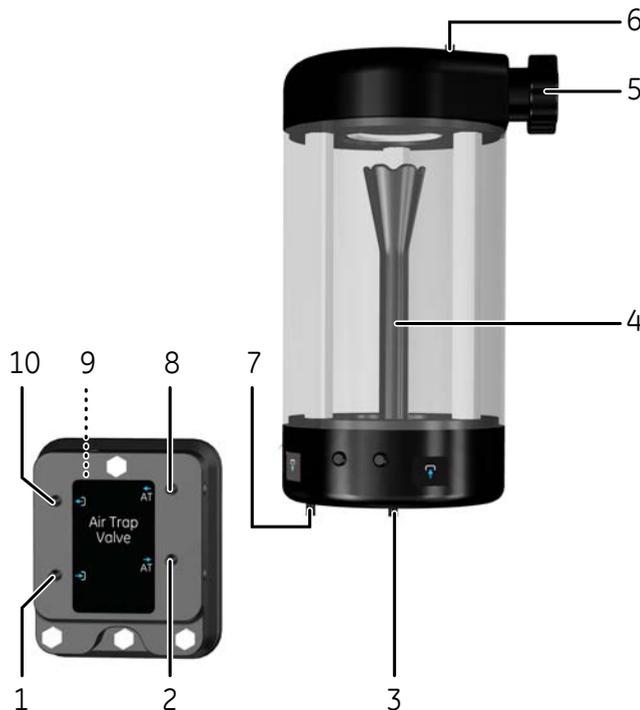
### Introduction

This section describes the air trap valve and the air trap. For information on how to fill and empty the air trap, see the *ÅKTA pilot 600 Operating Instructions*.

The purpose of the air trap is to trap air that has been introduced into the flow path. The purpose of the air sensor in the air trap valve is to detect and report all air in the flow path. Air reaching the column could decrease the column performance.

### Illustration

The illustration below shows the front of the air trap valve module (left) and the air trap (right).



| Part | Description                     |
|------|---------------------------------|
| 1    | Inlet, from the previous module |

## 3 Description of modules

### 3.6 Air trap valve and air trap

| Part | Description                |
|------|----------------------------|
| 2    | Outlet, to the air trap    |
| 3    | Air trap inlet             |
| 4    | Inlet funnel               |
| 5    | Manual vent valve knob     |
| 6    | Manual vent valve port     |
| 7    | Air trap outlet            |
| 8    | Inlet, from the air trap   |
| 9    | Position of the air sensor |
| 10   | Outlet, to the next module |

## Function

Air is trapped in the upper part of the air trap if the lower part contains liquid. The inlet funnel is designed to increase the mixing functionality and decrease foaming. During runs with gradients and low flow rate, make sure that the liquid level stays below the funnel top.

The air trap is in-line by default, but can be set in by-pass position using the air trap valve. The valve consists of a number of membrane valves that allow the flow to be directed through the air trap or to by-pass the air trap.

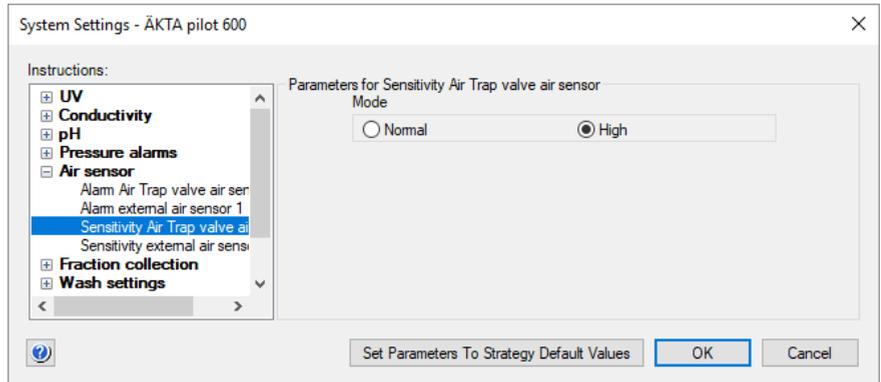
An air sensor is positioned near the outlet of the air trap valve. It detects air even when the air trap is by-passed. If air is detected in the flow path, its presence is indicated in the **Process Picture**. If air is detected in the flow path and the air sensor alarm is enabled, the run is paused.

When a large amount of air is trapped, the flow rate over the column is temporarily decreased. If the air trap is completely filled with air, air is detected by the sensor.

## Air sensor sensitivity

The air sensor has two sensitivity levels, **High** that detects down to approximately 30  $\mu\text{L}$  bubbles, and **Normal** that detects down to approximately 1 mL bubbles. The level **High** is default for the air sensor in the air trap valve.

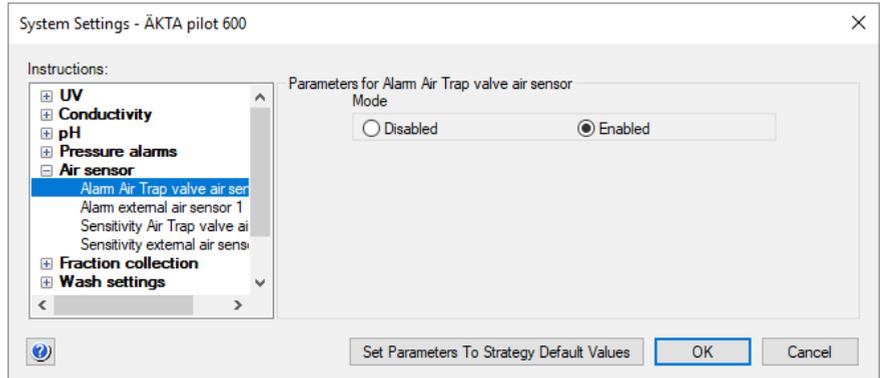
To change the sensitivity, select **System:Settings** in the **System Control** module to open the **System Settings** dialog box. Select **Air sensor** and **Sensitivity Air Trap sensor**. Set the sensitivity as required, see illustration below.



## Air sensor alarm

The alarm of this air sensor is default disabled. If the alarm is enabled, detected air pauses the pumps.

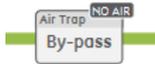
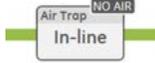
To enable the alarm, select **Alarm Air Tap valve air sensor** in the **System Settings** dialog box, select **Enabled** and click **OK**, see illustration below.



Any selections in a method run, for example in the method settings, overrides the selections made in system settings.

## Air trap indications in the Process Picture

The table below explains the four different states.

| Symbol  | Explanation   |
|---|---|
|  | <p>The air trap is by-passed, so air in the flow path is not captured.</p> <p>No air has been detected by the air sensor.</p>   |
|  | <p>The air trap is by-passed, so air in the flow path is not captured.</p> <p>Air has been detected by the air sensor. If the air sensor alarm is enabled, the run is paused.</p>   |
|  | <p>The air trap is in-line, so air in the flow path is captured.</p> <p>No air has been detected by the air sensor.</p>   |
|  | <p>The air trap is in-line, so air in the flow path is captured.</p> <p>Air has been detected by the air sensor. If the air sensor alarm is enabled, the run is paused.</p> <p><b>Note:</b><br/><i>Check if the air trap has run out of liquid.</i></p> |

**Note:** Make sure that the the air trap is filled before setting it in-line. The recommendation is to always have some liquid in the air trap because in-line position is default. For information on how to fill the air trap, see the ÄKTA pilot 600 Operating Instructions.

## When to use

Some examples when to use the air trap are shown below.

- to trap degassed bubbles released from cold solutions
- to catch any air introduced to the flow path during sample loading, for example when applying all sample using an external air sensor

At flow rates > 100 mL/min the air trap can always be in-line.

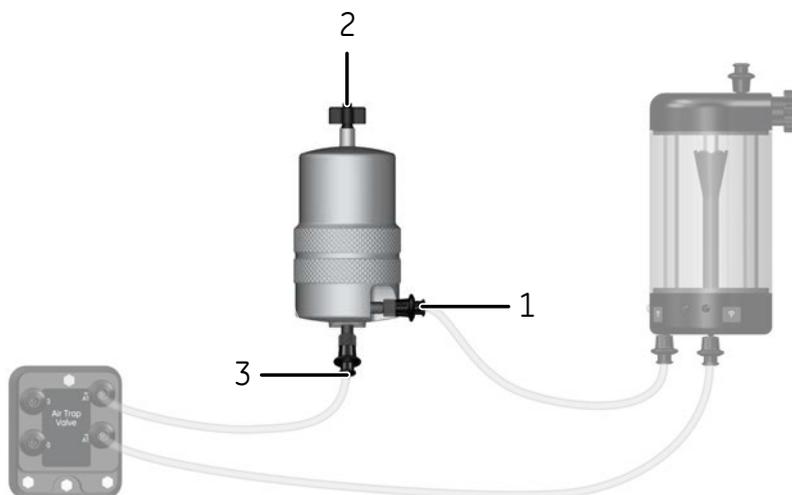
## 3.7 In-line filter (optional)

### Introduction

This section describes the optional in-line filters. The filters are used to prevent particles in solutions from reaching the column. For installation and replacement of a filter, see the *ÄKTA pilot 600 Operating Instructions*.

### Illustration

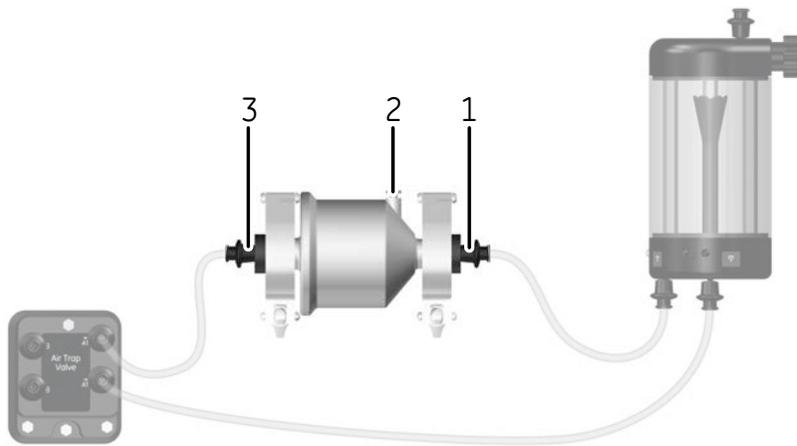
The illustrations below show the two in-line filter options, a stainless steel housing and an ULTA™ Capsule CG filter from GE.



| Part | Description                             |
|------|---|
| 1    | Inlet, stainless steel housing          |
| 2    | Air vent valve, stainless steel housing |
| 3    | Outlet, stainless steel housing         |

## 3 Description of modules

### 3.7 In-line filter (optional)



| Part | Description                            |
|------|--|
| 1    | Inlet, ULTA Capsule CG filter          |
| 2    | Air vent valve, ULTA Capsule CG filter |
| 3    | Outlet, ULTA Capsule CG filter         |

## Function

The stainless steel filter housing can be equipped with filter cassettes of different pore sizes, for example 0.2  $\mu\text{m}$  for sterile filtering and 3  $\mu\text{m}$  for particle removal. The housing can be used in the full system pressure range, up to 2 MPa.

The ULTA Capsule CG filter is mounted using an ULTA CG filter mounting kit, available from GE. This mounting kit is designed for ULTA Capsule CG filters. The pressure limit for these filters is 0.5 MPa, see the *ÄKTA pilot 600 Operating Instructions* for how to set system pressure limits.

**Note:** A small pore size will increase the back pressure and the system pressure.

## Air in filter

For instructions on how to fill the filter, see the *ÄKTA pilot 600 Operating Instructions*. If there is air inside the filter, the filter area is reduced. To remove any remaining air, carefully tilt the filter and tap on it.

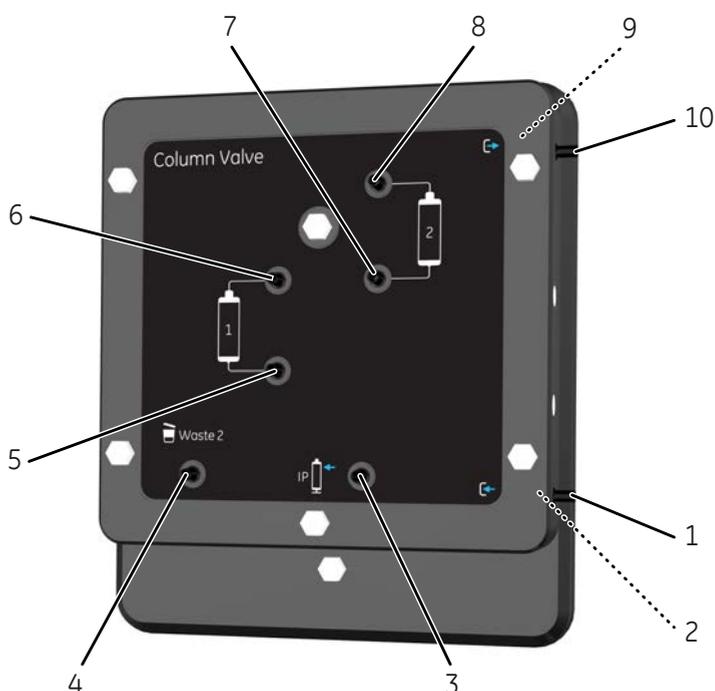
## 3.8 Column valve

### Introduction

This section describes the column valve module. The module is used to connect, control and monitor up to two columns, apply sample to a column using a Superloop, and to use Intelligent packing to automatically pack AxiChrom™ columns.

### Illustration

The illustration below shows the front of the column valve module.



| Part | Description                             |
|------|---|
| 1    | Inlet, from the previous module         |
| 2    | Pressure sensor just inside the inlet   |
| 3    | IP port for Intelligent packing and CIP |
| 4    | <b>Waste 2</b> port for waste           |

## 3 Description of modules

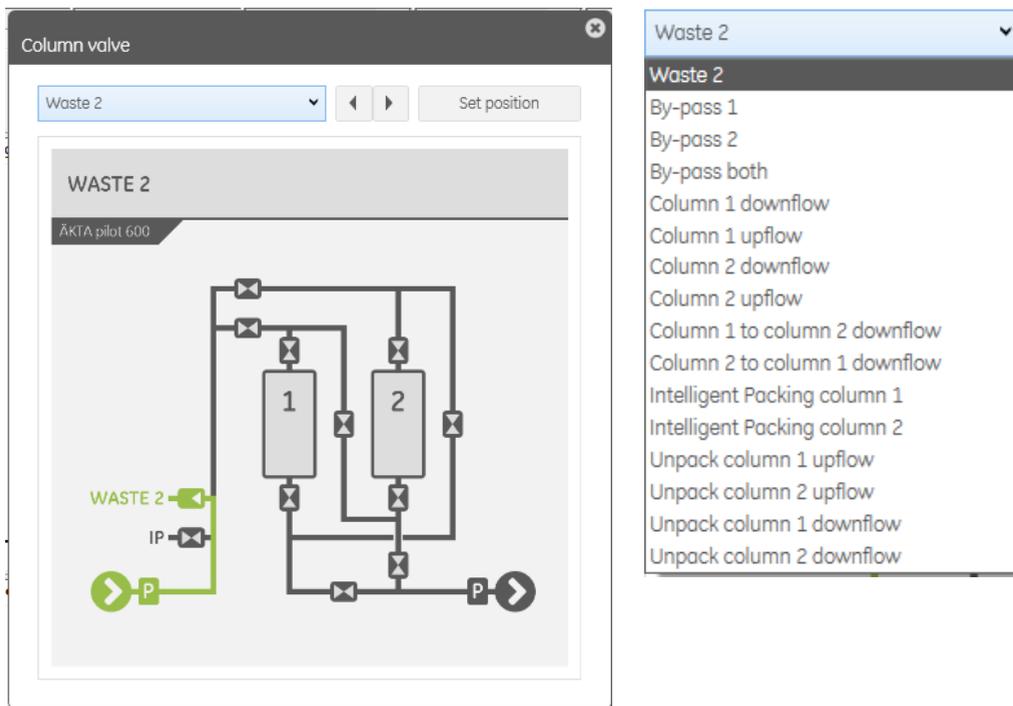
### 3.8 Column valve

| Part | Description  |
|------|--|
| 5    | Port for column position <b>1</b> , bottom of column |
| 6    | Port for column position <b>1</b> , top of column    |
| 7    | Port for column position <b>2</b> , bottom of column |
| 8    | Port for column position <b>2</b> , top of column    |
| 9    | Pressure sensor just inside the outlet               |
| 10   | Outlet, to the next module                           |

## Function

The column valve module consists of a number of membrane valves to control the flow path, and two integrated pressure sensors to monitor the pressure over the columns. The illustrations below show schematic flow paths inside the column valve module (left), and a list of options (right). This list is located in the **Column valve** dialog box accessible from the **Process Picture**.

The positions marked **P** in the left illustration below indicate the pre-column and post-column pressure sensors.



## Column valve options

See the list in the image above.

The **Waste 2** option is used, for example, during pump wash and air trap drain.

The **Bypass 1** and **Bypass 2** options represent different flow paths in the valve. When priming and cleaning the system, make sure that **Bypass 1** is selected first, followed by **Bypass 2**.

The **Bypass both** option is used during system wash.

The first four column run options make it possible to run a column separately with up or down flow.

The **Column 1 to column 2 downflow** and **Column 2 to column 1 downflow** options make it possible to run two columns in series, for example, to maximize column capacity. The two options can also be used to connect a Superloop, see [Section 4.1.3 Apply sample from a Superloop, on page 68](#).

The two packing options are used for Intelligent packing, see [Section 4.3 Intelligent packing, on page 77](#).

### 3 Description of modules

#### 3.8 Column valve

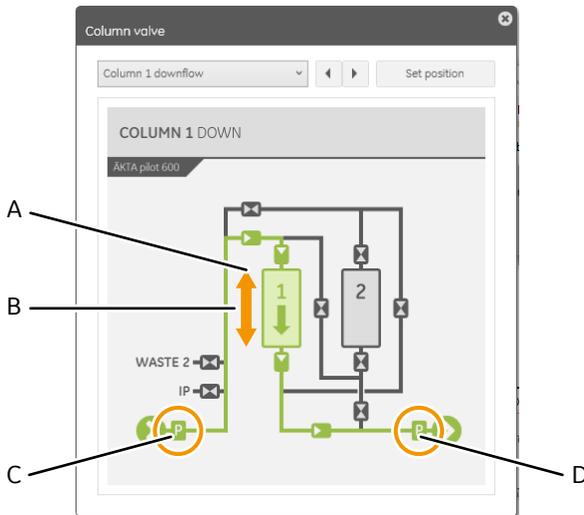
The four options for unpacking are used to simplify unpacking of columns. Before using an unpacking option, make sure to read each column instruction.

### Column pressure monitoring

The pre- and post-column pressure sensors are located at the inlet and outlet of the column valve.

The pressure drop between a sensor and a column is automatically compensated. This affects pressures at higher flow rates (>600 mL/min).

There are four pressure signals, see the image and table below.



| Part | Signal                      | Raw/compensated    | Purpose  |
|------|-----------------------------|--------------------|--|
| A    | Pre-column pressure         | Compensated signal | Used to protect the column hardware              |
| B    | Delta column pressure       | Compensated signal | Used to protect the column bed                   |
| C    | Pre-column sensor pressure  | Raw signal         | For reference                                    |
| D    | Post-column sensor pressure | Raw signal         | Used to protect the pH electrode and the UV cell |

## 3.9 Conductivity monitor

### Introduction

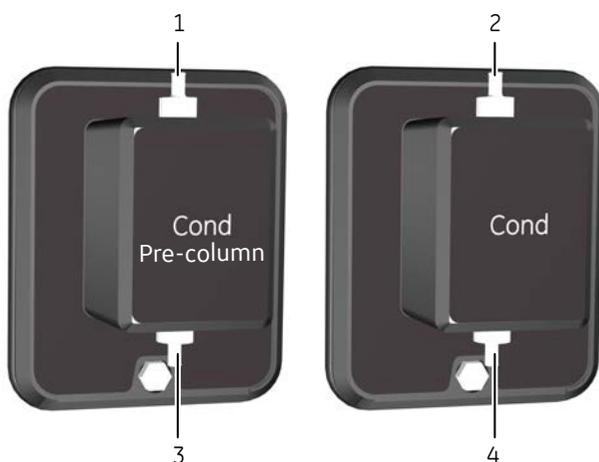
This section describes the conductivity monitor modules:

- Pre-column conductivity monitor (optional)
- Conductivity monitor (always installed in the system)

The purpose of these monitors is to continuously measure the conductivity of samples and buffers in the flow path.

### Illustration

The illustration below shows the front of the conductivity monitor modules. The two modules are physically identical.



| Part   | Description  |
|--------|--------------|
| 1 to 4 | Inlet/outlet |

### Function

The conductivity monitors can be used together with watch functions in UNICORN to control different steps in a chromatography or preparation run.

The individual cell constant for each monitor is calibrated on delivery but can be re-calibrated if needed, see [Section 5.4.2 Calibrate the conductivity monitor, on page 104](#).

## 3 Description of modules

### 3.9 Conductivity monitor

As variation in temperature influences conductivity readings, the conductivity flow cell is fitted with a temperature sensor that measures the temperature of the eluent. A temperature compensation factor is used to report the conductivity in relation to a set reference temperature. The measured temperature is also used for pH electrode compensation.

---

#### Pre-column conductivity monitor

If a conductivity monitor is installed in the flow path before the column valve, it can be used

- to follow conductivity during washing of pumps and valves before the column,
  - to monitor that the sample feed has suitable conductivity for the chromatography run,
  - to monitor that column equilibration is completed by comparing conductivity levels before and after the column,
  - to monitor that all sample has been wash out from the air trap by comparing conductivity of the sample and buffer used to finalize sample application (if using the air trap during sample application).
- 

#### Conductivity monitor (post-column)

The conductivity monitor after the column can be used

- to measure the conductivity of salt gradients during gradient elution,
  - to monitor washing of the system, with the column(s) by-passed,
  - to monitor that column equilibration is completed by comparing conductivity levels with column in-line and by-passed,
  - to follow peak positions relative to conductivity.
-

## 3.10 UV monitor

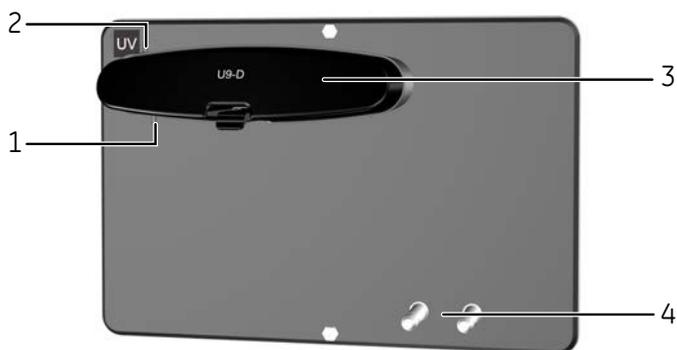
### Introduction

This section describes the UV monitor module. The UV monitor is used to detect proteins eluted from the column.

---

### Illustration

The illustration below shows the front of the UV monitor module.



| Part | Description                          |
|------|--------------------------------------|
| 1    | Inlet port                           |
| 2    | Outlet port                          |
| 3    | Detector unit                        |
| 4    | Mounting pins for the in-line filter |

### Function

The UV monitor is used to measure the UV/Vis absorbance at up to three wavelengths simultaneously, in the range 190 to 700 nm.

---

## Pressure protection

The UV monitor is sensitive to high pressure, but normally the pressure is low after the column. To protect sensitive monitors after the column, the post-column pressure has an alarm level of 0.8 MPa. This alarm is independent of other settings.

## UV cell size

The UV flow cell is available in two sizes:

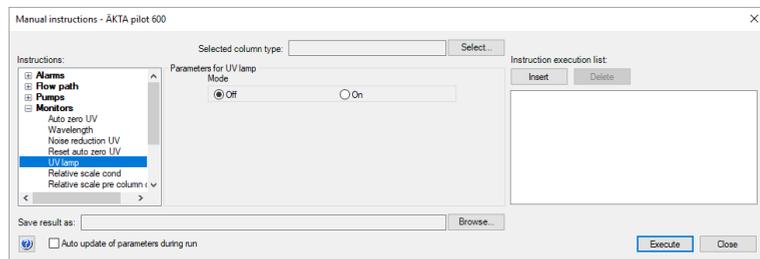
- 2 mm UV flow cell, can be used in all applications
- 5 mm UV flow cell, is recommended for peak volumes larger than 1000 mL

## Turning off the lamp

The UV lamp can be turned off manually if not needed during a run, for example during column or system conditioning or equilibration over long time, cleaning column or system, or sanitization. It will be turned on automatically for the next run.

Follow the steps below to turn off the lamp:

- | Step | Action   |
|------|--|
| 1    | Click <b>Manual:Execute manual instructions</b> in the <b>System Control</b> module to open the <b>Manual instructions</b> dialog box. |



- |   |   |
|---|---|
| 2 | Select <b>Monitors:UV lamp</b> , select <b>Off</b> and click <b>Execute</b> . |
|---|---|

## Cleaning

See [Section 5.3.2 Clean the UV flow cell, on page 98](#).

## 3.11 pH monitor (optional)

### Introduction

This section describes the optional pH monitor module. The module is used to continuously measure the pH of solutions used in the run.

### Illustration

The illustration below shows the front of the pH monitor module.



| Part | Description                      |
|------|----------------------------------|
| 1    | Dummy electrode                  |
| 2    | pH electrode in storage position |
| 3    | Inlet port                       |
| 4    | Manual injection port            |
| 5    | Outlet port                      |

## 3 Description of modules

### 3.11 pH monitor (optional)

#### Function

The pH module has two positions for the electrode: one position for measurements, calibration or cleaning in-line, and one for storage of the electrode. A dummy is placed in whichever position the electrode is not using. The module also has a manual injection port that facilitates the pH calibration. The pH electrode can both be calibrated before a run and cleaned after, without the need to open the flow path.

---

#### Pressure protection

The pH electrode is sensitive to high pressure, but normally the pressure is low after the column. To protect sensitive monitors after the column, the post-column pressure has an alarm level of 0.8 MPa. This alarm is independent of other settings.

---

#### Cleaning, storing, and calibration

For instructions on how to clean the pH electrode outside the flowpath, store or calibrate the pH monitor, see the *ÅKTA pilot 600 Operating Instructions*.

Before running a cleaning-in-place (CIP) method to clean the pH electrode in the flow path, make sure the electrode is compatible with the selected cleaning solution.

**Tip:** *Perform a system wash after the calibration to wash away the calibration buffer that was introduced into the flow path.*

---

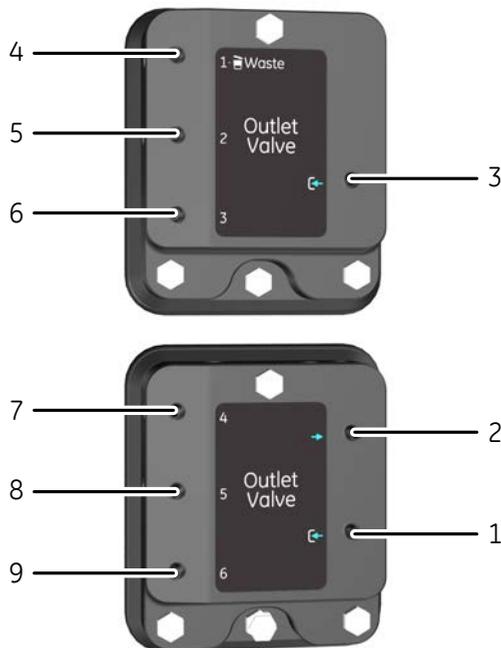
## 3.12 Outlet valves

### Introduction

This section describes the outlet valves, used for outlet fractionation and waste handling. It is optional to have more than one outlet valve.

### Illustration

The illustration below shows the front of two outlet valve modules, the mandatory module above and the first optional module below.



| Part | Description  |
|------|--|
| 1    | Inlet  |
| 2    | Outlet leading to the next outlet valve module (must be connected) |
| 3    | Inlet  |
| 4    | Outlet for waste solution  |

## 3 Description of modules

### 3.12 Outlet valves

| Part   | Description               |
|--------|---------------------------|
| 5 to 9 | Outlets for fractionation |

## Function

The outlet valve module contains a number of membrane valves to control the flow path during fractionation. For more information about fractionation, see [Section 4.2 Fractionation, on page 71](#). The first outlet port denoted **1-Waste** is used as waste outlet in all pre-defined methods.

Up to five outlet valves can be installed in the system, giving up to 15 outlets.

**Note:** *If additional outlet valve modules are installed, the outlets that lead to the next module must be connected to the inlet of next module inlet. Keep the tubing between the outlet valve modules as short as possible. For more information, see separate installation instructions for the outlet valve module.*

---

## Outlet tubing

The outlets are connected with SNAP connectors to outlet tubing with an inner diameter of 3.2 mm.

When running at low flow rates or collecting small fractions, it is recommended to use an adapter to be able to use outlet tubing with smaller diameter. If even smaller diameter is required, use suitable nipple and ferrule to further reduce the diameter.

**Note:** *Reduced tubing diameter increases the system pressure.*

---

# 4 Operation

## About this chapter

Basic instructions on how to prepare and perform a run are given in the *ÄKTA pilot 600 Operating Instructions*. This chapter considers additional topics related to operation of the instrument.

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## In this chapter

This chapter contains the following sections:

| Section                                   | See page |
|---|----------|
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| 4.2 Fractionation                         | 71       |
| 4.3 Intelligent packing                   | 77       |
| 4.4 Dual pump flow                        | 81       |
| 4.5 Pressure control                      | 84       |
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## 4.1 Sample application

### About this section

A basic description of how to apply a sample to the column in a ÄKTA pilot 600 system is given in *ÄKTA pilot 600 Operating Instructions*. This section gives more in-depth information on sample application techniques.

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### In this section

This section contains the following subsections:

| Section                                | See page |
|--|----------|
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| 4.1.2 Apply sample from a system inlet | 63       |
| 4.1.3 Apply sample from a Superloop    | 68       |

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## 4.1.1 Background considerations

### Chromatography techniques

Different chromatography techniques make different demands on sample application, as summarized in the table below. These considerations can influence the choice of sample application technique.

More information about chromatography techniques may be found in the *Life Science Handbooks*, available for download from the **Support** pages on [www.gelifesciences.com](http://www.gelifesciences.com).

| Technique  | Demands   |
|--|---|
| Size exclusion chromatography (SEC)  | A sharply defined sample band with constant sample concentration throughout. Any broadening of the sample band will be reflected in broadening of the elution peaks.<br><br>The sample volume applied to the column should ideally not be more than 10% of the column volume. Precise requirements will depend on the separation being performed. |
| Adsorption chromatography, (e.g., affinity or ion exchange chromatography) | Sample is concentrated by adsorption on to the chromatography resin during sample application. The volume and concentration of sample is not critical, provided that the capacity of the column is not exceeded.  |

### Sample application options

Samples may be applied to the column from a system inlet (**A** inlet) or from a Superloop 150 SNAP mounted on the column valve.

| Option                           | When?  | Description   |
|----------------------------------|--|---|
| Apply sample from a system inlet | Recommended for sample volumes larger than 50 mL and for column performance tests where sample is readily available. | The sample is pumped onto the column from one of the A inlets using system pump A. The sample passes through the system pump, flow restrictor and any other modules before the column valve in the flow path.<br><br>The sample volume that can be applied from a system inlet is in principle unlimited. |

## 4 Operation

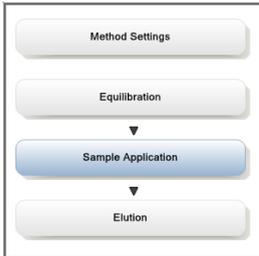
### 4.1 Sample application

#### 4.1.1 Background considerations

| Option                        | When?   | Description   |
|-------------------------------|---|---|
| Apply sample from a Superloop | Recommended for sample volumes in the range 10 to 150 mL. | <p>A Superloop (sold separately) is manually filled with sample and connected to one of the two column positions. The sample is injected onto the column by directing the system flow via the Superloop, and does not pass through any other modules in the system.</p> <p>Sample volumes applied from a Superloop are limited to the volume of the Superloop (150 mL).</p> |

## Sample application phase

A **Sample Application** phase is included in all predefined chromatography methods. The sample will be automatically applied to the column when the method is run. Selections in the **Sample Application** phase determine how the sample will be applied. The illustration below shows the phase in a method outline.



The following sections describe how to use the settings in the **Phase Properties** for the **Sample Application** phase. For detailed information on each setting, press the **F1** key to display the UNICORN help.

---

## 4.1.2 Apply sample from a system inlet

### Introduction

Samples may be applied from a system inlet using the system pump **A**. Up to three Inlet valve **A** modules are supported, giving up to nine inlets, labeled **A1** to **A9**. Inlet **A1** is normally used for buffer: other inlets may be used for sample or different buffers as required.

**Note:** *Sample application from **B** inlets is not recommended.*

There are up to three stages in sample application from a sample inlet.

| Stage | Description   |
|-------|---|
| 1     | <p>Prime the inlet tubing with sample. This will fill the inlet tubing up to the inlet valve with sample, giving more precise control over the amount of sample applied to the column.</p> <p>The inlet tubing may be primed automatically (see <a href="#">Sample Application phase properties, on page 63</a>) or manually (see <a href="#">Priming the inlet tubing manually, on page 66</a>).</p> |
| 2     | <p>Apply the sample according to the phase properties in the <b>Sample Application</b> phase.</p>   |
| 3     | <p>Finalize the sample application (if selected in the <b>Sample Application</b> phase). This will chase the sample remaining in the flow path on to the column, using the buffer inlet as specified in <b>Method Settings</b>.</p>   |

### When to use

Sample application from a system inlet is recommended for sample volumes larger than 50 mL.

### Sample Application phase properties

To apply sample from a system inlet, select **Inject sample directly onto column** in the **Phase Properties** for the **Sample Application** phase. Set the parameters as described in the table below. For details of settings in the **Phase Properties** that are not described here, see the UNICORN online help.

## 4 Operation

### 4.1 Sample application

#### 4.1.2 Apply sample from a system inlet

**Phase Properties** | **Text Instructions** | **IT**

### Sample Application

Use the same flow rate as in Method Settings  
Flow rate: 20.0 ml/min [0.0 - 600.0]

**Inject sample directly onto column.**  Inject sample from Superloop, manual load

Sample inlet: A2

Inject fixed sample volume: 0.00 ml

Inject all sample using ext. air sensor 1

Enable air sensor after: 25.00 ml

Set maximum volume to: 1000.00 ml

Prime sample inlet with: 30.00 ml

Pump wash after priming

Finalize sample injection: 50.00 ml

Air Trap valve: By-pass

Interrupt sample application at UV: 0.0 mAU [-6000.0 - 6000.0]

Fractionation settings

Continue ongoing

Outlet valve fractionation

No fractionation

Fractionation type: Fixed volume fractionation

Fractionation start position: Out 2

Fixed fractionation volume: 500 ml [20 - 1000000]

Peak fractionation volume: 10000 ml [20 - 1000000]

Stop fractionation in the end of this phase

Buttons: Advanced Settings..., Peak Frac Settings...

| Setting                                  | Description   |
|--|---|
| <b><i>Inject fixed sample volume</i></b> | Use this option to apply a precise sample volume. Use the following additional settings: <ul style="list-style-type: none"><li>• Select <b><i>Prime the sample inlet with</i></b> to ensure reproducible sample application.</li><li>• For adsorption chromatography, select <b><i>Finalize sample injection</i></b> to make sure that all sample is applied to the column before the next phase starts. For size exclusion chromatography, it is not necessary to finalize the sample injection.</li></ul> |

| Setting   | Description  |
|---|--|
| <b><i>Inject all sample using ext. air sensor 1</i></b> | <p>Use this option to apply all available sample. Sample application will stop when the External air sensor 1 detects air in the sample inlet tubing. See <a href="#">Section 3.1 External air sensors (optional)</a>, on page 30 for more information about external air sensors.</p> <p><b>Note:</b><br/> <i>The option in <b>Phase Properties</b> only supports External air sensor 1. Additional air sensors can be supported by editing the text instruction in the <b>Sample Application</b> phase.</i></p> <p>When this option is selected, the following options are available</p> <ul style="list-style-type: none"> <li>• <b>Enable air sensor after</b> enables the air sensor when the specified volume has been pumped. Use this option if the sample inlet tubing has not been primed with sample or buffer and may contain air. Enter a volume larger than the volume of the inlet tubing.</li> <li>• <b>Set maximum volume to</b> sets a maximum limit on the amount of sample applied.</li> </ul> |
| <b><i>Prime sample inlet with</i></b>                   | <p>This option fills the inlet tubing with sample by pumping the specified volume of sample. Enter a volume larger than the volume of the inlet tubing.</p> <p>When this option is selected, select <b>Pump wash after priming</b>. This will flush the flow path up to the column valve with buffer.</p> <p><b>Note:</b><br/> <i>The flow path can be primed manually if desired, see <a href="#">Priming the inlet tubing manually</a>, on page 66.</i></p>  |
| <b><i>Finalize sample injection</i></b>                 | <p>This option pumps the specified volume of buffer directly after the sample. Buffer is taken from the buffer inlet specified in <b>Method Settings</b>. The recommended volume is 5 to 10 times larger than the flow path volume up to the column valve. If the Air Trap valve is set to <b>In-line</b>, the minimum recommended volume is 300 mL.</p>   |
| <b><i>Air trap valve</i></b>                            | <p>Set the air trap valve to <b>In-line</b> if there is a risk that air may enter the inlet tubing. This applies, for example, if the inlet tubing has not been primed, or if an external air sensor close to the inlet valve port is used to minimize sample loss.</p>  |

| Setting                                   | Description   |
|---|---|
| <b>Interrupt sample application at UV</b> | If this option is selected, sample application will be interrupted when the UV signal reaches the specified level. This feature is typically used in adsorption chromatography, to detect sample in the flowthrough.  |
| <b>Fractionation settings</b>             | Settings specified here determine how flowthrough during sample application will be collected. See <a href="#">Section 4.2 Fractionation, on page 71</a> for details of fractionation settings. Fractionation during sample application is generally relevant only for adsorption chromatography. |

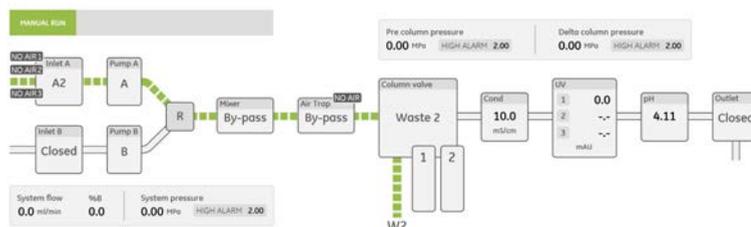
## Priming the inlet tubing manually

The sample inlet tubing can be primed manually if desired, using manual instructions or the **Process Picture**.

Follow the steps below to prime one sample inlet tubing manually using the **Process Picture**.

| Step | Action |
|------|--------|
|------|--------|

- |   |  |
|---|--|
| 1 | <p>Set the following modules:</p> <ul style="list-style-type: none"> <li>• <b>Inlet A</b> to inlet used for sample (for example, <b>A2</b>).</li> <li>• Air trap to <b>By-pass</b>.</li> <li>• Mixer (if installed) to <b>By-pass</b>.</li> <li>• Column valve to <b>Waste 2</b>.</li> </ul> |
|---|--|



**Result:** The flow path is opened as shown.

| Step | Action   |
|------|--|
| 2    | Insert the end of the sample inlet tubing into the sample container.<br><br><b>Tip:</b><br><i>If the tubing is filled with buffer, introduce a small air bubble into the sample tubing between the buffer and the sample. This will mark the progress of the sample front along the flow path tubing.</i>  |
| 3    | Set a suitable pump flow rate, for example 100 mL/min. Follow the sample front in the tubing. (The sample front may be difficult to follow if the flow rate is too high.)  |
| 4    | When the sample front has passed through the inlet valve, click <b>Pause</b>  .   |
| 5    | Switch the inlet valve to buffer inlet.  |
| 6    | Click <b>Continue</b>  , change the flow rate to maximum (600 mL/min), and wait until all air bubbles have been removed through the <b>Waste 2</b> outlet on the column valve.<br><br><i>Result:</i> The flow path contains sample up to the inlet valve and buffer from the inlet valve to the column valve. |
| 7    | Click <b>End</b>  .   |

## 4 Operation

### 4.1 Sample application

#### 4.1.3 Apply sample from a Superloop

## 4.1.3 Apply sample from a Superloop

### Introduction

Samples may be applied to the column from a Superloop, connected to the column valve directly before the column. Superloop 150 SNAP (capacity 150 mL) is fitted with SNAP connections.

Instructions for handling the Superloop, including filling with sample and cleaning, are given in the *Superloop 150 SNAP Instructions*, supplied with the Superloop.



#### CAUTION

Do not use a Superloop without its protective jacket. The Superloop may crack if exposed to overpressure.

### When to use

Use of a Superloop is recommended for:

- sample volumes in the range 10 to 150 mL
- Size exclusion chromatography and desalting (to minimize band broadening)

**Note:** *Sample application from a Superloop can only be used with downflow through the column.*

### Mounting the Superloop

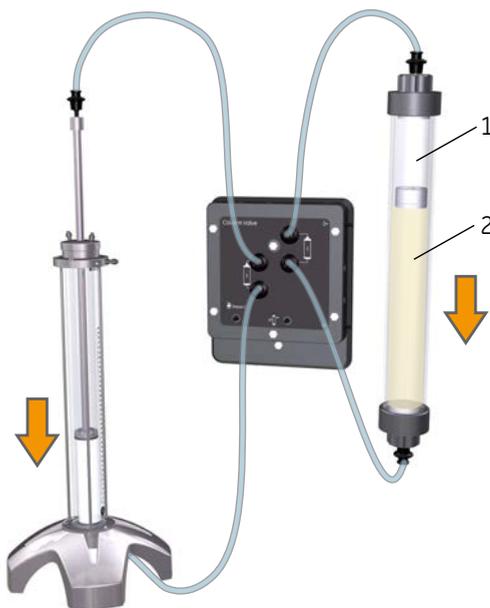
The Superloop should be mounted close to the column valve to keep connecting tubing short. Mounting in an ÄKTA accessory holder on a module front panel with rails (see [Module front panel with rails, on page 25](#)) or an Extension Stand (see [Extension stand for columns and accessories, on page 24](#)) is recommended.

Follow the steps below to mount and connect the Superloop.

| Step | Action  |
|------|---|
| 1    | Fill the Superloop eluent chamber with buffer and sample chamber with sample (see the separate <i>Superloop 150 SNAP Instructions</i> )       |
| 2    | Mount the Superloop on or close to the ÄKTA pilot 600 instrument, with the sample chamber downwards.  |
| 3    | Connect the Superloop eluent inlet (top connection) using SNAP connectors to the top port for the unused column position on the column valve. |

| Step | Action   |
|------|--|
| 4    | Connect the Superloop sample outlet (bottom connection) using SNAP connectors to the bottom port for the unused column position on the column valve. |

The illustration below shows a column mounted as **Column 1** and a Superloop mounted as **Column 2**. The arrows indicate flow direction.



| Part | Description    |
|------|----------------|
| 1    | Eluent chamber |
| 2    | Sample chamber |

## Sample Application phase properties

To apply sample from a Superloop, select **Inject sample from Superloop, manual load** in the **Phase Properties** for the **Sample Application** phase. Set the parameters as described in the table below. For details of settings in the **Phase Properties** that are not described here, see the UNICORN online help.

## 4 Operation

### 4.1 Sample application

#### 4.1.3 Apply sample from a Superloop

**Phase Properties** | Text Instructions | T

### Sample Application

Use the same flow rate as in Method Settings  
Flow rate: 20.0 ml/min [0.0 - 600.0]

Inject sample directly onto column |  **Inject sample from Superloop, manual load**

Loop position: Column 2  
Empty loop with: 10.00 ml

Use the same inlets as in Method Settings  
Inlet A: A1  
Inlet B: B1 0.0 % B [0.0 - 100.0]

Fill the system with the selected buffer  
Wash flow path to: Waste/Out1

Interrupt sample application at UV: 0.0 mAU [-6000.0 - 6000.0]

Fractionation settings

Continue ongoing  
 Outlet valve fractionation  
 No fractionation

Fractionation type: Fixed volume fractionation  
Fractionation start position: Out 2  
Fixed fractionation volume: 500 ml [20 - 1000000]  
Peak fractionation volume: 10000 ml [20 - 1000000]  
 Stop fractionation in the end of this phase

Advanced Settings...  
Peak Frac Settings...

| Setting                                       | Description   |
|---|---|
| <b>Loop position</b>                          | Shows the column valve position used by the Superloop. This setting can only be changed by changing the <b>Column position</b> in <b>Method Settings</b> .  |
| <b>Empty loop with</b>                        | The specified volume will be pumped through the Superloop on to the column. If it is important that all of the sample is applied to the column, enter a volume approximately 10 mL greater than the volume of sample in the Superloop.  |
| <b>Use the same inlets as Method Settings</b> | This option controls the source of the buffer pumped into the Superloop eluent chamber during sample application.   |
| <b>Fill the system with selected buffer</b>   | Select this option to fill the system flow path with the buffer before applying the sample to the column.   |
| <b>Fractionation settings</b>                 | Settings specified here determine how flowthrough during sample application will be collected. See <a href="#">Section 4.2 Fractionation, on page 71</a> for details of fractionation settings. Fractionation during sample application is generally relevant only for adsorption chromatography. |

## 4.2 Fractionation

### Introduction

Fractionation in ÄKTA pilot 600 system is performed using the outlet valves. Up to five outlet valves can be installed in the system, giving up to 15 outlets. Outlet **1-Waste** is used by default as a waste outlet.

Fractionation is set in the **Phase Properties** of the UNICORN method, and can be used in the following phases:

- **Column Wash**
- **Sample Application**
- **Conditional Fractionation**
- **Elution**

### Fractionation settings

Fractionation settings determine the type of fractionation.

Whether and how fractionation will be performed is determined by the three options to the left of the panel:

| Setting                           | Description  |
|-----------------------------------|--|
| <b>Continue ongoing</b>           | Continues fractionation using the settings from the previous phase.<br><br><b>Note:</b><br><i>If <b>Stop fractionation at the end of this phase</b> is checked in the previous phase, fractionation will not continue. If there is no previous phase with fractionation settings, fractionation will not be performed.</i> |
| <b>Outlet valve fractionation</b> | Performs fractionation according to the specified type and settings.   |
| <b>No fractionation</b>           | Outflow will be directed to outlet <b>1-Waste</b> .  |

The remaining settings apply to **Outlet valve fractionation**:

| Setting  | Description  |
|--|--|
| <b>Fractionation type</b>                          | Choose the fractionation type. See below for details.  |
| <b>Fractionation start position</b>                | Choose the outlet position for the first fraction. Subsequent fractions will be directed to the next outlet position in numerical order. Outflow after the last outlet position (determined by the settings in <b>Advanced Settings</b> ) will be directed to outlet <b>1-Waste</b> .  |
| <b>Fixed fractionation volume</b>                  | Applies to fractionation types with fixed volume fractions. Enter the fraction volume.   |
| <b>Peak fractionation volume</b>                   | Applies to fractionation types with peak fractionation. Specify the peak detection conditions in <b>Peak Frac Settings</b> . If the collected volume for one fraction reaches the value set in <b>Peak fractionation volume</b> , outflow will be directed to the next fraction. Set a large value for this parameter to avoid splitting peaks into several fractions. |
| <b>Stop fractionation at the end of this phase</b> | Fractionation will stop at the end of the phase, even if fractionation in the next phase is set to <b>Continue ongoing</b> .<br><br><b>Note:</b><br><i>This setting is not available in <b>Conditional Fractionation</b> phases.</i>   |

## Fractionation types

The following fractionation types can be used for **Outlet valve fractionation**:

| Fractionation type                         | Description  |
|--|--|
| <b>Fixed volume fractionation</b>          | Fixed volume fractions will be collected.  |
| <b>Peak fractionation</b>                  | Fractions will be collected according to the peak detection conditions in <b>Peak Frac Settings</b> . When no peak is detected, flow is directed to Outlet <b>1-Waste</b> .  |
| <b>Fixed volume and peak fractionation</b> | Combines fixed volume and peak fractionation.<br>Fixed volume fractionation applies as long as a peak is not detected. Peaks are collected as separate fractions according to the peak detection conditions in <b>Peak Frac Settings</b> . |

| Fractionation type  | Description   |
|---------------------|---|
| <b>Fixed outlet</b> | <p>Outflow will be directed to the specified outlet position until fractionation ends.</p> <p><b>Note:</b><br/><i>Make sure that <b>Stop fractionation at the end of this phase</b> is checked in the current or a subsequent phase as appropriate, to avoid directing all subsequent outflow to the specified outlet position.</i></p> |

## Advanced settings

**Advanced settings** determines the maximum number of fractions that will be collected. Any outflow after the maximum number of fractions has been collected will be directed to Outlet **1-Waste**.



Check **Use all available outlets** to use all the outlet positions installed on the instrument except for Outlet **1-Waste**.

Alternatively, remove the checkmark and enter the maximum number of fractions.

## Peak fractionation settings

**Peak Frac Settings** determine the conditions for peak detection.

Set the parameters as follows:

| Fractionation type                  | Description   |
|-------------------------------------|---|
| <b>Signal</b>                       | Choose the signal to be used for peak detection.  |
| <b>Mode</b>                         | Choose the detection mode: <ul style="list-style-type: none"> <li>• <b>Level</b> refers to the magnitude of the signal.</li> <li>• <b>Slope</b> refers to the value of the signal slope of the signal.</li> <li>• <b>Level and slope</b> triggers fractionation when both the magnitude and the slope reach the specified value.</li> <li>• <b>Level or slope</b> triggers fractionation when either the magnitude or the slope reach the specified value.</li> </ul> |
| <b>Start level/<br/>Start slope</b> | Set the threshold value for detection of peak start. The peak start is detected when the signal exceeds the specified value.  |
| <b>End level/<br/>End slope</b>     | Set the threshold values for detection of peak end. The peak end is detected when the signal falls below the specified value.<br><br><b>Note:</b><br><i>The value for <b>End slope</b> is implicitly negative. The minus sign is not shown. The peak end is detected when the slope value is negative and rises arithmetically above the specified value.</i>   |
| <b>Minimum peak width</b>           | Enter the minimum peak width value in minutes. Peak end will not apply until the peak has reached the minimum width.  |

## Conditional fractionation

The **Conditional Fractionation** phase sets conditions for fractionation start and stop that will apply in subsequent phase(s). The conditions will apply until fractionation is stopped, either because the **Stop Condition** is met or the **Stop fractionation at the end of this phase** is checked in a subsequent phase.

**Note:** **Conditional Fractionation** is not included in predefined methods but can be added if required from the phase library.

The screenshot shows the 'Phase Properties' dialog box for a 'Conditional Fractionation' phase. It has two tabs: 'Phase Properties' (selected) and 'Text Instructions'. The 'Conditional Fractionation' section contains two checked checkboxes: 'Start Condition' and 'Stop Condition'. For the 'Start Condition', the 'Signal' is 'Conductivity', the 'Mode' is 'Greater than', and the 'Value' is '20.00 mS/cm [0.00 - 1000.00]'. For the 'Stop Condition', the 'Signal' is 'Conductivity', the 'Mode' is 'Less than', and the 'Value' is '10.00 mS/cm [0.00 - 1000.00]'. Below this is the 'Fractionation settings' section, which includes 'Fractionation type' set to 'Peak fractionation', 'Peak fractionation start position' set to 'Out 2', 'Fixed fractionation volume' set to '500 ml [20 - 1000000]', and 'Peak fractionation volume' set to '10000 ml [20 - 1000000]'. There are two buttons on the right: 'Advanced Settings...' and 'Peak Frac Settings...'.

**Conditional Fractionation** can be used to set conditions for fractionation start and stop based on one signal (e.g., conductivity) while performing peak fractionation based on another signal (e.g., UV absorbance).

To use **Conditional Fractionation**, insert the phase before the one where the conditions are required. Set the fractionation settings in the **Conditional Fractionation** phase, and set the next phase to **Continue ongoing**.

**Note:** To stop conditional fractionation, include a **Fractionation Stop** phase from the phase library.

## Fractionation in practice

When fractionation is complete or if you re-use outlet positions, empty the outlet tubing by releasing the SNAP connectors on the outlets and allowing the tubing contents to drain into the fraction containers.

For unattended fractionation, the maximum number of fractions that can be collected is 14 (using five installed outlet valves). Plan your fractionation settings carefully to avoid creating more than the maximum number of fractions. Use **Conditional Fractionation** and **Peak fractionation** to optimize the number of fractions.

If the number of fractions exceeds the number of available outlets, it is possible to re-use outlet positions for fractionation in different phases (for example, set **Fractionation start position** to **Out 2** in all phases that use fractionation). This requires that you change the outlet tubing and fraction containers on re-used positions between phases. It may be advisable include a **Miscellaneous** phase to set a **Pause timer** with a message, to remind you to change the containers.

---

## 4.3 Intelligent packing

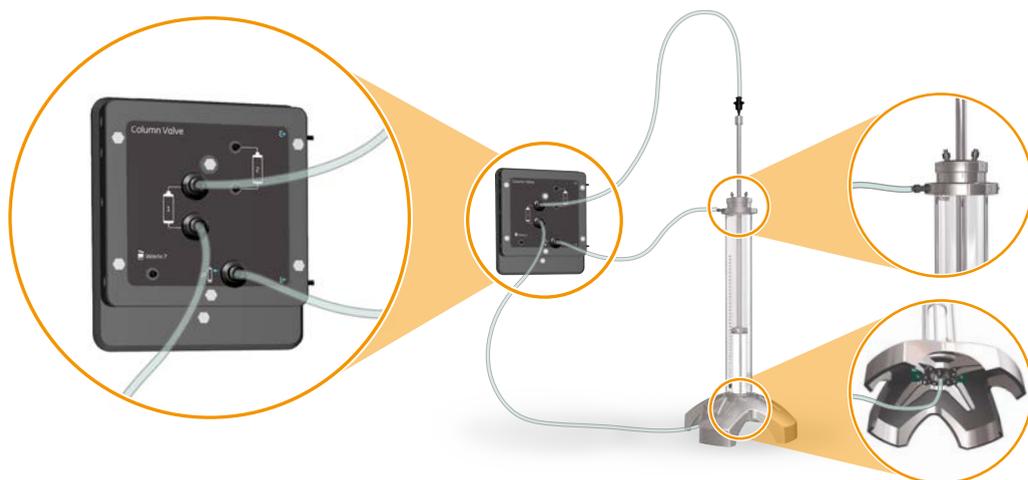
### Introduction

The ÄKTA pilot 600 system can pack AxiChrom columns with a pre-defined **Intelligent Packing** method that uses the **IP** port. When column hardware, bed support, resin and target bed height has been entered in the method, default values will automatically be set in the method to provide a complete method.

In this section, you will get general information for how to handle the packing procedures. For more information, read about the **Intelligent Packing** phase in the UNICORN help and documentation.

### Illustration

The illustration below shows an AxiChrom 50 column connected to the **IP** port and the column position **1** ports.



### Prepare the packing

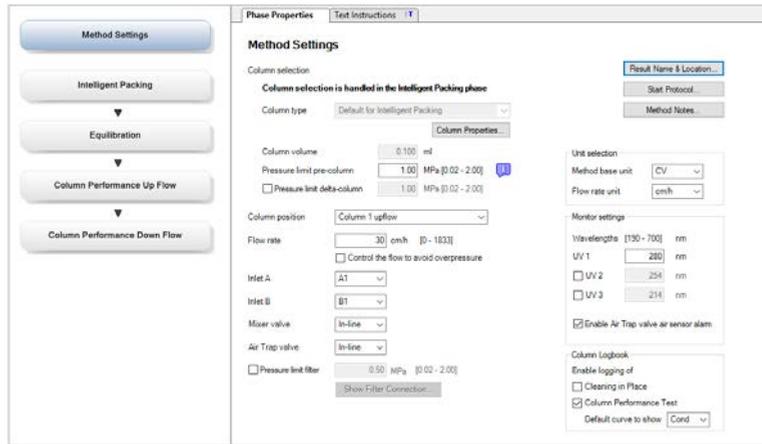
Follow the steps below to create your packing method.

| Step | Action |
|------|--------|
|------|--------|

- |   |  |
|---|--|
| 1 | In the <b>File</b> menu of the <b>Method Editor</b> module, select <b>New Method</b> . |
|---|--|

**Step**      **Action**

- In the **New Method** dialog box, select **Intelligent Packing** from the **Predefined Method** list. Click **OK**.  
*Result:* A new method based on the predefined **Intelligent Packing** method is created and opened.
- Verify the method settings. Make sure that **Column position** and **Default curve to show** are correct.



**Tip:**

For information about the settings, click on the **Phase Properties** tab and then press **F1**.

**Note:**

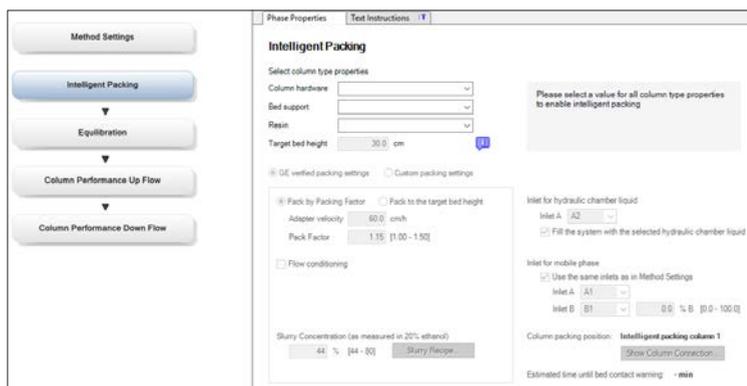
Text editing in **Method Settings** or the **Intelligent Packing** phase of an intelligent packing method is not recommended.

- Click on the **Intelligent Packing** phase.

**Step Action**

5 Set the following parameters:

- **Column hardware**
- **Bed support**
- **Resin**
- **Target bed height**



*Result:* Default values are automatically set to provide a complete method.

6 Select **GE verified packing settings** to get a packing method for GE resins verified by GE. If you use a custom resin, the only option is **Custom packing settings**.

**Tip:**

When **GE verified packing settings** is selected, you can enter the slurry concentration and click **Slurry Recipe** to get a slurry recipe calculated to give your target bed height.

7 Verify the inlets for hydraulic chamber liquid and mobile phase.

**Tip:**

Click **Show Column Connection** to find an illustration of the connections to the column, and read the time estimation.

8 Verify the phase properties for the remaining phases.

9 Save the method.

## Perform the packing

Follow the steps below to perform the packing. Use the UNICORN method created above.

| Step | Action |
|------|--------|
|------|--------|

- |   |   |
|---|---|
| 1 | Connect position <b>1</b> or <b>2</b> according to the method on the <b>Column valve</b> module to the top (on the adaptor) and bottom of the AxiChrom column. Connect the <b>IP</b> port to the horizontal nipple on the column top (not on the adaptor). See <a href="#">Illustration, on page 77</a> . Note that the illustration shows connections to position <b>1</b> . |
|---|---|



### WARNING

Before connecting a column, read the instructions for use of the column.

**Tip:**

In the **Intelligent Packing** phase of your packing method, click on **Show Column Connection** to find an illustration of the connections.

Show Column Connection...

- |   |   |
|---|---|
| 2 | Run your packing method in the <b>System Control</b> module, follow the instructions in dialog boxes on the screen. |
|---|---|

**Note:**

Make sure that you press **Continue** when the adapter meets the consolidated bed surface. If **Continue** is not pressed in time, the resin can be damaged, and the delta column pressure alarm pauses the packing method.

- |   |   |
|---|---|
| 3 | Evaluate and save the packing of the column in the <b>Evaluation</b> module, see the <b>Getting started</b> page in the <b>Evaluation</b> module, or the <i>UNICORN Evaluation Manual</i> . |
|---|---|

## 4.4 Dual pump flow

### Introduction

It is possible to use both pumps to obtain a flow rate in the range 600 to 1200 mL/min. This function is called **Dual pump flow**.

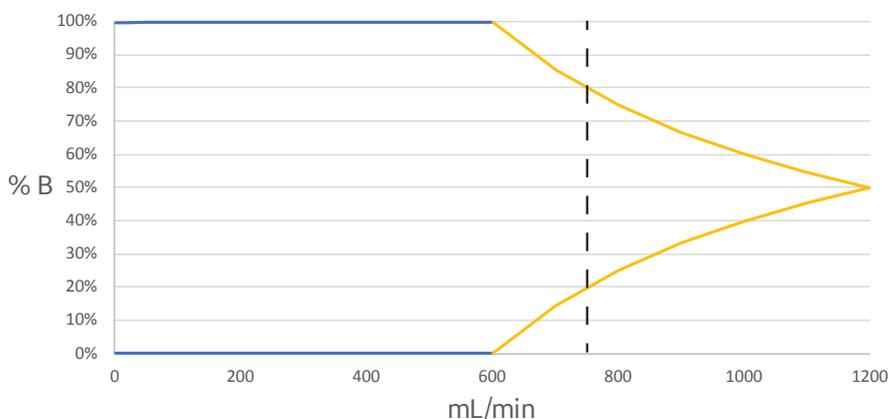
### Isocratic applications

When **Dual pump flow** is used during isocratic applications, the flow rate can be set to up to 1200 mL/min.

**Note:** *In isocratic applications the same buffer is used for pump A and B. This can be achieved by placing the A and B inlets in the same buffer container.*

### Gradient applications

It is possible to use **Dual pump flow** to make gradients above 600 mL/min. The illustration below shows the gradient range for ÄKTA pilot 600. Above 600 mL/min (yellow part of the curve), the gradient range is limited due to each pump maximum flow rate of 600 mL/min. For example, at 720 mL/min (dashed line in the illustration) a gradient can be run between 20% and 80% B.



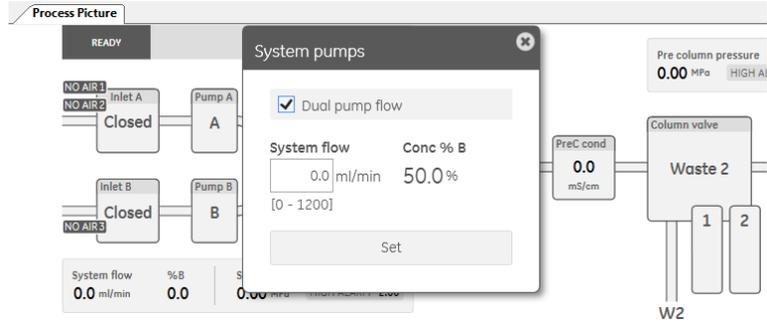
**Note:** *Gradient applications require that the inlets for pumps A and B are placed in different buffers.*

## Using the Process Picture

Follow the steps below to start dual pump flow using the **Process Picture**.

### Step Action

- 1 Click on one of the pumps to open the **System pumps** dialog box.



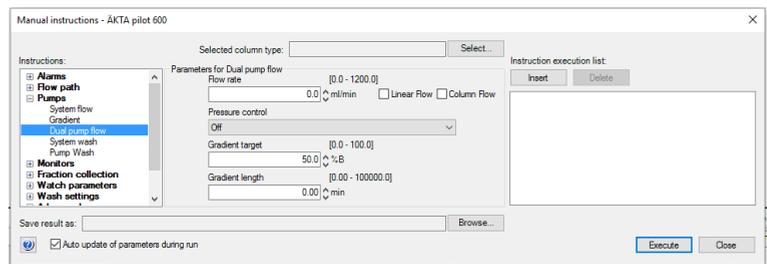
- 2 Tick the **Dual pump flow** check box, enter **System flow** value and click **Set**.

## Using dual pump flow manually

Follow the steps below to start dual pump flow using manual instructions.

### Step Action

- 1 Select **System Control:Manual:Execute manual instructions** to open the **Manual instructions** dialog box.

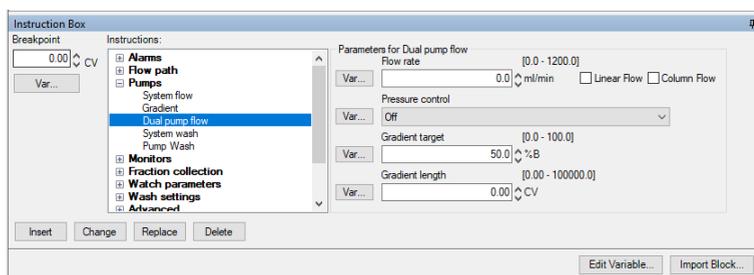


- 2 Select **Pumps: Dual pump flow**, enter your parameters and click **Execute**.

## Using dual pump flow in a method

Follow the steps below to use dual pump flow in a UNICORN method. These steps must be performed for each phase that uses dual pump flow.

- | Step | Action  |
|------|---|
| 1    | Open the <b>Text Instructions</b> tab for the phase.  |
| 2    | Select the <b>System flow</b> instruction.  |
| 3    | In the <b>Instruction Box</b> pane of the <b>Text Instructions</b> tab, select <b>Pumps: Dual pump flow</b> . |



- 4 Enter your parameters. Verify in the graph shown in [Gradient applications, on page 81](#) that the combination of %B and flow is compatible, and click **Change**.

**Tip:**

When **Pumps: Dual pump flow** is selected, press the **F1** key to access information about the parameters.

## 4.5 Pressure control

### Introduction

Pressure control is an optional feature that may be used to regulate the flow rate in order to avoid the risk of method stop due to pressure alarms. If the pressure approaches the limit, for example if the sample has higher viscosity than the buffer, the flow rate is automatically lowered. Pressure control uses PI (proportional-integral) regulation.

This section describes how to set the **Pressure Control** parameters and activate pressure control. Refer to the UNICORN online help and documentation for more detailed recommendations.

---

### When to adjust pressure control

Pressure control requirements in the ÄKTA pilot 600 system depend on whether the Air trap is bypassed or in-line as well as on flow rate. Pressure control is most important at high flow rates when the Air trap is in-line and contains a significant volume of air.

Pressure control parameters may need to be adjusted in the following cases:

- When the flow rate increases too slowly to the set value.
- When the flow rate increases too rapidly, causing overpressure in the system.

Suitable values for the parameters may need to be determined empirically.



#### NOTICE

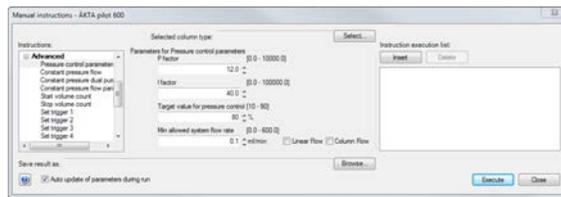
Do not change pressure control parameters if you are not familiar with the principles of PI regulation.

## Optimize pressure control settings

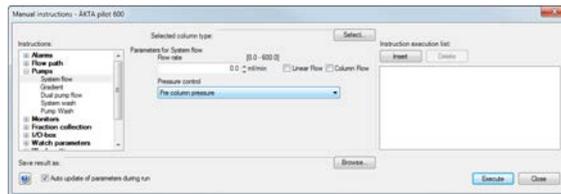
To optimize pressure control parameters, change the settings manually and test the new settings with a manual run. Follow the steps below:

### Step Action

- 1 Open the **Advanced:Pressure control parameters** section of **Manual instructions** in **System Control**.



- 2 Adjust the parameters as required.
- 3 Activate pressure control and select the appropriate **Pressure control** parameter in the **Pumps:System Flow** section of **Manual instructions**.



- 4 Run **Pumps:System flow** at a suitable flow rate to test the effect of your adjustments.
- 5 When you are satisfied, update the pressure control parameters in **System Settings** so that the optimized settings will apply automatically in the future.

## Set pressure control parameters

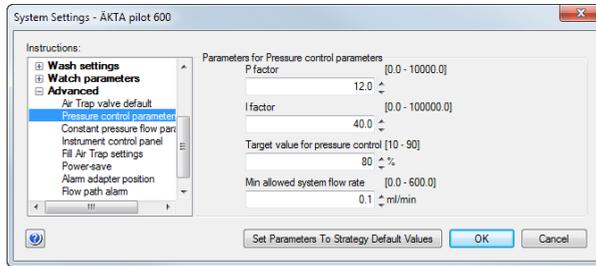
Follow the steps below to change the pressure control parameters in **System:Settings**. Settings changed in this way will apply as default for both manual and method runs.

### Step Action

- 1 Open **System:Settings** in the UNICORN **System Control** module.

**Step**      **Action**

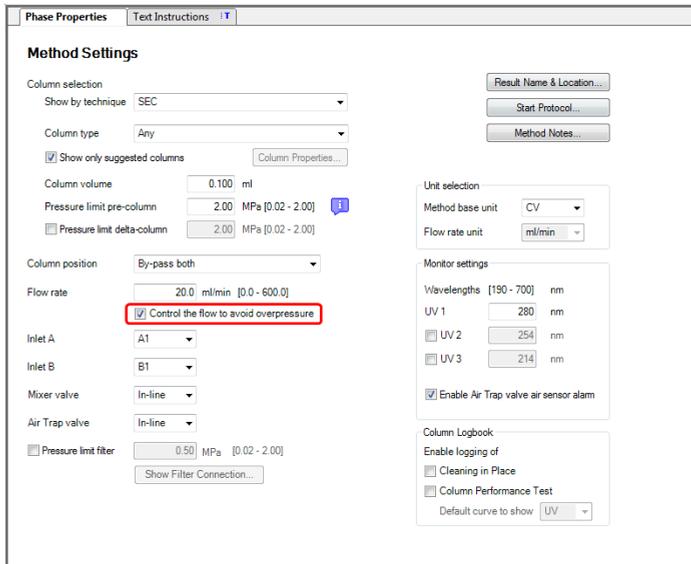
- 2      Open **Pressure control parameters** in the **Advanced** section of the **System Settings** dialog.



- 3      Set the parameters as required. Click  or press **F1** to access the UNICORN online help for the pressure control parameters.
- 4      Click **OK**.

## Activate pressure control in a method

To activate pressure control in a method, select **Control the flow to avoid overpressure** in the **Phase properties** of **Method Settings**.



The pressure control applies by default to the pre-column pressure.

## 4.6 Performing runs in a cold environment

### Introduction

The instrument can be placed and run in a cold cabinet or room. When running the instrument in a cold environment, take the precautions listed in this section.

---

### Precautions concerning runs in a cold cabinet



#### NOTICE

- **Avoid condensation.** If ÄKTA pilot 600 is kept in a cold room, cold cabinet or similar, keep it switched on in order to avoid condensation.
- **Avoid overheating.** If ÄKTA pilot 600 is kept in a cold cabinet and the cold cabinet is switched off, also switch off ÄKTA pilot 600 and keep the cold cabinet open to avoid overheating.
- **Place the computer in room temperature.** If the ÄKTA pilot 600 instrument is placed in a cold room, use a cold room compatible computer or place the computer outside the cold room and use the Ethernet cable delivered with the instrument to connect to the computer.

### Configure at room temperature

Configure the modules at room temperature, to prevent leaks in the system and kinks on the tubing.

---

### Moving to/from a cold environment

Reset the pressure sensors, see [Section 5.4.1 Reset the pressure sensors, on page 101](#), and calibrate the pH electrode, see the *ÄKTA pilot 600 Operating Instructions*, when the instrument has equilibrated to the new temperature.

---

## 4 Operation

### 4.6 Performing runs in a cold environment

#### Use equilibrated solutions

Always use solutions equilibrated to the ambient temperature, for example, sample solutions, buffers, and standard buffers for pH calibration. Temperature differences might for example increase outgassing.

---

#### Higher viscosity

Generally, cold solutions have higher viscosity than warm solutions. The flow rate might need to be lowered to keep an acceptable back pressure.

---

# 5 Maintenance

## About this chapter

Regular maintenance of the ÄKTA pilot 600 system is essential for reliable function.

This chapter describes the recommended maintenance schedules and provides detailed instructions for maintenance operations.

---

## In this chapter

This chapter contains the following sections:

| Section                    | See page |
|----------------------------|----------|
| 5.1 Maintenance manager    | 92       |
| 5.2 Maintenance schedule   | 93       |
| 5.3 Cleaning procedures    | 95       |
| 5.4 Calibration procedures | 100      |
| 5.5 Replacement procedures | 109      |

---

## Maintenance tasks

Refer to the *ÄKTA pilot 600 Operating Instructions* for instructions concerning the following maintenance tasks:

- Cleaning the instrument
  - Cleaning the flow path
  - Cleaning and storing the pH electrode
  - Changing the pump rinsing solution
  - Replacing the pump rinsing tubing
  - Replacing flow path tubing and connectors
  - Replacing valve membranes
  - Replacing the main fuses
-

### Precautions



#### WARNING

**Electrical shock hazard.** All repairs should be done by service personnel authorized by GE. Do not open any covers or replace parts unless specifically stated in the user documentation.



#### WARNING

**Use only approved parts.** Only spare parts and accessories that are approved or supplied by GE may be used for maintaining or servicing the product.



#### WARNING

For continued protection against injury risks due to fluid jets, burst pipes or potentially explosive atmosphere, the user must test the piping system for leakage at maximum operating pressure.

- Always perform a leakage test after assembly or maintenance.
- Always perform a leakage test before operation or CIP.



#### WARNING

**Hazardous chemicals during run.** When using hazardous chemicals, flush the entire system tubing with distilled water, before service and maintenance.



#### WARNING

**Decontaminate before maintenance.** To avoid personnel being exposed to potentially hazardous substances, make sure that the column is properly decontaminated and sanitized before maintenance or service.



**NOTICE**

Remove all columns from the instrument before performing maintenance.



**NOTICE**

Do not allow solutions which contain dissolved salts, proteins or other solid solutes to dry out in the UV flow cell.

## 5.1 Maintenance manager

### Introduction

The **Maintenance Manager** in UNICORN software displays general information and operational statistics for the ÄKTA pilot 600 system and modules. It also allows creation of automated notifications for maintenance actions, based on calendar time and operational statistics. A set of predefined notifications is provided, and custom notifications may be created.

---

### Using the *Maintenance Manager*

For details of how to use the **Maintenance Manager**, refer to the *UNICORN Administration and Technical Manual*.

---

## 5.2 Maintenance schedule

### Introduction

Maintenance procedures to be performed by the user are outlined below.

### Periodic maintenance

The following periodic maintenance should be performed by the user of the ÄKTA pilot 600 system.

| Interval                 | Maintenance action  | Instructions  |
|--------------------------|---|---|
| Daily or before each run | Calibrate the pH monitor.   | See the <i>ÄKTA pilot 600 Operating Instructions</i>                  |
| Daily or weekly          | Change pump rinsing solution.<br><b>Note:</b><br><i>Change the pump rinsing solution daily if buffer is used, or weekly if 20% ethanol is used.</i> | See the <i>ÄKTA pilot 600 Operating Instructions</i>                  |
|                          | Reset the pressure sensors.   | <a href="#">Section 5.4.1 Reset the pressure sensors, on page 101</a> |
| Every 6 months           | Clean the UV flow cell.   | <a href="#">Section 5.3.2 Clean the UV flow cell, on page 98</a>      |
|                          | Replace the pH electrode.   | See the <i>ÄKTA pilot 600 Operating Instructions</i>                  |
| Yearly                   | Replace valve membranes.  | See the <i>ÄKTA pilot 600 Operating Instructions</i>                  |

### Maintenance when required

The following maintenance should be performed when required.

| Maintenance action            | Instructions   |
|-------------------------------|--|
| Clean the instrument surfaces | See the <i>ÄKTA pilot 600 Operating Instructions</i> |

## 5 Maintenance

### 5.2 Maintenance schedule

| Maintenance action                        | Instructions  |
|---|---|
| Run System CIP (System cleaning-in-place) | See the <i>ÄKTA pilot 600 Operating Instructions</i>                          |
| Sanitize the instrument                   | See the <i>ÄKTA pilot 600 Operating Instructions</i>                          |
| Clean and store the pH electrode          | See the <i>ÄKTA pilot 600 Operating Instructions</i>                          |
| Clean the conductivity flow cell          | <a href="#">Section 5.3.1 Clean the conductivity flow cell, on page 96</a>    |
| Calibrate the conductivity monitor        | <a href="#">Section 5.4.2 Calibrate the conductivity monitor, on page 104</a> |
| Reset pressure sensors                    | <a href="#">Section 5.4.1 Reset the pressure sensors, on page 101</a>         |
| Replace tubing and connectors             | See the <i>ÄKTA pilot 600 Operating Instructions</i>                          |
| Replace the UV flow cell                  | <a href="#">Section 5.5.3 Replace the UV monitor flow cell, on page 114</a>   |
| Replace pump rinsing system tubing        | See the <i>ÄKTA pilot 600 Operating Instructions</i>                          |
| Replace mains fuses                       | See the <i>ÄKTA pilot 600 Operating Instructions</i>                          |
| Run performance tests                     | <a href="#">Chapter 6 Performance tests, on page 119</a>                      |

## 5.3 Cleaning procedures

### About this section

This section describes procedures for cleaning the ÄKTA pilot 600 instrument and components. See the *ÄKTA pilot 600 Operating Instructions* for additional cleaning instructions.

**Note:** *For column cleaning and storage procedures, refer to the column instructions. Refer to the computer documentation for recommendations for cleaning the computer equipment.*

---

### In this section

This section contains the following subsections:

| Section                                | See page |
|--|----------|
| 5.3.1 Clean the conductivity flow cell | 96       |
| 5.3.2 Clean the UV flow cell           | 98       |

---

## 5 Maintenance

### 5.3 Cleaning procedures

#### 5.3.1 Clean the conductivity flow cell

## 5.3.1 Clean the conductivity flow cell

### Maintenance interval

Clean the conductivity flow cell when the signal is unstable or when unexpected readings are obtained.

---

### Required material

The following materials are required:

- Luer-to-SNAP connector (see [Luer-SNAP 3.2 connectors, on page 25](#))
  - Waste container (for example, 250 mL flask or beaker)
  - Syringe, 25 mL
  - 1 M NaOH
  - Distilled water
- 

### Procedure

Follow the steps below to clean the conductivity flow cell. The same procedure applies to both pre- and post-column conductivity monitors.



#### WARNING

**Corrosive substance.** NaOH is corrosive and therefore dangerous to health. When using hazardous chemicals, avoid spillage and wear protective glasses and other suitable Personal Protective Equipment (PPE).

| Step | Action  |
|------|---|
| 1    | Disconnect the tubing from the bottom of the conductivity monitor and replace it with a Luer-to-SNAP connector (see <a href="#">Luer-SNAP 3.2 connectors, on page 25</a> ). |

| Step | Action   |
|------|--|
| 2    | Disconnect the tubing from the top of the conductivity monitor and replace it with a piece of tubing leading to the waste container. |



|   |  |
|---|--|
| 3 | Flush the conductivity flow cell with 25 to 30 mL distilled water, using a syringe attached to the Luer-to-SNAP connector. |
| 4 | Flush the conductivity flow cell about five times with 10 mL 1 M NaOH.   |
| 5 | Allow the 1 M NaOH to remain in the flow cell for about 15 minutes.  |
| 6 | Flush the flow cell again with 25 to 30 mL distilled water.  |
| 7 | Disconnect the Luer-to-SNAP connector and waste tubing from the conductivity monitor, and reconnect the system tubing.     |

**Note:** Calibration of the conductivity monitor (see [Section 5.4.2 Calibrate the conductivity monitor, on page 104](#)) is recommended after cleaning, particularly if the flow cell was very dirty and correct conductivity values are important.

## 5.3.2 Clean the UV flow cell

### Maintenance interval

Clean the UV flow cell every six months, or when required.



#### NOTICE

**Keep UV flow cell clean.** Do not allow solutions containing dissolved salts, proteins or other solid solutes to dry out in the flow cell. Do not allow particles to enter the flow cell, as damage to the flow cell may occur.

### Required material

The following materials are required:

- Luer-to-SNAP connector (see [Luer-SNAP 3.2 connectors, on page 25](#))
- Waste container
- Syringe, 25 to 30 mL
- Detergent solution (e.g., Decon™ 90, Deconex™ 11, or RBS™ 25), diluted according to the manufacturer's instructions.
- Distilled water

### Procedure

Follow the steps below to clean the UV flow cell.

| Step | Action   |
|------|--|
| 1    | Disconnect the tubing from the bottom of the UV monitor and replace it with a Luer-to- SNAP connector. |

| Step | Action   |
|------|--|
| 2    | Disconnect the tubing from the top of the UV monitor and replace it with a piece of tubing leading to the waste container. |



- 3 Flush the flow cell with 25 to 30 mL distilled water, using a syringe attached to the Luer-to-SNAP connector.
- 4 Flush the flow cell about five times with 10 mL detergent solution.  
**Tip:**  
*Warm the detergent solution to 40°C for enhanced cleaning effect.*
- 5 Allow the detergent solution to remain in the flow cell for about 15 minutes.
- 6 Flush the flow cell twice with 25 to 30 mL distilled water.
- 7 Disconnect the Luer-to-SNAP connector and waste tubing from the UV monitor, and reconnect the system tubing.

## 5.4 Calibration procedures

### About this section

This section provides instructions for calibration procedures that can be performed using the **System Control** module in UNICORN software.

---

### In this section

This section contains the following subsections.

| Section                                  | See page |
|--|----------|
| 5.4.1 Reset the pressure sensors         | 101      |
| 5.4.2 Calibrate the conductivity monitor | 104      |

---

## 5.4.1 Reset the pressure sensors

### Introduction

The ÄKTA pilot 600 system is fitted with three pressure sensors, for monitoring system pressure, pre-column pressure and post-column pressure.

The pressure sensors should be reset at intervals by setting the reading for atmospheric pressure to zero in UNICORN software. The pressure sensors themselves are not accessible to the user.

This section gives instructions for resetting the pressure sensors.

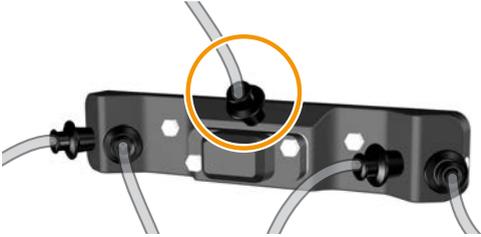
### Maintenance interval

The pressure sensors should be tested every week, or when the the ambient temperature has changed by more than 5°C. Reset the pressure sensors if the atmospheric pressure reading is outside the range  $\pm 0.02$  MPa (0.2 bar, 2.9 psi).

### Tubing connections

Resetting pressure sensors involves disconnecting tubing from the appropriate module according to the table below.

**Note:** Take care to avoid spillage when disconnecting tubing.

| Monitored pressure | Placing                                | Disconnect tubing  |
|--------------------|--|--|
| System pressure    | At the outlet from the flow restrictor | Flow restrictor outlet<br> |

| Monitored pressure                    | Placing                             | Disconnect tubing  |
|---------------------------------------|-------------------------------------|--|
| Pre-column ( <b>PreC</b> ) pressure   | At the inlet to the column valve    | Column valve inlet<br>   |
| Post-column ( <b>PostC</b> ) pressure | At the outlet from the column valve | Column valve outlet<br> |

## Reset the pressure sensors

Follow the steps below to reset the pressure sensors. Reset the pressure sensors one at a time.

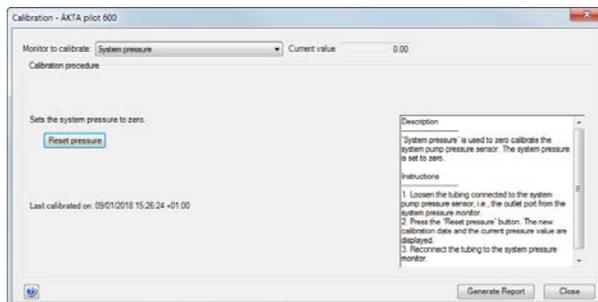
| Step | Action |
|------|--------|
|------|--------|

- |   |  |
|---|--|
| 1 | Make sure that the appropriate tubing (see <a href="#">Tubing connections, on page 101</a> ) is disconnected so that the pressure sensor is exposed to atmospheric pressure. |
| 2 | Choose <b>Calibrate</b> from the <b>System</b> menu in UNICORN <b>System Control</b> .   |

**Step**      **Action**

---

- 3      In the **Calibration** dialog, choose the pressure sensor in the **Monitor to calibrate** list.



- 4      If the **Current value** is outside the range  $\pm 0.02$  MPa (0.2 bar, 2.9 psi), click **Reset pressure**.  
*Result:* The pressure reading is reset to zero.
- 5      Reconnect the tubing to the pressure sensor.
-

## 5.4.2 Calibrate the conductivity monitor

### Introduction

Two types of calibration can be performed:

- **Conductivity monitor - user calibration:** Calibrates the conductivity cell constant.
- **Conductivity monitor - factory calibration:** Restores the conductivity cell constant to the factory default value.

This section gives instructions for both calibration procedures. The instructions apply to both pre- and post-column conductivity monitors.

---

### Maintenance interval

When to perform the two types of calibrations is as follows:

| Calibration type    | When to perform   |
|---------------------|---|
| User calibration    | When the signal is unstable, or when you suspect that the value is incorrect. |
| Factory calibration | When user calibration fails.  |

### Required material

The following materials are required:

- Luer-to-SNAP connector (see [Luer-SNAP 3.2 connectors, on page 25](#))
  - Waste container
  - Syringe, 25 mL
  - Calibration solution, either a certified conductivity standard solution (1.0 M KCl) or your own accurately prepared 1.00 M NaCl.
  - Distilled water
- 

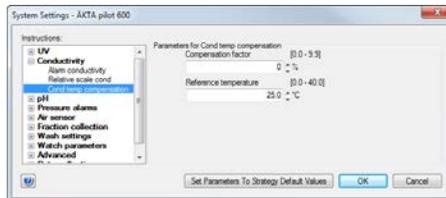
### User calibration procedure

Follow the steps below to calibrate the conductivity monitor.

- | Step | Action   |
|------|--|
| 1    | Clean the conductivity flow cell before calibration (see <a href="#">Section 5.3.1 Clean the conductivity flow cell, on page 96</a> ). |
| 2    | Disconnect the tubing from the bottom of the conductivity monitor and replace it with a Luer-to-SNAP connector.                        |
| 3    | Disconnect the tubing from the top of the conductivity monitor and replace it with a piece of tubing leading to the waste container.   |



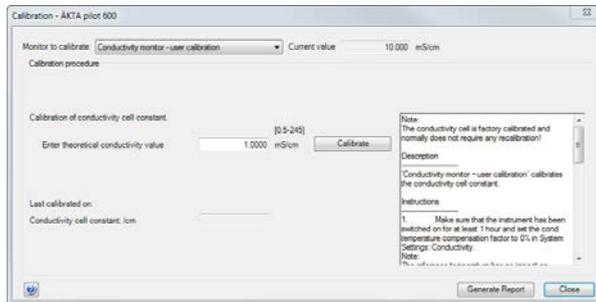
- 4 Select **System:Settings** in UNICORN **System Control** module.
- 5 In the **Instructions** list, select **Conductivity:Cond Temp Compensation**.



- 6 Set the **Compensation factor** to 0 and click **OK**.
- 7 Select **System:Calibrate** from the main **System control** menu.

**Step**      **Action**

- 8 In the **Calibration** dialog, choose **Conductivity monitor - user calibration** in the **Monitor to calibrate** list.

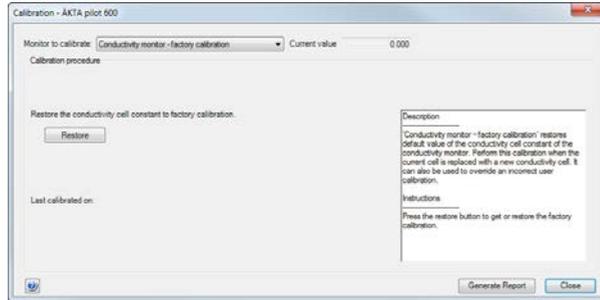


- 9 Flush the conductivity flow cell twice with 25 to 30 mL distilled water, using a syringe attached to the Luer-to-SNAP connector. Leave the syringe attached to the connector so that solution does not drain from the flow cell.
- 10 Flush the conductivity flow cell with conductivity standard solution, using a syringe attached to the Luer-to-SNAP connector, then wait until the conductivity reading is stable.
- 11 In the **Calibration** dialog box, enter the theoretical conductivity value at the current conductivity temperature in the **Enter theoretical conductivity value** input field.
- If a certified conductivity standard solution is used, use the supplied theoretical conductivity value.
  - If a manually prepared 1.00 M NaCl calibration solution is used, see the graph for conductivity value at the current temperature [Conductivity values of 1.00 M NaCl, on page 107](#).
- 12 In the **Calibration** dialog, click **Calibrate**.  
*Result:* The new conductivity cell constant is displayed in the **Conductivity cell constant/cm** box.
- 13 In the **System Settings** dialog box, select **Conductivity:Cond temp compensation** and set the **Compensation factor** back to the desired value, default 2.1%. Click **OK**.
- 14 Reconnect the system tubing to the conductivity monitor.

## Factory calibration procedure

Follow the steps below to restore the conductivity cell constant to the factory default value.

- | Step | Action  |
|------|---|
| 1    | Select <b>System:Calibrate</b> from the UNICORN <b>System control</b> menu.   |
| 2    | In the <b>Calibration</b> dialog, choose <b>Conductivity monitor - factory calibration</b> in the <b>Monitor to calibrate</b> list. |

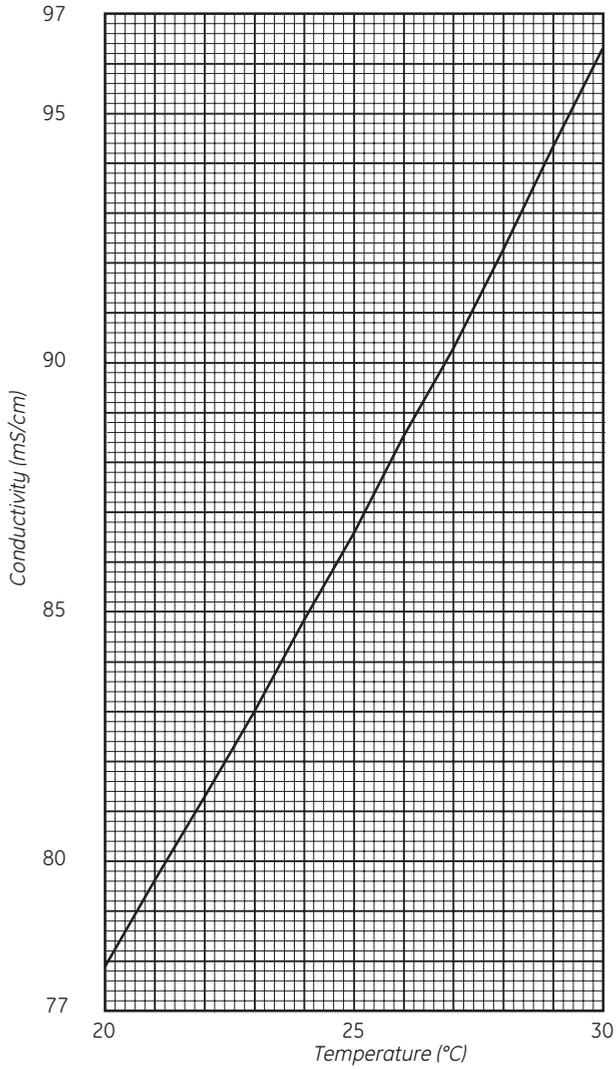


- |   |   |
|---|---|
| 3 | Click <b>Restore</b> .<br><i>Result:</i> The conductivity cell constant is restored to the factory default value. The conductivity cell constant is printed on the packaging of the Conductivity monitor. |
|---|---|

## Conductivity values of 1.00 M NaCl

The graph below shows the conductivity value for 1.00 M NaCl solution as a function of temperature.

Conductivity of 1.00 M NaCl at 20–30°C



## 5.5 Replacement procedures

### About this section

This section gives instructions for the replacement procedures to be performed by the user of ÄKTA pilot 600 instrument.

---

### In this section

This section contains the following subsections:

| Section                                | See page |
|--|----------|
| 5.5.1 Replace the pH electrode         | 111      |
| 5.5.2 Replace the Mixer module         | 112      |
| 5.5.3 Replace the UV monitor flow cell | 114      |
| 5.5.4 Replace valve fronts             | 118      |

---

### Precautions



#### WARNING

**Disconnect power.** Always disconnect power from the instrument before replacing any component on the instrument, unless stated otherwise in the user documentation.



#### WARNING

**Hazardous chemicals during run.** When using hazardous chemicals, flush the entire system tubing with distilled water, before service and maintenance.



**WARNING**

**Hazardous chemicals.** Always empty the system of liquids before service.



**WARNING**

**Decontaminate before maintenance.** To avoid exposing personnel to potentially hazardous substances, clean and sanitize the ÄKTA pilot 600 system before maintenance or service.

## 5.5.1 Replace the pH electrode

### Maintenance interval

Replace the pH electrode every six months, or when required.

---

### Required material

The following materials are required:

- pH electrode
  - distilled water
  - pH electrode storage solution (1:1 pH 4 standard buffer:1 M KNO<sub>3</sub>)
  - pH 4 and pH 7 standard buffers
- 

### Procedure

Follow the steps below to replace the pH electrode.



#### CAUTION

**pH-electrode.** Handle the pH-electrode with care. The glass tip may break and cause injury.

Follow the steps below to replace the pH electrode.

| Step | Action  |
|------|---|
| 1    | Disconnect the pH electrode cable from the connection on the front of the pH monitor.     |
| 2    | Unscrew the locking ring nut of the pH electrode and remove the electrode.                |
| 3    | Fit a new pH electrode as described in the <i>ÄKTA pilot 600 Operating Instructions</i> . |
| 4    | Allow the electrode to remain in storage solution for at least 1 h before use.            |
| 7    | Calibrate the new pH electrode (see the <i>ÄKTA pilot 600 Operating Instructions</i> ).   |

---

## 5.5.2 Replace the Mixer module

### Maintenance interval

Replace the Mixer module when the existing module malfunctions.

---

### Required material

The following is required:

- Mixer module (available as a spare part from GE)
  - 7 mm socket tool
- 

### Procedure

Follow the steps below to replace the Mixer module.

| Step | Action |
|------|--------|
|------|--------|

---

- |   |  |
|---|--|
| 1 | Disconnect the tubing from the mixer chamber.      |
| 2 | Pull the mixer chamber away from the module front. |



- |   |   |
|---|---|
| 3 | Remove the retaining screw that holds the module in the instrument chassis (see the <i>Mixer Kit Installation Instruction</i> supplied with the replacement mixer). |
| 4 | Install the new module as described in the instructions provided with the Mixer Kit.  |

| Step | Action   |
|------|--|
| 5    | Reconnect the tubing to the mixer chamber.                                   |
| 6    | Flush the system with water or buffer to remove the air from the new module. |

## 5.5.3 Replace the UV monitor flow cell

### Introduction

The UV monitor may need replacement in two situations:

- Changing between 2 mm and 5 mm path length flow cells
- Replacing a defective flow cell

The replacement procedure is the same in both cases. However, if you are changing between different path lengths, handle the replaced flow cell with care.



#### CAUTION

The system uses high intensity ultra-violet light that is harmful to the eyes. Before changing or cleaning the UV cell optical fiber, make sure that the UV lamp is disconnected or that the power is disconnected.

### Maintenance interval

Replace the UV flow cell when a flow cell with a different path length is required, or if the cell is damaged. Clean the optical fiber connectors if they are accidentally touched during flow cell replacement.

---

### Required material

The following materials are required:

- UV flow cell
  - Lens paper
  - Isopropanol
- 

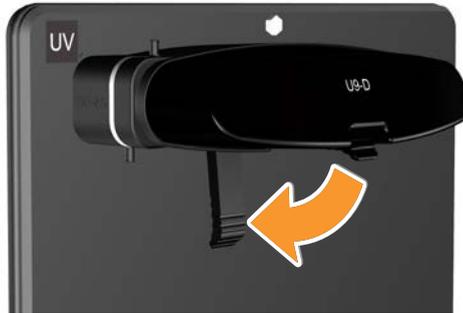
### Replace the flow cell

Follow the steps below to replace the UV flow cell.

| Step | Action                                       |
|------|--|
| 1    | Switch off the instrument.                   |
| 2    | Disconnect the tubing from the UV flow cell. |

| Step | Action |
|------|--------|
|------|--------|

- |   |   |
|---|---|
| 3 | Push the latch on the UV detector to disconnect the detector. |
|---|---|



**Note:**

*The UV lamp does not operate while the UV detector is disconnected.*

- |   |  |
|---|--|
| 4 | Pull off the detector with the flow cell off the instrument. |
|---|--|



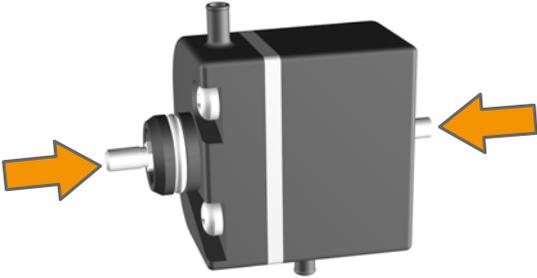
**Note:**

*Make sure that the flow cell does not come into contact with any liquid, and that no liquid enters the UV detector or the front panel.*

## 5 Maintenance

### 5.5 Replacement procedures

#### 5.5.3 Replace the UV monitor flow cell

| Step | Action   |
|------|--|
| 5    | <p>Remove the flow cell from the detector. Do not touch the exposed optical fiber connectors.</p> <p><b>Note:</b><br/><i>If you do accidentally touch the optical fiber connectors, clean them according to <a href="#">Clean the optical fiber connectors</a>, on page 116.</i></p>  |
| 6    | <p>If you intend to reuse the flow cell that you have removed, move the protective caps from the replacement flow cell to the optical fiber connectors on the removed flow cell to protect them from dust. Take care not to damage the flow cell.</p>  |
| 7    | <p>Fit the replacement UV flow cell to the detector.</p>   |
| 8    | <p>Fit the detector with the replacement flow cell to the instrument front panel. Pull the latch upwards to secure the detector.</p>   |
| 9    | <p>Connect the tubing to the new flow cell.</p>  |
| 10   | <p>Switch on the instrument.</p> <p><i>Result:</i> The flow cell path length is automatically recognized by the monitor when a new flow cell is connected.</p>   |

## Clean the optical fiber connectors

Follow the steps below to clean the optical fiber connectors.



**WARNING**

**Hazardous substances.** When using hazardous chemicals, take all suitable protective measures, such as wearing protective clothing, glasses and gloves resistant to the substances used. Follow local and/or national regulations for safe operation and maintenance of the product.

| Step | Action  |
|------|---|
| 1    | Wipe the optical fiber connectors with a lens paper moistened with isopropanol. |
| 2    | Wipe the optical fiber connectors dry with lens paper.                          |

## 5.5.4 Replace valve fronts

### When to replace the valve fronts

The valve fronts only need replacing if they are damaged.

---

### Procedure

Obtain a new valve front from GE, then follow the procedure for replacing valve membranes (described in the *ÄKTA pilot 600 Operating Instructions*).

---

# 6 Performance tests

## About this chapter

Performance tests allow the user to test the integrity and functional aspects of the whole ÄKTA pilot 600 system or of individual components.

This chapter describes in general terms how to access, run, and assess performance tests. Detailed on-screen instructions are provided in the UNICORN software for each test.

---

## In this chapter

This chapter contains the following sections:

| Section                               | See page |
|---------------------------------------|----------|
| 6.1 When to run performance tests     | 120      |
| 6.2 How to run performance tests      | 122      |
| 6.3 How to evaluate performance tests | 126      |

---

## 6.1 When to run performance tests

### Schedule

Performance tests may be run at any time, as required. Recommendations are given in the following table.

| Occasion                             | Test   |
|--------------------------------------|--|
| After system installation            | Run the system tests in the following order<br>1 <b>System leakage test</b><br>2 <b>System backpressure test</b><br>3 <b>System test</b><br>If any of the tests fails, correct the cause of the failure before continuing. |
| After module addition or replacement | Run the test(s) associated with the specific module.   |
| After valve membrane replacement     | Run the test for the specific valve.   |
| When a problem is suspected          | Test the modules that may be involved in the problem. <sup>1</sup>   |

<sup>1</sup> **Note:**

*Valve modules are tested for leakage only, not for valve switching function.*

## Performance test run order

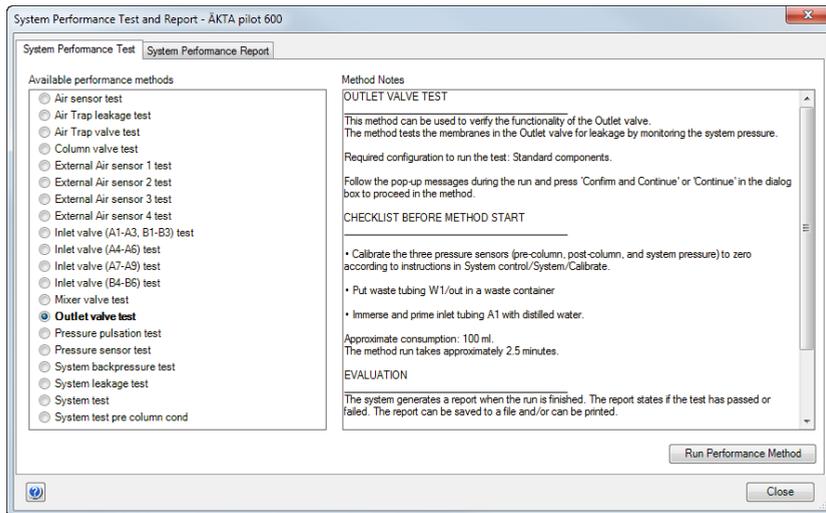
Under certain conditions, some performance tests should be run in a specific order according to the table below. In other circumstances (for example, when membranes are changed on a specific valve), tests may be run independently of each other.

| Condition                                   | Test order  |
|---|---|
| System leakage test fails                   | Run valve module tests in the following order: <ul style="list-style-type: none"><li>• Inlet valve</li><li>• Outlet valve</li><li>• Column valve</li><li>• Air trap valve</li><li>• Mixer valve</li></ul> |
| Before running <b>Air Trap leakage</b> test | Ensure that <b>Air Trap valve</b> and <b>Column valve</b> tests are passed. The <b>Air Trap leakage</b> test will fail if either of these tests fails, even if the Air Trap itself does not leak.         |

## 6.2 How to run performance tests

### Introduction

This section describes the general procedure for running performance tests. Details of the individual tests including purpose and required materials are given in the method notes for each test method. An example is shown below.



#### NOTICE

Valve module tests and other leakage tests expose the system to pressures close to the system pressure limit by running the system pumps at a low flow rate with the flow path closed at some point. Ensure that the system pressure limit is set to the default value (2.0 MPa) before running performance tests.

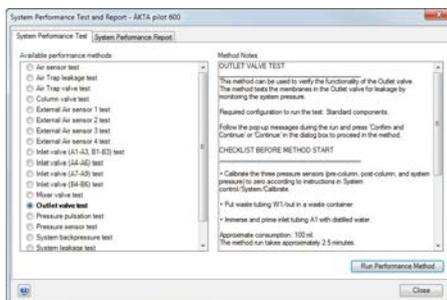
### Procedure

Performance tests are provided with the instrument configuration in UNICORN software. Follow the general steps below to run a performance test. Detailed requirements and procedures are shown on the screen when the test is run.

| Step | Action |
|------|--------|
|------|--------|

|   |   |
|---|---|
| 1 | Open <b>System Control</b> in the UNICORN software if it is not already open. |
|---|---|

- | Step | Action   |
|------|--|
| 2    | Select <b>Performance Test and Report...</b> from the <b>System</b> menu.                              |
| 3    | Select the test you want to run. Method notes for the selected test are shown in the right-hand panel. |



**Note:**

This dialog lists tests for all modules that can be installed in the ÄKTA pilot 600 system. Attempting to run a test for a module that is not installed will generate an error message.

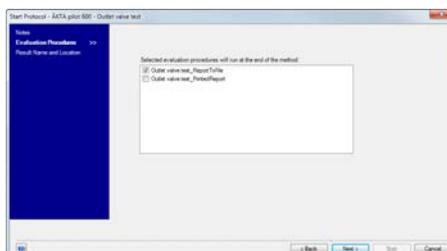
- 4 Click **Run Performance Method**.



- 5 Read the **Method Notes** carefully, and make sure that all requirements are met.

See the UNICORN Software Manual for more information regarding the other **Notes** tabs for a method run.

Click **Next>** to continue.



## 6 Performance tests

### 6.2 How to run performance tests

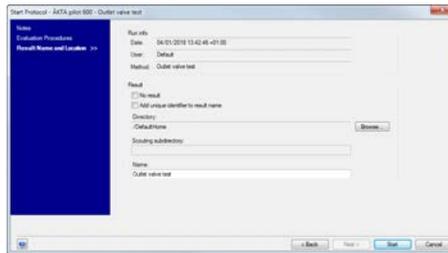
| Step | Action |
|------|--------|
|------|--------|

- |   |  |
|---|--|
| 6 | Select whether the test report should be saved to a file and/or printed. |
|---|--|

**Note:**

*Sending the report to a printer requires that a system printer is installed on the UNICORN Instrument Server (see the UNICORN Administration and Technical Manual for details).*

Click **Next>** to continue.



- |   |   |
|---|---|
| 7 | Specify the details of the result file from the performance test. |
|---|---|

**Note:**

*The result file is separate from the test report. The report will be generated even if **No result** is selected.*

Click **Start** to start the performance test.

- |   |   |
|---|---|
| 8 | Follow any instructions that are shown on the screen. |
|---|---|

The progress of the performance test is shown in the chromatogram pane and can be customized in the same way as any chromatogram.

## Required materials

Materials required for performance tests, in addition to distilled water, are specified in the method notes for the test and summarized in the table below.

| Test   | Required material  |
|--|--|
| <b>System test/System test pre column cond</b> | 2 L 1% acetone (v/v) in 1.0 M NaCl<br>Reference capillary 1                                    |
| <b>Column valve test</b>                       | Reference capillary 2<br>Short lengths of i.d. 3.2 mm with SNAP connectors to replace columns. |
| <b>Pressure pulsation test</b>                 | Reference capillary 2  |

Reference capillaries are provided with the system, and may also be obtained separately from GE.

---

## 6.3 How to evaluate performance tests

### Introduction

This section describes how to evaluate the outcome of performance tests.

---

### Inspect for leaks

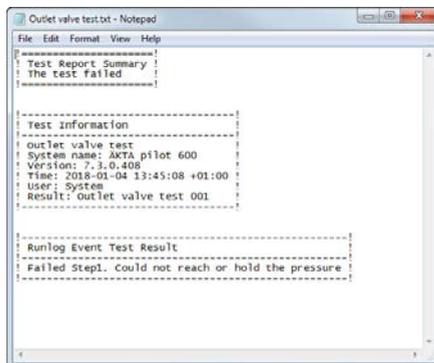
Valve module tests and other leakage tests expose the system to pressures close to the system pressure limit by running the system pumps at a low flow rate with the flow path closed at some point. Make sure that the system pressure limit is set to the default value (2.0 MPa) before such tests are run. The tests may fail if the system pressure limit is set to a lower value.

Inspect the system visually for leaks while the test is running. If there is significant leakage, the test report will indicate that the maximum pressure cannot be reached or held.

---

### Test reports

Running a performance test generates a test report that specifies whether the test has passed or failed. The report is a text file that can be opened in Windows Notepad or any generic text editor. The illustration below shows the report from a failed Outlet valve test.

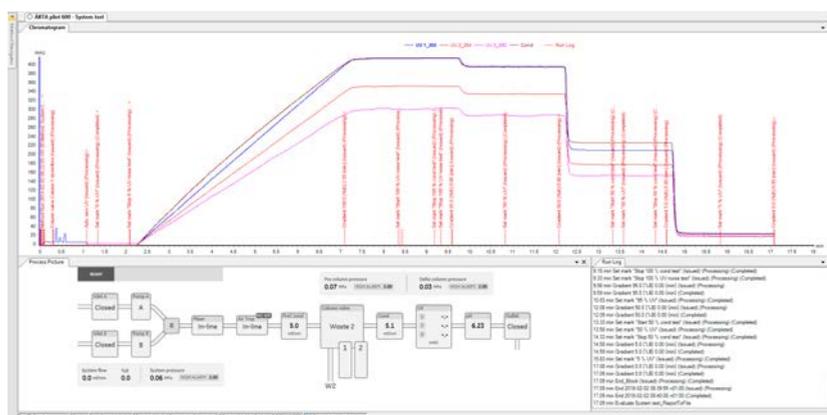


The reports are saved in the folder C:\Program Files (x86)\GE Healthcare\UNICORN\UNICORN <version number>\Temp. Each test generates a text file with the name of the test as filename. An incremented serial number is added if a file with the same name already exists.

**Note:** The dialog for selecting and running a performance test includes a tab with heading **System Performance Report** (see [Procedure, on page 122](#)). This tab does not contain the reports for performance tests.

## Test results

The performance test data are displayed in the **System Control** module as the test progresses, and are saved in the same way as results from chromatography runs. Examination of the results can help in assessing the outcome of the tests, particularly for tests that fail. The illustration below shows the results of a **System test**, displayed in the **System Control** module as the test was run.



You can choose not to save the test results when a test is run (see [Procedure, on page 122](#)). This does not affect generation of the text report.

# 7 Troubleshooting

## About this chapter

This chapter describes actions that should be taken when performance tests fail, indicating a malfunction in the system. See [Chapter 6 Performance tests, on page 119](#) for information on how to run performance tests.

Basic troubleshooting is described in the *ÄKTA pilot 600 Operating Instructions*.

If you have problems that cannot be resolved using the information provided here, contact your GE service organization.

---

## In this chapter

This chapter contains the following sections:

| Section                              | See page |
|--------------------------------------|----------|
| 7.1 System leakage test failure      | 129      |
| 7.2 System backpressure test failure | 130      |
| 7.3 System test failure              | 131      |
| 7.4 Other performance tests          | 133      |

---

## 7.1 System leakage test failure

### Description

The **System leakage test** tests the system flow path for leakage. Leakage from the tubing connections and valve membranes is tested by monitoring the system pressure. The first step tests the whole flow path up to the outlet valve at a lower pressure level. The second step tests the flow path up to the column valve at a higher pressure level. During each test step, the system pressure increases slowly and is then maintained for a limited time. The outcome of the two steps is listed separately in the performance test report.

### Corrective actions

If the **System leakage test** fails, inspect all tubing connections and valves in the flow path. Correct the problem according to the table below, and rerun the **System leakage test** to make sure the leakage is corrected. If the problem persists after corrective action has been applied, contact your GE representative.

| Cause of failure                      | Corrective action   |
|---------------------------------------|---|
| Visible leakage at a tubing connector | Make sure that the SNAP connectors are correctly fitted.<br>Replace the tubing.   |
| Visible leakage at a valve            | Replace the valve membranes (see the <i>ÅKTA pilot 600 Operating Instructions</i> ).<br>If the problem persists, replace the valve module.  |
| No visible leakage                    | <ol style="list-style-type: none"> <li>1 Make sure that both inlets <b>A1</b> and <b>B1</b> have been primed with water.</li> <li>2 Repeat the test.</li> <li>3 If the test still fails, run individual module tests until the leakage is located.</li> </ol> |

## 7.2 System backpressure test failure

### Description

The **System backpressure test** tests that the backpressure in the system does not exceed a specified value. Increased backpressure may result from an obstruction in the flow path such as kinked tubing.

---

### Corrective action

If the **System backpressure test** fails, make sure that tubing is not kinked or obstructed. Replace any kinked tubing.

Locate the source of increased backpressure by moving the outlet tubing to the outlet port of successive modules, starting with the outlet valve(s) and working systematically towards the beginning of the flow path. The backpressure will drop when the blocked module is excluded from the flow path.

If the cause of the failure cannot be located, contact your GE service organization.

---

## 7.3 System test failure

### Description

The **System test** checks the function of the liquid delivery and the UV and conductivity monitors. Correct gradient formation is tested by producing a linear gradient and a series of concentration steps of acetone. Correct UV monitoring is tested by monitoring the absorbance ratios 265/254 nm and 265/280 nm with 1% acetone concentration.

The outcome of each component of the test is listed individually in the performance test report.

### Corrective actions

Corrective actions in the case of failure are listed in the table below. If the problem persists after corrective action has been applied, contact your GE representative.

**Note:** *It is important that the correct concentrations (1% (v/v) acetone and 1 M NaCl) are used in the **System test** and that the solutions are thoroughly mixed.*

| Failed test                    | Corrective action   |
|--------------------------------|---|
| Gradient test                  | <p>Make sure that tubing to and from <b>Pump A</b> and <b>Pump B</b> is not obstructed.</p> <p>Restart the instrument to perform automatic wavelength calibration and light intensity check for the UV monitor.</p>   |
| Step response test             | <p>Make sure that tubing to and from <b>Pump A</b> and <b>Pump B</b> is not obstructed.</p> <p>Errors in all values may indicate air in the pump. Errors in the 5% or 95% values indicate a problem with <b>Pump B</b> or <b>Pump A</b> respectively. Run the <b>Pressure pulsation test</b> to test the pump status.</p> <p>Restart the instrument to perform automatic wavelength calibration and light intensity check for the UV monitor.</p> |
| UV response test               | <p>Clean the UV flow cell.</p> <p>Restart the instrument to perform automatic wavelength calibration and light intensity check for the UV monitor.</p>  |
| Curve amplitude stability (UV) | <p>Restart the instrument to perform automatic wavelength calibration and light intensity check for the UV monitor.</p>   |

7 Troubleshooting  
7.3 System test failure

| Failed test                              | Corrective action   |
|--|---|
| Curve amplitude stability (conductivity) | <p>Make sure that the temperature compensation is turned on (the temperature compensation factor is not zero). If the test is performed in a cold-room, the temperature compensation factor should be 2.1.</p> <p>The temperature compensation factor is accessed through <b>System Settings:Conductivity:Cond temp compensation</b>.</p> |

## 7.4 Other performance tests

### Corrective actions

The Instrument Configuration software installed with the system (see [Section 2.4 Instrument Configuration software, on page 27](#)) provides an extensive set of performance tests for individual modules. The table below gives some guidance concerning corrective actions when individual module tests fail.

| Test failure                 | Corrective action   |
|------------------------------|---|
| Valve tests indicate leakage | Replace the valve membranes (see the <i>ÅKTA pilot 600 Operating Instructions</i> ).<br>If the problem persists, replace the valve module.  |
| Sensor malfunction           | Where appropriate, clean the flow cell in the module.<br>If the problem persists, replace the module.   |
| UV test failure              | Power off and restart the instrument before running the test. This will enable automatic wavelength calibration and light intensity check for the UV monitor.<br>If the problem persists, replace the module. |

# 8 Reference information

## About this chapter

System specifications, chemical resistance information and other basic reference information are given in the *ÄKTA pilot 600 Operating Instructions* and the *ÄKTA pilot 600S Product Documentation* for ÄKTA pilot 600S systems. Detailed information specific to the individual system is provided separately with ÄKTA pilot 600R systems. This chapter gives additional specification details.

---

## In this chapter

This chapter contains the following sections:

| Section                  | See page |
|--------------------------|----------|
| 8.1 Component volumes    | 135      |
| 8.2 Ordering information | 136      |

---

## 8.1 Component volumes

### Module volumes

The table below lists the internal volumes of the modules in ÄKTA pilot 600 system.

| Module                        | Internal volume (mL) |
|-------------------------------|----------------------|
| External air sensor           | 0.7                  |
| Inlet valve                   | 4.3                  |
| Pump (two pump heads)         | 7.1                  |
| Pump rinse system             | 4.9                  |
| Flow restrictor               | 3.9                  |
| Air trap valve                | 3.9                  |
| Air trap                      | 73.7                 |
| Mixer valve                   | 3.4                  |
| Mixer chamber                 | 5.4                  |
| Column valve                  | 15.0                 |
| Conductivity monitor          | 0.4                  |
| UV monitor (2.0 mm flow cell) | 0.2                  |
| UV monitor (5.0 mm flow cell) | 0.5                  |
| pH monitor                    | 0.8                  |
| Outlet valve 1                | 3.6                  |
| Outlet valves 2 to 5          | 4.0                  |

## 8.2 Ordering information

### Visit [gelifesciences.com](http://gelifesciences.com)

Refer to our website [www.gelifesciences.com/aktapilot](http://www.gelifesciences.com/aktapilot) or GE representative for further information about the following topics:

- System recommendations
  - Literature
  - Service
  - Training
  - Accessories
  - Ordering information
- 

### Spare parts

Your local GE representative can suggest recommended spare parts.

---

### Contact information

Contact information can be found on the back page of this document.

---

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