

# **ÄKTA** go User Manual





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# 1 Introduction

## About this chapter

This chapter contains important user information, the intended use of ÄKTA<sup>™</sup> go, and lists the available user documentation.

## In this chapter

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1.1	About this manual	6
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1.3	User documentation	9

# 1.1 About this manual

## **Purpose of this manual**

The User Manual provides you with a detailed description of the ÄKTA go system, the function of the different parts, and how to maintain and replace them. The User Manual also provides tips on how to get the most out of your system when running it. Basic instructions, including important safety information, are given in ÄKTA go Operating Instructions (29360951). For information about chromatography techniques and columns, refer to the respective handbook or instruction.

The instrument is controlled by a PC running  $\mathsf{UNICORN}^{\mathsf{M}}$  system control software version 7.4 or later.

## **Typographical conventions**

Software items are identified in the text by **bold italic** text.

Hardware items are identified in the text by **bold** text.

# 1.2 Important user information

# Read the *Operating Instructions* before operating the product

All users must read the entire ÄKTA go 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 go system is intended for the purification of bio-molecules, in particular proteins, for research purposes. It is intended to be used by trained laboratory staff members in research laboratories within academia and industry.

The ÄKTA go system must not be used in any clinical procedures, or for diagnostic purposes.

### **Prerequisites**

In order to operate the system according to the intended purpose, it is important that the following prerequisites are followed:

- you have a general understanding of how the computer and the Microsoft<sup>®</sup> Windows<sup>®</sup> operating system work.
- you understand the concepts of liquid chromatography.
- you have read and understood the Safety instructions chapter in ÄKTA go Operating Instructions.
- a user account has been created according to UNICORN Administration and Technical Manual.

### Definitions

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.



## **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 proce- dures.

## 1.3 User documentation

### Introduction

This section describes the available user documentation for ÄKTA go.

## **Associated documentation**

Document	Main contents
ÄKTA go Cue Cards	This document contains step-by-step instructions for basic use of the ÄKTA go system.
ÄKTA go Operating Instructions	This document contains instructions needed to install, operate and maintain ÄKTA go in a safe way. Translations of the original instructions are given in several languages.
ÄKTA go Product Documentation	Specifications and material conformity.
ÄKTA go Site Preparation Guide	Instructions on how to prepare the installation site for the ÄKTA go system.
ÄKTA go Unpacking Instructions	This document describes how to handle the delivery package and unpack the ÄKTA go instrument.

# UNICORN online help and user documentation

The UNICORN user documentation is available in the **UNICORN Online Help and Documentation** section of the online help.

Online help in UNICORN may be accessed in three ways:

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

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

The UNICORN user documentation is listed in the following table.

Document	Main contents
UNICORN Help	Descriptions of UNICORN dialog boxes (available from the <b>Help</b> menu).
Getting started with Evaluation	<ul> <li>Video clips showing common workflows in the <i>Evaluation</i> module.</li> <li>Overview of features of the <i>Evaluation</i> module.</li> </ul>
UNICORN Method Manual	<ul> <li>Overview and detailed descriptions of the method creation features in UNICORN.</li> <li>Workflow descriptions for common operations.</li> </ul>
UNICORN Evaluation Manual	<ul> <li>Overview and detailed descriptions of the <i>Evaluation Classic</i> module in UNICORN.</li> <li>Description of the evaluation algorithms used in UNICORN.</li> </ul>
UNICORN Administration and Technical Manual	<ul> <li>Overview and detailed description of network setup and complete software installation.</li> <li>Administration of UNICORN and the UNICORN database.</li> </ul>
UNICORN System Control Manual	<ul> <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 Manual	<ul> <li>Technical descriptions of the UNICORN OPC server.</li> <li>Suggestions on security settings for remote access to UNICORN OPC server.</li> <li>Reference to UNICORN OPC Data Access, Alarms &amp; Events and Historical Data Access address space.</li> </ul>

## **Additional literature**

For practical tips on chromatography, refer to the handbooks available at *cytiva.com/handbooks*.

## About this chapter

This chapter provides an overview of the  $\ddot{\mathsf{A}}\mathsf{KTA}$  go system and describes the instrument modules and components.

## In this chapter

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2.2	Standard modules and components	14
2.3	Optional modules and components	27
2.4	General system settings	65

# 2.1 System overview

## Introduction

This section provides an overview of the system and its available modules and components.

# Illustration of the ÄKTA go instrument

The illustration below shows the ÄKTA go instrument with all standard components denoted. The instrument in the illustration also has an optional column valve and a column connected.



Part	Function
1	Pump
2	Pump rinsing solution tube
3	Pressure monitor
4	Mixer
5	Injection valve
6	UV monitor
7	Top tray
8	Holder rails
9	Instrument control panel
10	Conductivity monitor
11	Flow restrictor
12	Outlet valve
13	Inlet valve
14	Bottom tray

## Illustration of the flow path



Part	Description
1	Inlet valve
2	Pump
3	Pressure monitor
4	Mixer
5	Injection valve
6	Column
7	UV monitor
8	Conductivity monitor
9	Flow restrictor
10	Outlet valve
11	Fraction collector

# 2.2 Standard modules and components

## In this section

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2.2.2	Pump and pump rinsing system	16
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2.2.4	Mixer	19
2.2.5	Injection valve	20
2.2.6	UV monitor	22
2.2.7	Conductivity monitor	23
2.2.8	Flow restrictor	24
2.2.9	Outlet valve	25
2.2.10	Module panel	26

## 2.2.1 Inlet valve

### Introduction

Inlet valves are used to select which buffers or samples to use in a run. The inlet valve **K9** is a membrane valve with four inlet ports: two buffer inlets, one sample inlet, and one inlet for cleaning solutions. By default, the inlet valve is closed. Once a flow is started, the software automatically opens the **A** inlet. Another inlet can be specified before the flow is started.

The inlet valve can be used to create step or linear gradients, by mixing desired proportions of buffers from the **A** and **B** inlets. When used for gradient formation the valve opens one inlet at a time, and the buffers are mixed in the inlet, pump, and mixer.

The number of inlets to the system can be increased by installing extra optional inlet valves. For information on optional inlet valves, refer to *Section 2.3.3 Optional inlet valves, on page 35*.

## **Location and illustration**

The inlet valve **K9** is the first module in the flow path of ÄKTA go. The following illustration shows the location and parts of inlet valve **K9**.



Part	Description
1	<b>A</b> inlet, with tubing to buffer A
2	<b>Sample</b> inlet, with tubing to sample
3	<b>To Pump</b> outlet, with tubing to the pump
4	<b>B</b> inlet, with tubing to buffer B
5	<b>C</b> inlet, with tubing to cleaning solutions

2 System description
 2.2 Standard modules and components
 2.2.2 Pump and pump rinsing system

## 2.2.2 Pump and pump rinsing system

## Introduction

This section describes the design and main functions of the pump and the pump rinsing system.

## Function of the pump

The ÄKTA go system is fitted with one high precision pump. The pump consists of two pump heads that work alternately to give a continuous, low pulsation liquid delivery.

To ensure delivery of correct liquid volume, the pump must be free from air. Each pump head is equipped with a purge valve that is used for this purpose. For instructions on how to purge the pump heads, refer to *ÄKTA go Operating Instructions (29360951)*.

## Location and illustration of the pump

The pump is installed after the inlet valve and before the pressure monitor in the flow path. The illustration below shows the location and parts of the pump.



Part	Description
1	Outlet port with check valve
2	Purge valve
3	Pump head
4	Inlet port with check valve
5	Connections to pump rinsing system

## Function of the pump rinsing system

The pump rinsing system flushes the low pressure chambers behind the pistons with a low flow of 20% ethanol or aqueous buffer to rinse the pistons and lubricate the piston seals. This prolongs the lifetime of the pump by preventing the deposition of salts from buffers on the pistons and leakage at the seals between the pump chamber and the drive mechanism.

For instructions on how to fill the pump rinsing system, refer to ÄKTA go Operating Instructions (29360951).

# Illustration of the pump rinsing system



Part	Description
1	Inlettubing
2	Outlet tubing
3	Rinsing solution tube holder
4	Rinsing solution tube
5	Tubing connecting pump heads

2.2 Standard modules and components

2.2.3 Pressure monitor

## 2.2.3 Pressure monitor

## Introduction

The pressure monitor measures the pressure in the system. To measure the highest pressure in the system, the pressure monitor is placed after the pump. A pump restrictor is incorporated in the pressure monitor module, to make sure that the liquid delivery is accurate at low flow rates.

Additional pressure sensors can be included in the system by installing the advanced column valve **V9-C**.

## **Location and illustration**

The pressure monitor is installed after the pump and before the mixer in the flow path. The illustration shows the pressure monitor and the connected tubing in the ÄKTA go instrument.



Part	Description
1	Outlet tubing, to the mixer
2	Pressure monitor
3	Tubing connecting the pump restrictor to the pressure monitor
4	Pump restrictor
5	Inlet tubing, from right pump head
6	Inlet tubing, from left pump head

## 2.2.4 Mixer

## Introduction

The ÄKTA go mixer is a static mixer with a volume of 1mL. The mixer uses a titanium membrane to mix buffers and does not contain a filter inside.

*Tip:* Add an online filter to prevent particles from entering the flow path and clogging the column.

Depending on sample constituents, the membrane can become clogged. The membrane can be cleaned manually or using a system Cleaning-In-Place (CIP) method. See Section 5.2.3 Maintenance of the mixer, on page 137 for instructions.

## Location and illustration of the mixer

The mixer is installed after the pressure monitor and before the injection valve. The illustration shows the location and parts of the mixer.



Part	Description
1	Outlet, with tubing to the injection valve
2	Mixer
3	Mixer holder
4	Inlet, with tubing from the pressure monitor

2 System description2.2 Standard modules and components2.2.5 Injection valve

## 2.2.5 Injection valve

## Introduction

The injection valve is used to direct sample onto the column and enables a number of different sample application techniques.

## **Location and illustration**

The injection valve is installed after the mixer in the flow path, and before a column or column valve. The illustration below shows the location and parts of the injection valve.



Part	Description
1	Column port <b>Col</b> , for connection to a column or column valve.
2	Loop port <b>LoopF</b> , for connection and filling of a loop.
3	Syringe port <b>Syr</b> , for connection of a syringe to fill a loop.
4	Waste port <b>W2</b> , for loop waste.
5	Pump port <b>Pump</b> , for connection with the mixer.
6	Loop port <b>LoopE</b> , for connection and emptying of a loop.
7	Waste port <b>W1</b> , for pump waste.
8	Loop

### 2 System description 2.2 Standard modules and components 2.2.5 Injection valve

Position	Flow path illustration	Description
Load	Col LoopF Pump Syr LoopE W2 W1	The flow is directed onto the column or column valve. Sample can be loaded manually into the loop through the syringe port <b>Syr</b> . Excess sample leaves through the waste port <b>W2</b> .
Inject	Col LoopF Syr W2 W1	The flow is directed through the loop and onto the column or column valve. The syringe port can be washed manually in this position.
Waste	Col LoopF Pump Syr LoopE W2 W1	The flow is directed to waste through the <b>W1</b> port. This flow path is used for performing a pump wash.

## **Flow paths**

**Note:** Make sure all ports in the injection value are plugged with either tubing or stop plugs, to avoid liquid spurting out during value turns.

2 System description
 2.2 Standard modules and components
 2.2.6 UV monitor

## 2.2.6 UV monitor

## Introduction

The UV monitor measures the UV absorbance, of buffers and eluted proteins, at the fixed wavelength of 280 nm. The UV monitor includes a monitor unit with a UV flow cell. The standard flow cell path length is 2 mm, but a 5 mm flow cell is also available. The 5 mm flow cell is better suited for high resolution SEC runs due to its lower internal volume of 20  $\mu$ L, compared to 30  $\mu$ L internal volume of the 2 mm flow cell. For instructions on how to replace the UV flow cell, see Section 5.2.4 Maintenance of the UV flow cell, on page 140.

The UV lamp is a LED lamp that does not need warming up before use and does not heat the sample. The lamp is automatically turned on when a run starts, and turned off when the run ends. It also turns off during a run when in state **Pause** or **Errors, alarms and warnings** for more than 30 min and automatically turns on after changing these states.

If running long runs, where the UV lamp is not required, the lamp can be turned off using a manual instruction. See Section 7.6.5 Manual instructions - Monitors, on page 256.

## **Location and illustration**

The UV monitor is installed after the column or column valve and before the conductivity monitor in the flow path. The illustration below shows the UV monitor and the UV flow cell.



Part	Description
1	UV monitor
2	Inlet, with tubing from a column or column valve
3	UV flow cell
4	Outlet, with tubing to the conduc- tivity monitor

Note:

## 2.2.7 Conductivity monitor

## Introduction

The conductivity monitor measures the conductivity of buffers and eluted proteins. The conductivity monitor is factory-calibrated on delivery but can be re-calibrated if needed, see Section 5.3.3 Calibrate the conductivity monitor, on page 159.

As the conductivity of a liquid is dependent on temperature, the conductivity flow cell is fitted with a temperature sensor. To adjust for changes in temperature, the temperature sensor is used in UNICORN together with a temperature compensation factor. Always keep the conductivity temperature compensation on. Turning it off may result in fluctuating conductivity signals, especially if the run is done in a refrigerator where the temperature changes.

## **Location and illustration**

The conductivity monitor is installed after the UV monitor. A flow restrictor is included in the flow path after the conductivity monitor. If a pH valve is installed in the system, the flow restrictor should be moved to the pH valve. See Section 2.2.8 Flow restrictor, on page 24.

The illustration below shows the conductivity monitor with the flow restrictor.



Part	Description
1	Conductivity monitor
2	Flow restrictor
3	Inlet, with tubing from the UV monitor
4	Outlet, with tubing to the flow restrictor or pH valve

2 System description
 2.2 Standard modules and components
 2.2.8 Flow restrictor

## 2.2.8 Flow restrictor

## Introduction

The flow restrictor generates a steady back pressure in the flow path of approximately 0.2 MPa. This prevents formation of air bubbles in the UV flow cell.

**Note:** There is a risk that air bubbles cause large disturbances in the UV signal. Use the automatic pressure control function to avoid pressure alarms, instead of removing the flow restrictor. See Section 3.8 Pressure control, on page 96.

## **Location and illustration**

The flow restrictor is included in the flow path after the conductivity monitor. The conductivity monitor is equipped with a holder for the flow restrictor. If ÄKTA go is fitted with a pH valve, the flow restrictor should be moved from the conductivity monitor to the pH valve.

The illustrations below show the flow restrictor fitted on the conductivity monitor and on the pH valve.

Conductivity monitor with flow restrictor	pH valve with flow restrictor	Part	Description
		1	Flow restrictor
Conduction		2	Outlet
		3	Inlet
4	3 2 2	4	Holder

## 2.2.9 Outlet valve

### Introduction

The outlet valve is used to direct the flow to a Fraction collector, to outlet 1, or to waste.

## Location and illustration

The outlet valve is installed last in the flow path on the instrument. The illustration below shows the location and parts of the outlet valve.



Part	Description
1	<b>In</b> port, with tubing from the flow restrictor or pH valve
2	<b>Frac</b> port, with tubing to the Frac- tion collector
3	Out1 outlet port
4	<b>W</b> waste port

2 System description
 2.2 Standard modules and components
 2.2.10 Module panel

## 2.2.10 Module panel

## Introduction

All positions in the chassis of the instrument must be occupied. Positions not used for standard or optional modules must be fitted with a module panel.

Module panels are installed in the same way as other modules, and must be connected to the cable inside the instrument. See *Install optional modules, on page 28*.

When a module is replaced by a module panel, the removed module has to be deselected in **System Properties** in UNICORN.

## **Location and illustration**

The illustration shows a module panel (indicated in orange) installed on an ÄKTA go instrument equipped with an optional column valve.



# 2.3 Optional modules and components

## In this section

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2.3 Optional modules and components

2.3.1 Installation of optional modules

## 2.3.1 Installation of optional modules

## Introduction

The modules compatible with ÄKTA go are easy to install in the instrument. The installation procedures are similar for most modules. Up to four external modules can also be connected to the instrument via UniNet-9 cables at the back of the system.

## Install optional modules

Follow the steps below to install or replace a module or module panel. Module panels must be used in positions not occupied by modules.

**Note:** The illustrations show the principle of how to replace and install a module. The position of the module on the instrument will depend on the module being installed.

Step	Action
1	Disconnect power from the instrument by using the instrument power button.
2	If a module is to be replaced, loosen the tubing connectors and remove the tubing from the existing module.
З	Loosen the screw in the module or module papel with a Tory T20 screw-

3 Loosen the screw in the module or module panel with a Torx T20 screwdriver.



4

Remove the module or module panel and disconnect the cable at the back.



#### Step Action

5 Connect the cable to the new module or module panel.



Insert the module or module panel and fasten it with a Torx T20 screwdriver.



**Note:** When an optional module is removed or a new module is installed, the system configuration must be updated in UNICORN. See the following section for more information.

### **Install external modules**

6

External modules such as a Fraction collector, an air sensor and an I/O box are not installed in the instrument chassis, but are connected via a UniNet-9 cable at the back of the system. It is possible to install up to four external modules with F-type connectors. Optional inlet valves, if not installed in the instrument chassis, can also be connected via UniNet-9 cables at the back of the system.

Follow the steps below to install or replace an external module.



#### Step Action

1

Disconnect the ÄKTA instrument in the UNICORN system control software, and turn off the ÄKTA instrument.

2.3 Optional modules and components

2.3.1 Installation of optional modules

# Step Action 2 On the back of the ÄKTA instrument, remove the jumper from the UniNet-9

#### Note:

port to be used.

Keep the removed jumper safely. All contacts must have a connected module or jumper.



3 Connect the UniNet-9 cable between the UniNet-9 port on the external module and on the back of the ÄKTA instrument.

#### Tip:

The rounded side of the connector is facing the ÄKTA instrument.

4 Make sure that all unused UniNet-9 ports on the ÄKTA instrument are plugged with jumpers.

## System configuration

When an optional module is removed or a new module is installed, the system configuration must be updated in UNICORN. Follow the steps below to update the system configuration in UNICORN.

#### Step Action

1

In the **Tools** menu in the **Administration** module, click **System Properties** or click the **System Properties** icon to open the dialog.

Result:

The System Properties dialog is displayed.

System Properties		×
Systems	Properties	
DKTA99	AKTA go         AKTA Chromatography system Instrument configuration           System is        ACTA go (1.0.0.8)           Instrument Server        ACTA go (1.0.0.8)           Connection based on        Instrument IP address           Enabled Components        Instrument IP address           Outlet valve (V9-0)         UV monter (C9)           Instrument (C9)         Fraction collector (F9-R)           Presure mondor (P9)         Presure mondor (P9)           Inter valve (K3)         Igection valve (V9-J)	
Print     Edit     Activate	Define System Instrument Configurations	ose

2.3 Optional modules and components

2.3.1 Installation of optional modules

Step	Action							
2	Select the systen <b>Edit</b> . <i>Result:</i>	Select the system of interest in the <b>System Properties</b> dialog and click <b>Edit</b> . Result:						
	The <b>Edit</b> dialog is	displayed.						
	Edit - ÄKTA go				×			
	Instrument configuration	ÄKTA go (1.0.0.8)	~	Information	Import			
	UNICORN Control PC	G1T4MSM2E	~	Rename				
	Real-Time Unit	123456						
	Connect by:	Fixed IP address: 10.1.1.69						
	Component types:	O Instrument serial no.	Test Connection					
	Valves Monitors and sensors Fraction collectors Other Core components (always	present)	Component selection Column valve (V9-Cm) Column valve (V9-C) pH valve (V9-PH) Outlet valve (V9-Ph) A inlet valve (V9-ImA) B inlet valve (V9-ImB) Sample inlet valve (V9-Im)	Property				
	Advanced Setting	s Extensions		ОК	Cancel			

3 Select the type of module installed/uninstalled from the **Component types** list.

#### Result:

The available modules are shown in the **Component selection** list.

#### Note:

Instrument modules are referred to as **Components** in UNICORN.

4 Select the appropriate checkbox and click **OK** to apply the changes.

#### Note:

If applicable, choose the appropriate option in the **Property** column.

## 2.3.2 Air sensor

## Introduction

An air sensor is used to prevent air from entering the liquid flow path of the system. By detecting the presence of air, the air sensor can be used for complete sample loading or to detect if running out of buffer.

## **Location and illustration**

One of two different air sensors, **L9-1.5** or **L9-1.2**, can be installed in ÄKTA go. The communication cable in the air sensor connects to a UniNet-9 port at the back of the instrument. The air sensor can be installed in the following positions:

- L9-1.5 or L9-1.2 before inlet valve on the sample inlet tubing,
  - or
- L9-1.5 air sensor between inlet valve and pump.



When installed before the inlet valve, the air sensor can be used to load sample onto a column until air is detected. In this location, the air sensor can be attached to the rails on the instrument using a suitable clamp or holder, see *Adapter for air sensor, on page 62*.

2.3 Optional modules and components

2.3.2 Air sensor

When installed after the inlet valve, the air sensor **L9-1.5** can detect if a buffer is running out.



Part	Description
1	Tubing connectors
2	Communication cable
3	Holder adapter

## 2.3.3 Optional inlet valves

## Introduction

Three optional inlet valves can be installed on ÄKTA go. The optional inlet valves available for ÄKTA go and their functions are described in the table below.

Inlet valve	Label	Function
A inlet valve	V9-ImA	Enables automatic change between six buffer inlets. Connects to port <b>A</b> of inlet valve <b>K9</b> .
B inlet valve	V9-ImB	Enables automatic change between six buffer inlets. Connects to port <b>B</b> of inlet valve <b>K9</b> .
Sample inlet valve	V9-ImS	Enables automatic loading from five sample inlets and one buffer inlet. Connects to port <b>Sample</b> of inlet valve <b>K9</b> .

## Location

Optional inlet valves must be installed before inlet valve **K9**. The optional inlet valves can either be installed in the instrument chassis or in an extension box. Extension boxes can be installed on the instrument rails or placed on the bench, to the left of the instrument.

# Illustration of V9-ImA and V9-ImB inlet valves

The illustration below shows the ports of **V9-ImA** and **V9-ImB** inlet valves, in this example with label **V9-ImA**. Corresponding **B** inlets are found in the **V9-ImB** inlet valve.



Part	Description
1 to 6	A1 to A6 inlets
7	Out port

2.3 Optional modules and components

2.3.3 Optional inlet valves

## Illustration of inlet valve V9-ImS

The illustration below shows the ports of the **V9-ImS** sample inlet valve.



Part	Description
1 to 5	S1 to S5 inlets
6	Buffer inlet
7	Out port
# 2.3.4 Online filter

#### Introduction

The online filter prevents particles from entering the flow path and clogging the column.

#### Location and illustration

The online filter can be installed before the mixer in the flow path. Use the male-male union connector with inner diameter of 0.75 mm (union 1/16"M / 1/16"M) supplied in the accessories box, to screw the online filter directly onto the bottom of the mixer. The illustration shows the location and parts of the online filter.



Part	Description
1	Top nut
2	Holder
3	Support net
4	Filter
5	Bottom nut

2.3 Optional modules and components

2.3.5 Column valves

# 2.3.5 Column valves

#### Introduction

Column valves can be used to connect several columns to the system. The optional column valves **V9-Cm** and **V9-C** allow the user to choose the column, the flow direction through the column, or to bypass the columns.

The column valves available for ÄKTA go and their functions are described in the table below.

Label	Description
V9-Cm	Up to three columns can be connected to the valve.
V9-C	Up to five columns can be connected to the valve.
	Contains two pressure sensors, one before the column and one after the column. These measure the pre- and post-column pressure, which are used to calculate the delta-column pressure.

#### **Location and illustration**

The column valve is installed after the injection valve and before the UV monitor. The illustration below shows the location and parts of the **V9-Cm** and **V9-C** column valves.



Part	Description
1	<b>1A</b> port, with tubing to the top of a column.
2	In port, with tubing from the injection valve.
3	<b>Out</b> port, with tubing to the UV monitor.

Part	Description
4	<b>1B</b> port, with tubing to the bottom of a column.

**Note:** If the system is running in downflow mode, the liquid flow path is directed from the column valve onto the column through the **A** ports, and from the column onto the column valve through the **B** ports.

If the system is running in upflow mode, the liquid flow path runs in the opposite direction.

2 System description2.3 Optional modules and components2.3.6 pH valve

# 2.3.6 pH valve

#### Introduction

The pH valve is used to direct the flow to a pH electrode when in-line monitoring of pH is desired during a run. For instructions on how to install the pH electrode, see *Replace the pH electrode, on page 147*.

When using a pH valve, move the flow restrictor from the conductivity monitor to the pH valve. The flow restrictor generates back pressure to prevent the formation of air bubbles. The pH valve directs the flow to the pH electrode and to the flow restrictor, or by-passes one or both.

#### **Location and illustration**

The pH valve should be installed last in the flow path, right before the outlet valve. This prevents the pH electrode in the valve from being exposed to high pressure. During a normal run, the electrode should not be exposed to more than 0.5 MPa but can with-stand transient pressure pulses of 0.8 MPa.

The illustration below shows the location and parts of the pH valve with the flow restrictor.



Part	Description
1	pHelectrode
2	<b>Cal</b> calibration port
3	<b>ToR</b> port, with tubing to the flow restrictor
4	W3 waste port
5	<b>In</b> port, with tubing from the conductivity monitor
6	<b>FrR</b> port, with tubing from the flow restrictor
7	<b>Out</b> port, with tubing to the outlet valve
8	Flow restrictor

#### **Flow paths**

The table below describes the different flow paths through the pH valve.

2 System description 2.3 Optional modules and components 2.3.6 pH valve

Position	Flow path illustration	Description
By-pass Both	Cal ToR W3 Out FrR	Both the pH electrode and flow restrictor are by-passed.
Restrictor Only	Cal ToR W3 Out MPa	The flow restrictor is in use and the pH electrode is by-passed.
Restrictor and pH	Cal ToR W3 Out FrR Out	Both the pH electrode and flow restrictor are in use.

2.3 Optional modules and components

2.3.6 pH valve

Position	Flow path illustration	Description
pH Only	Cal TOR W3 Out FrR	The pH electrode is in use and the flow restrictor is by-passed.
Calibration	ToR W3 Out MPa	The calibration port <b>Cal</b> is used to inject solution into the flow cell using a syringe. Excess solution leaves the valve through waste port <b>W3</b> . This flow path is used when calibrating the pH monitor and when filling the pH flow cell with storage solution.

2 System description 2.3 Optional modules and components 2.3.7 Fraction collector F9-T

## 2.3.7 Fraction collector F9-T

#### Introduction

A Fraction collector is used for the collection of fractions from purification runs. The F9-T Fraction collector can be used for the following functions:

- Fixed volume fractionation
- Peak fractionation
- Combined fixed volume fractionation and peak fractionation.

For a more detailed description of the fractionation functions see Section 3.3 Fractionation overview, on page 83.

The fractions can be collected in plates or tubes, see the table in *Tubes, on page 49* for supported plates and tubes.

The Fraction collector can be placed in a tunnel below an ÄKTA go instrument or beside the ÄKTA instrument.

The Fraction collector F9-T can use the drop sync function to reduce risk of spillage during movement between fractions. Technical details are found in *Section 3.3 Fractionation overview, on page 83*.

#### Illustrations

#### **Front view**



The illustration below shows the main parts of Fraction collector F9-T.

2.3 Optional modules and components

2.3.7 Fraction collector F9-T

Part	Description
1	Nozzle
2	Fractionation arm
3	Tube rack for 50 mL tubes
4	Home position
5	Plate position 1
6	Plate position 2

#### **Back view**

The illustration below shows the back view of Fraction collector F9-T.

The Fraction collector is connected to the ÄKTA instrument via the UniNet-9 F-type connector port, both for communication and power supply.



Part	Description
1	UniNet-9 F-type connector port
2	Node ID
3	Status LED

# Connect Fraction collector without tunnel

The Fraction collector can be placed beside ÄKTA go. The recommended tubing length for such a setup is 40 cm.

**Note:** Longer tubing increases back pressure and peak broadening in the chromatographic process.

Follow the steps below to connect the tubing from the ÄKTA go instrument to the Fraction collector F9-T.

Step	Action
1	Connect one end of the tubing to the <b>Frac</b> port on the outlet valve on the ÄKTA go instrument using a fingertight connector.
2	Connect the other end to the selected nozzle.

3 Insert the nozzle into the fractionation arm.



4

Adjust the delay volume setting in the UNICORN software, see Section 7.9 *Delay volumes, on page 269*.

#### Note:

The default delay volume setting in UNICORN is defined for the 40 cm tubing with inner diameter 0.5 mm (orange tubing).

2 System description
 2.3 Optional modules and components
 2.3.7 Fraction collector F9-T

# Connect Fraction collector using a tunnel

To reduce the footprint, the Fraction collector F9-T can be positioned under the ÄKTA go instrument by using a tunnel.

Follow the steps below to place the ÄKTA go instrument on top of the tunnel and install the fraction collector F9-T inside the tunnel.

Step	Action

- 1 Place the tunnel where you want your installation.
- 2 Use two persons to lift the ÄKTA go instrument onto the tunnel in a safe manner.



#### CAUTION

**Heavy object.** Use proper lifting equipment, or use two or more persons when moving the instrument. All lifting and moving must be performed in accordance with local regulations.

- 3 Make sure that the front feet of ÄKTA go are placed on top of the pins at the front of the tunnel.
- 4 Connect the UniNet-9 cable to the back of ÄKTA go and pull the cable into the tunnel from the back.
- 5 Connect the other end of the UniNet-9 cable to the fraction collector and put the fraction collector securely on the tunnel tray.
- 6 Connect 80 cm tubing to the **Frac** port on the outlet valve of ÄKTA go.
- 7 Guide the tubing inside the tunnel using the tubing guide on the left-hand side inside the tunnel.



8 Attach the tubing to the nozzle and attach the nozzle guide to it.



#### **Dispenser head**

The illustration below shows the dispenser head of Fraction collector F9-T.



Part	Description
1	Dispenser head
2	Nozzle

2.3 Optional modules and components

2.3.7 Fraction collector F9-T

Part	Description
3	Drop sync sensor
4	LED light

#### **Drop sync function**

To reduce sample spillage during fractionation, the fraction collector can use the Drop sync function. Technical details are found in *Section 3.3 Fractionation overview, on page 83*.

#### **LED** light

A LED light in the dispenser head indicate ongoing fractionation and illuminate the fractionation area. It can be turned on or off in **System settings** in UNICORN.

#### **Plates**

The tables below list available plate types and recommended plate manufacturers. Plates from other manufacturers can work if they are of the same quality and dimensions.

Plates	Maximum volume	Default fractionation volume
96 well microplate <sup>123</sup>	0.3 mL	0.1 mL
96 deep well plate <sup>4</sup>	2 mL	2 mL
48 deep well plate <sup>5</sup>	4.5 mL	4 mL
24 deep well plate <sup>4</sup>	9 mL	8 mL

<sup>1</sup> Microplate holder F9-T and micro nozzle are required.

<sup>2</sup> If microplates with maximum volume less than 0.3 mL is used, change to blue (i.d. 0.25 mm) tubing between UV detector and Fraction collector F9-T.

- <sup>3</sup> Adjust delay volume accordingly.
- <sup>4</sup> Only standard height 44 mm with square wells.
- <sup>5</sup> Only standard height 44 mm with rectangular wells **A1** to **H6**.

Plates form the following manufacturers are recommended:

- Whatman™
- Corning<sup>™</sup>
- Greiner<sup>™</sup>
- Nunc<sup>™</sup>

#### **Tubes**

The table below lists tubes and suitable holders.

For tube sizes 0.5 mL, 1.5 mL, and 2 mL, the manufacturer Eppendorf<sup>™</sup> is recommended. For 15 mL and 50 mL tube sizes, the manufacturer Falcon™ is recommended.

Tubes from other manufacturers can be used if they are of the same quality and dimensions.

Tube size	Suitable holder
0.5 mL tubes	48 position rack
1.5 mL tubes	24 deep well plate <sup>1</sup>
2 mL tubes	24 deep well plate <sup>1</sup>
15 mL tubes <sup>2</sup>	Tube rack for 50 mL tubes
50 mL tubes	Tube rack for 50 mL tubes

<sup>1</sup> Only for tubes with an attached lid.
 <sup>2</sup> Only for the Home position, not for fractionation.

2 System description
 2.3 Optional modules and components
 2.3.7 Fraction collector F9-T

# Correct tube position in a 24 deep well plate

1.5 mL and 2.0 mL tubes are placed in a 24 deep well plate with their lids bent backward, and inserted in 45 degrees from the well structure with the lids in the upper right corners. For example, a tube in the **A1** position have the tube facing the outer corner, and the lid facing the opposite corner.



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#### Correct tube position in a rack

0.5 mL tubes are placed in a rack with 48 positions with their lids bent backward. The tube is positioned in the round part of the hole, and the lid is positioned in the lid shaped part of the hole



#### **Dispensing position and adjustment**

For all plates and tubes, except for the 0.5 mL tubes, the default positioning of the nozzle is not at the center of the well/tube.

Depending on the running conditions, or if non-standard plates are used, the default position might need to be adjusted. There is an adjustment setting available in the **System Control**  $\rightarrow$ **System**  $\rightarrow$ **Adjust drop position (F9-T)**. See below.

- 2.3 Optional modules and components
- 2.3.7 Fraction collector F9-T

Adjust Drop Positi	ion (F9-T) ÄKTA go	
<u>P</u> late Type	96 deep well plate	~
Adjust <u>L</u> eft / Right	-1.0 mm [-2.0 - 2.0]	
Adjust <u>D</u> own / Up	-1.0 mm [-2.0 - 2.0]	
R	estore to Default	
Preview		
2 1	efault ➡	
	A B	
0	Apply	

#### **Default dispensing position**

The default dispensing position is chosen for optimal liquid behavior in the wells/tubes. The values in the table below represent the nozzle distance (in mm) from the center of wells/tubes in left/right and down/up direction, respectively.

Plate type	Default offset from center of well Left/ Right [mm]	Default offset from center of well Down/Up [mm]
96 deep well plate	-1.0	-1.0
96 microplate	-0.5	-0.5
48 deep well plate	-0.8	-5.3

2 System description 2.3 Optional modules and components

Plate type	Default offset from center of well Left/ Right [mm]	Default offset from center of well Down/Up [mm]
24 deep well plate	-5.5	-5.5
0.5 mL tubes	0.0	0.0
1.5 mL tubes	-0.8	-0.8
2 mL tubes	-0.8	-0.8
50 mL tubes	0.0	8.0

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# 2.3.8 Fraction collector F9-R

#### Introduction

A Fraction collector is used for the collection of fractions from purification runs. The F9-R Fraction collector can be used for the following functions:

- Fixed volume fractionation
- Peak fractionation

For a more detailed description of the fractionation functions see Section 3.3 Fractionation overview, on page 83.

In Fraction collector F9-R fractions can be collected in tubes of the following sizes:

Tube length	Tube diameter
50 to 180 mm	12, 18, and 30 mm

To reduce sample spillage during fractionation, the Fraction collector F9-Rcan use the **Drop sync** function, for a system flow up to 2 mL/min. Technical details are found in *Fraction collector F9-R Operating instructions (29656880)*.

#### Illustration



The illustrations below shows the main parts of the F9-R Fraction collector.

Part	Description
1	Lock knob
2	Stationary part of delivery arm
3	Delivery arm
4	Tubing connector
5	Tube sensor
6	Collection tubes
7	Tuberack
8	Base unit
9	Node ID switch
10	UniNet-9 F-type connector (for communication and power supply)

2.3 Optional modules and components

2.3.8 Fraction collector F9-R

#### **Connect tubing**

Step	Action
1	Remove the tubing connector from the delivery arm and insert the tubing through the tubing connector.



2 Use the tube adjustment cavity on the delivery arm to expose the correct length of tubing from the tubing connector. Insert the tubing into the tube adjustment cavity and slide the connector down towards the delivery arm. Tighten the tubing connector when correct tubing length is exposed.



- 3 Re-install the tubing connector on the delivery arm.
- 4 Adjust the position and height of the delivery arm. Use the adjustment knob to place the tube sensor in the correct position for the used tubes. Refer to *Fraction collector F9-R Operating instructions (29656880)* for detailed instructions.



- 5
- Connect the 40 cm precut tubing from the Fraction collector to the **Frac** port on the outlet valve.

#### Step Action

6 Adjust the delay volume setting in UNICORN to the volume of the tubing. See *Section 7.9 Delay volumes, on page 269* for more details.

#### Note:

The default delay volume is correct and does not need to be set if standard tubing (40 cm) is used.

2 System description2.3 Optional modules and components2.3.9 I/O-box

## 2.3.9 I/O-box

#### Introduction

The I/O-box is used to interface other equipment in order to measure parameters such as refractive index, light scattering, and fluorescence as analog signals. The I/O-box can control external equipment by a digital output signal, and also detect the equipment state by digital input. It is also possible to send out internal detector signals to external equipment.

For information on requirements of the equipment that can be connected to the I/Obox, refer to the *Install I/O-box E9* document.

#### **Location and illustration**

One I/O-box can be installed on ÄKTA go. The illustration below shows the I/O-box and its recommended location.



Part	Description
1	I/O-box
2	UniNet-9 F-type cable
3	D-sub cable
4	Multi-purpose holder
5	Clip

# 2.3.10 Accessories

#### Introduction

This section describes the available accessories for ÄKTA go. Holders and clamps are used to attach or organize columns, tubing and bottles to the instrument. These can be attached to the rails of the instrument.

Two column clamps and one multidirectional column clamp are delivered with the standard system. The remaining accessories listed in this section can be ordered separately. For ordering information, see *Chapter 8 Ordering information, on page 282*.

#### **Column clamp**

The column clamp can be used to attach small sized columns. Use two clamps to attach long columns.

The illustration below shows the column clamp.



Part	Description
1	Position for a column
2	Inner end tabs

#### Multidirectional column clamp

The multidirectional column clamp can be used to attach small sized columns directly to the UV monitor. The direction of the clamp can be changed, for use on other systems, to connect a column directly to the multi-wavelength UV monitor **U9-M**. In systems with long rails it is possible to use two base clamps to place a column horizon-tally on the system.

The illustration below shows the multidirectional column clamp.

2.3 Optional modules and components

2.3.10 Accessories



Part	Description
1	Base clamp with snap-in to holder rails
2	Position for a column

#### **Column holder**

The column holder has one position for medium sized columns and one position for small sized columns. The column holder can also be used for bottles. Use two holders to attach long columns.

The illustration below shows the column holder.



Part	Description
1	Position for a medium sized column or bottle
2	Position for a small sized column
3	Tab for holder attachment/ detachment
4	Snap-in to holder rails

#### Column holder rod

The column holder rod is used to attach several HiTrap<sup>™</sup> columns. The holder has threaded ports for HiTrap columns and tubing connectors. Push the button of the holder to attach the holder to a holder rail.

The illustration below shows the column holder rod.



Part	Description
1	Positions for columns
2	Button for holder attachment/ detachment
3	Snap-in to holder rails

#### **Tubing holder spool**

The tubing holder spool is used to hold and arrange tubing. It can be useful when using additional inlet valves in the instrument.

The illustration below shows the tubing holder spool.



#### **Tubing holder comb**

The tubing holder comb is used to hold and arrange tubing. The illustration below shows the tubing holder comb.



Par t	Description
1	Positions for tubing
2	Tab for holder attachment/ detachment
3	Snap-in to holder rails

2.3 Optional modules and components

2.3.10 Accessories

#### **Bottle holder**

The bottle holder can be used for holding bottles when attached to the holder rails. For example, to hold a sample bottle. It can also be used to hold an air sensor fitted with an adapter.

The illustration below shows the bottle holder.



#### Adapter for air sensor

The adapter for air sensor is used to fit an external air sensor into the bottle holder. The illustrations below show how to install the adapter onto the air sensor, and bottle holder.



Part	Description
1	Airsensor
2	Air sensor adapter
3	Bottle holder

#### Multi-purpose holder

The multi-purpose holder can be used for different functions. Secure the I/O-box cable to the multi-purpose holder when the unit is attached to the ÄKTA go instrument; or hold the holder for flat 10 mL sample loops. The multi-purpose holder can be attached to a holder rail on the instrument.

The illustration below shows the multi-purpose holder.

2 System description 2.3 Optional modules and components 2.3.10 Accessories



#### **Inlet filters**

Inlet filters can be attached to inlet tubing for the filtering of buffers. Attach inlet filters to inlet tubing, by inserting the tubing into the inlet filter holder and rotating it until securely attached. The inlet filters are also useful to keep the tubing at the bottom of buffer bottles.

The illustration below shows an inlet filter.



#### **Rail extension**

The rail extension rod can be used to attach accessories, such as column holders or a multi-purpose holder. The rod has extra rails on both sides. Push the button of the rod to attach it to a holder rail.

The illustration below shows the rail extension rod.

2.3 Optional modules and components

2.3.10 Accessories



Part	Description
1	Button for holder attachment/ detachment
2	Extension rod

#### **Extension box**

The extension box can be used to install extra valves on the ÄKTA go instrument outside the system chassis, if all positions in the chassis are filled.

The illustration below shows an extension box with an optional inlet valve mounted on the side of ÄKTA go.



The extension box can be mounted in two ways.

- On the side of ÄKTA go.
- Standing next to ÄKTA go.

# 2.4 General system settings

#### Introduction

System settings can be accessed through the **System Control** module in UNICORN, by selecting **System**  $\rightarrow$  **Settings**.

This section describes the system settings related to delay volume, instrument control panel, power-save mode, and wash settings. For more system settings information see *Section 7.5 System settings, on page 231*.

#### **Delay volume**

The delay volume is the retention volume that has to be dispensed before collecting the fractions indicated in the chromatogram. It corresponds to the volume in the flow path from the UV monitor to the Fraction collector. There is a default value set for the delay volume, if no changes are done in the flow path after the UV monitor this value is correct. If a module has been installed after the UV monitor in the flow path or if the tubing dimensions have been changed, the delay volume has to be adjusted to make sure that the fractions are dispensed in the correct tubes. See Section 7.9 Delay volumes, on page 269, for information on standard delay volumes and instructions to determine delay volumes.

System Settings - ÄKTA go		$\times$
Instructions: UV Conductivity P pH Pressure alarm Fraction collection Fraction collection Fractionation settings Peak frac parameters UV Fraction numbering mode Delay volume W adsh settings Watch parameters Advanced Data collection	Parameters for Delay volume Detector - Frac [0 - 7999] 223 ↓ µl Restrictor volume [0 - 999] 48] ↓ µl pH cell volume [0 - 999] 76 ↓ µl	
0	Set Parameters To Strategy Default Values OK Cancel	

#### Instrument control panel

The **Pause** and **Continue** buttons of the **Instrument control panel** can be locked or unlocked. To do this in UNICORN, select *Advanced* →*Instrument control panel* in the *System Settings* dialog, in *System control*.

#### 2.4 General system settings

System Settings - ÄKTA go				×
Instructions:	^	Parameters for Instrument control panel Mode		
Pressure alam     Fraction collection     Wash settings     Watch parameters     Advanced     Power-save     Instrument control panel     Pressure control panel     Pressure control panel     Max flow during valve tum     Data collection     <				
<b>@</b>		Set Parameters To Strategy	Default Values C	Cancel

#### **Power-save**

ÄKTA go has a power-save mode. The instrument enters **Power-save** after having been in **Ready** state for a set period of time. The system enters the **Ready** state when a method run, a method queue, or a manual run ends.

To enable **Power-save** in UNICORN, select **Advanced**  $\rightarrow$  **Power-save** in the **System Settings** dialog.

#### Note: To activate Power-save, a system must be connected and in Ready state.

System Settings - ÄKTA go		×
Instructions: Conductivity PH Pressure alarm Fraction collection Wash settings Watch parameters Advanced Pressure control panel Pressure control panelers Max flow during valve turn Parts collocation Valve State	Parameters for Power-save Mode	
<b>@</b>	Set Parameters To Strategy Default Values OK	Cancel

**Note: Power-save** lowers the power consumption of the instrument to < 20 W when the instrument is not in use, by turning off power to all modules except the instrument control panel.

#### Wash settings

Wash settings, including wash volumes and flow rates, are defined by default and optimized for the system. See Section 3.6 System wash and pump wash, on page 91, for reasons to change the default settings for system wash and pump wash.

#### **Pump wash**

To change the volume and the flow rate for pump wash and system wash in UNICORN, select **Wash settings** in the **System Settings** dialog.

System Settings - ÄKTA go Instructions: UV Conductivity PH Pressure alarm Fraction collection Wash settings System wash settings Watch parameters Advanced Data collection	Parameters for Pump wash settings Pump wash flow rate [1.00 - 65.00] 65.00] ☆ mi/min Pump wash volume [10 - 1000] 65] ☆ ml	×
	Set Parameters To Strategy Default Values OK Cancel	

#### System wash

To change the flow rate and post wash volume for system wash in UNICORN, select *Wash settings* in the *System Settings* dialog.

nstructions	Parameters for System wash setti	ngs
tV     Conductivity     pH	Row rate 25.	[1.00 - 25.00] 00] 🗘 ml/min
Pressure alarm     Fraction collection     Wash settings     Pump wash settings	Post wash volume 6.0	[0.000 - 1000.000] 00 ♀ ml
System wash settings Watch parameters Advanced Data collection		

**Post wash volume** sets the volume to wash the system with after System wash has been performed. It includes a 6 mL post wash volume by default with the flow rate used when System wash was executed. The post wash volume can be changed. If the most recently used flow rate was 0 mL/min before System wash was issued, the **Flow rate** set in **System wash settings** will be used for Post wash volume.

# 3 Considerations when running the system

#### About this chapter

Basic instructions on how to prepare and perform a run are given in *ÄKTA go Operating Instructions*. This chapter contains additional information along with tips related to operation of the system.

#### In this chapter

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# 3.1 Sample application

#### About this section

This section gives an overview of the different sample application techniques available for ÄKTA go, and includes considerations to make when choosing sample application technique.

### In this section

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3 Considerations when running the system

3.1 Sample application

3.1.1 Sample application techniques

# 3.1.1 Sample application techniques

There are three techniques for sample application on ÄKTA go: using a sample loop, a Superloop, or the pump. Several factors should be considered in order to choose the most appropriate sample application technique.

The table below shows the suitable sample volumes for the different sample application techniques.

Sample application technique	Volume
Sample loop injection	25 µL to 10 mL
Superloop injection	1 to 150 mL
Pump injection	>5mL

The table below shows additional considerations regarding the different sample application techniques.

Sample application technique	Pros	Cons
Sample loop injection	<ul> <li>Only option for small sample volumes (25 µL to 5 mL)</li> <li>No sample in the pump or mixer</li> <li>All sample is loaded onto the column</li> </ul>	<ul> <li>Manual loading of sample loop from syringe</li> <li>Need to empty the loop 3 to 5 times the loop volume to load all sample. Sample is diluted.</li> </ul>
Superloop injection	<ul> <li>Suits repeated application of the same sample</li> <li>No sample dilution</li> <li>Little sample loss for medium volumes</li> <li>No sample in the pump or mixer</li> <li>Transparent graded sample container, possible to check quality and monitor injected volume</li> </ul>	<ul> <li>Manual loading of the Superloop</li> <li>Needs to be cleaned after use</li> </ul>

3 Considerations when running the system 3.1 Sample application 3.1.1 Sample application techniques

Sample application technique	Pros	Cons
Pump injection	<ul> <li>Loading of large amount of sample</li> <li>Easy (only requires priming of the sample inlet with correct sample)</li> <li>Possibility to use air sensor to load all sample</li> <li>No extra equipment needed</li> </ul>	<ul> <li>Not possible to load small sample volumes due to the flow path volume</li> <li>Sample in the flow path from inlet valve to column needs to be pushed onto the column</li> <li>Not possible to load the sample left in the inlet tubing without an air sensor</li> <li>Sample has to travel through the entire flow path up to the column, which has to be cleaned after use</li> </ul>

3 Considerations when running the system

3.1 Sample application

3.1.2 Sample application using a sample loop

# 3.1.2 Sample application using a sample loop

#### Introduction

A sample loop is recommended for injection of small sample volumes onto a column.

#### Filling a sample loop

There are two ways to fill a sample loop: partial filling and overfilling. With partial filling, there is no sample loss but reproducibility is lower if the same procedure is repeated. With overfilling, a better volume accuracy is obtained. For a complete fill, load three to five times the loop volume to obtain high accuracy. The needed volume depends on the loop dimensions (length and i.d.). Generally, the larger the loop volume the less overfill is needed.

The image below illustrates these concepts, in this example using a 100  $\mu L$  loop with an inner diameter of 0.5 mm.



**Note:** For partially filled sample loop, do not fill more than half of the total loop volume. If more is applied, a portion of the sample might pass through and out of the loop.

#### Emptying a sample loop

To avoid dilution when emptying a sample loop, empty it in the opposite direction from which it was filled. This is done by design in ÄKTA systems.

The volume needed to achieve complete recovery will vary with the flow rate, loop dimensions, and the properties of the sample, but usually three to five times the loop volume is sufficient.

The image below shows an example of the recovery achieved at different volumes when emptying a 100  $\mu L$  loop at 0.5 mL/min. To empty the loop completely, in this example a buffer volume corresponding to three times the loop volume was needed.


To achieve high sample recovery, use a large volume to empty the loop. For nonbinding techniques, such as desalting and SEC, there are sample volume limitations due to the size of the column used.

The chromatogram below shows how the separation in an SEC column is affected by different volumes used to empty the loop during sample injection. Here resolution is increased by using a lower volume to apply the sample onto the column.



3 Considerations when running the system

3.1 Sample application

3.1.3 Sample application using a Superloop

## 3.1.3 Sample application using a Superloop

#### Introduction

A Superloop can be used to load medium to large volumes of sample onto a column. The Superloop is connected to the injection valve and filled with a syringe.

A Superloop can also be used for multiple injections, for example in a scouting experiment when the same application conditions are required.

When a Superloop is used in-line, its pressure limit should be considered. Set a pressure alarm to the Superloop that corresponds to the specification of the Superloop in use. Refer to *Measured pressure, on page 93* for more information.

#### **Prepare the Superloop**

To avoid injecting air into the flow path of the instrument, the Superloop should be prefilled with buffer manually before attaching the Superloop to the system. See the corresponding Superloop instructions for information on how to assemble and prefill a Superloop with buffer.

#### **Connect the Superloop**

Follow the steps below to connect the Superloop to the injection valve:

Step	Action
1	Attach the Superloop to the instrument using a column holder.
2	Connect the top tubing from the Superloop to the <b>LoopE</b> port on the injec- tion valve.
3	Connect the bottom tubing from the Superloop to the <b>LoopF</b> port on the injection valve.

#### **Fill the Superloop**

Follow the steps below to fill the Superloop using a syringe:

Step	Action
1	In the <b>Process Picture</b> , make sure that the injection valve is in position <b>Load</b> .
	<b>Note:</b> Load is the default position for the valve.
2	Fill a syringe with sample.

**Note:** If using a predefined method, a suitable pressure limit for the Superloop is automatically set.

Step	Action
3	Connect the syringe to the <b>Syr</b> port in the injection valve.
	<b>Note:</b> Make sure that there is no air in the syringe before connecting the syringe to the injection valve.
4	Load the sample by emptying the syringe into the injection valve.
5	Disconnect the syringe and plug the <b>Syr</b> port with a stop plug.
	Note:
	a stop plug.

3 Considerations when running the system

3.1 Sample application

3.1.4 Sample application using pump injection

## 3.1.4 Sample application using pump injection

#### Introduction

Use the pump to load large volumes of sample onto the column. Sample is loaded using the **Sample** inlet on inlet valve **K9**.

The smallest recommended volume to load using the pump is 5 mL. This is because the instrument has a dead volume between the inlet valve and the injection valve of approximately 4 mL. The dead volume is illustrated below.



Make sure to load the sample remaining in the flow path between the inlet valve and the injection valve onto the column, especially when loading small sample volumes. This is done by pumping buffer from the **A** inlet onto the column after loading sample, and before continuing the run. When using predefined methods, this is done by clicking the **Finalize Sample injection** tick box.



To prepare for pump injection, prime the sample manually to the inlet valve. For instructions, refer to ÄKTA go Operating Instructions.

# Multiple sample injections using the pump

It is possible to use the pump to inject up to five samples after each other, for instance in a scouting run. For this you have to install an optional sample inlet valve and connect it to the **Sample** inlet of inlet valve **K9**. Load all sample in the flow path, from the optional sample inlet valve to the injection valve, with buffer from the optional sample inlet valve. This is done by selecting the *Finalize Sample injection* option in the predefined methods. 3 Considerations when running the system

3.2 Air sensor

## 3.2 Air sensor

#### About this section

This section describes how the air sensors can be used on ÄKTA go.

#### Introduction

ÄKTA go can be equipped with one of two different air sensors, **L9-1.5** or **L9-1.2**. Air sensor **L9-1.5** can be used either for complete sample loading or to detect when a buffer is finished. **L9-1.5** is placed before the inlet valve for complete sample loading and between the inlet valve and the pump to detect when a buffer is finished. The two location options are illustrated below.

Air sensor **L9-1.2** can only be placed before the inlet valve for complete sample loading. It is not recommended to place it between the inlet valve and the pump.



Note:

The location of the air sensor must be set in UNICORN during system configuration.

### In this section

Section		See page
3.2.1	Air sensor for complete sample loading	80
3.2.2	Air sensor L9-1.5 to detect finished buffers	82

3 Considerations when running the system

3.2 Air sensor

3.2.1 Air sensor for complete sample loading

### 3.2.1 Air sensor for complete sample loading

#### Introduction

For complete sample loading, place the air sensor L9-1.5 or L9-1.2 on the sample tubing connected to the **Sample** inlet on inlet valve K9. Disable the air sensor alarm in the method and program the method so that when air is detected in the flow path, sample loading stops and the sample remaining between the inlet valve and the column is loaded onto the column with the buffer from the **A** inlet. This is done by selecting *Inject all sample using air sensor* and then *Finalize sample injection* in the predefined method.

**Note:** Do not connect the **L9-1.2** air sensor with narrow tubing o.d. 1/16" since the high flow rate during a pump wash might cause cavitation. Use connectors Union 1/16" Male, 5/16" Female fitting tubing connector, 5/16" + Ferrule (yellow), 1/8".

It is recommended to install the air sensor at a minimum distance of 150 mm from the inlet valve, when working at the maximum flow rate. This provides sufficient time for the system to stop the liquid flow before air enters the inlet valve.

**Note:** If using a low flow rate, of 5 mL/min or less, a distance of 100 mm is enough.



#### Air sensor for complete sample loading with optional sample inlet valve

It is possible to use the air sensor for complete sample loading with an additional sample inlet valve as well. This is useful when up to five different samples are loaded in a scouting run. When this function is used, the air between the additional sample inlet valve and inlet valve **K9** needs to be removed before the next sample is loaded. This is done using two pump washes, which are included in the predefined methods. One pump wash from the **Buffer** inlet in the sample inlet valve, followed by another pump wash from the **A** inlet. It is important that the distance from the sample inlet valve to inlet valve **K9** is not more than 400 mm, to make sure that the pump can remove the air in this flow path.



3 Considerations when running the system

3.2 Air sensor

3.2.2 Air sensor L9-1.5 to detect finished buffers

### 3.2.2 Air sensor L9-1.5 to detect finished buffers

To detect when a buffer is finished, place the air sensor **L9-1.5** between the inlet valve and the pump.



To use the air sensor L9-1.5 to detect when a buffer is finished, the air sensor alarm needs to be activated. If using a predefined method, the air sensor alarm is activated by default. If performing a manual run, the *Air sensor alarm* is activated by the user in *System Settings*. See *Section 7.5.5 System settings - Air sensor, on page 237* for details.

After replacing the empty bottle of buffer, prime the inlet tubing and perform a pump wash to remove the air from the flow path before continuing the run.

**Note:** It is not recommended to place the air sensor between inlet valve **K9** and the pump for complete sample loading. This is because all sample in the flow path between the air sensor and the injection valve (up to 4 mL) is lost when the air is removed using a pump wash. If the air sensor is placed before inlet valve **K9**, only the amount of sample present between the air sensor and inlet valve **K9** will be lost (approximately 0.3 mL).

## 3.3 Fractionation overview

#### Introduction

ÄKTA go can be equipped with a Fraction collector, to collect fractions of the chromatographic separation.

If a Fraction collector is installed, the system can perform the following types of fractionation:

- Fixed volume fractionation
- Peak fractionation
- Combined fixed volume fractionation and peak fractionation. (F9-T only).

During fractionation there is a delay in retention between when the fractionation mark is seen in the UV to when the same liquid is dispensed in the Fraction collector. This socalled delay volume corresponds to the volume of the flow path from the UV monitor to the Fraction collector and must be dispensed before what is detected in the UV reaches the Fraction collector. The volume present in the flow path from the UV monitor to the Fraction collector when the first fractionation mark is shown in the chromatogram is called the pre-fractionation volume. The pre-fractionation volume has the same size as the delay volume. A default delay volume is set for the standard system. If anything is changed in the flow path from the UV monitor to the Fraction

The Fraction collectors have a Drop sync (drop synchronization) function that is used during low flow rates to synchronize movement after drop release. The Drop sync settings differ between the two Fraction collectors.

#### **Drop sync in F9-T**

The available settings are **On**, **Off**, and **Auto**. The flow rate limit for when drop synchronization works depends on the properties (for example viscosity) of the liquid, of the plate type used (distances between wells), and type of nozzle used (size of the drops). The **Auto** setting turns the drop synchronization on for flows below 3 mL/min, and turns it off for flows above 3 mL/min. This is done for all plate types except 96 deep well, where the limit is 5 mL/min.

**Note:** These flow rates are optimized for water-based buffers and the standard nozzle. Do not use the **Auto** setting together with a micro nozzle because the micro nozzle only form drops up to 1.5 mL/min.

#### **Drop sync in F9-R**

The available settings are, **On** and **Off**. It is recommended to use Drop sync for flow rates below 2 mL/min. Higher flow rates can however be used, depending on the properties (for example viscosity) of the liquid.

- 3 Considerations when running the system
- 3.3 Fractionation overview

#### **Fixed volume fractionation**

In fixed volume fractionation, the Fraction collector collects the set liquid volume and moves to the next tube.

The illustration below shows an example chromatogram from fixed volume fractionation using Fraction collector F9-R The fractions collected and the numerical marking of fractions are indicated.



- **Note:** The pre-fractionation volume is collected in the first tube of the Fraction collector F9-R. This is not indicated with a numerical fraction mark on the chromatogram.
- **Note:** In F9-T the collection of pre-fractionation volume can be set in system settings. See Section 7.5.6 System settings Fraction collection F9-T, on page 238 for more information.

#### **Peak fractionation**

In peak fractionation, a peak detection algorithm uses the UV signal to determine when to start and stop fractionation. To allow for different parts of the peaks to be analyzed, the fraction size during elution can be set to a value smaller than the expected peak volume.

There are three different modes to perform peak fractionation – at level, at slope, or in a combination of the two. When separating a number of peaks that do not have a baseline separation, it is often useful to perform peak fractionation at slope. Use the default values for the first run. Adjust the method by examining the chromatogram from the run in the **Evaluation** module, refer to UNICORN Evaluation manual. In the **Evaluation Classic** module, it is possible to differentiate a curve to get the values of the slope.

**Note:** When peak fractionation is used in F9-T, the fraction collector moves to start position and waits for the peak in position. Collection of pre-fractionation volume is done using the settings chosen.

The illustration below shows an example chromatogram of peak fractionation in F9-R. The fractions collected and the numerical marking of fractions are indicated.



#### Combined fixed volume fractionation and peak fractionation

In combined fractionation, Fraction collector F9-T moves to the next position when a peak in the UV signal is detected, independently of the volume set in fixed volume fractionation. After the peak, fixed volume fractionation continues.

To allow for different parts of the peaks to be analyzed, the fraction size during elution can be set to a value smaller than the expected peak volume.

#### Collection of flow-through in Out1

To collect the liquid flow-through of a run, direct the liquid flow to the **Out1** outlet in the outlet valve.

**Note:** Predefined methods do not support collection of a peak or fixed volume fraction through the **Out1** outlet. However, this can be done using a watch in text programming.

# Collection of pre-fractionation volume

The liquid volume present in the flow path from the UV detector to the Fraction collector when a fractionation command is issued is called the pre-fractionation volume. There is no waste available in either Fraction collector F9-T or F9-R. In F9-R this pre-fractionation volume is collected in the first tube in the Fraction collector. In F9-T the pre-fractionation volume can be collected either together with the first fraction or in the tube/well before the first fraction. For available settings for F9-T see Section 7.5.6 System settings - Fraction collector F9-T, on page 238. The size of the pre-fractionation volume equals that of the delay volume. See Section 7.9 Delay volumes, on page 269 for examples of delay volumes and how to calculate delay volume.

3.4 Performance optimization

## 3.4 Performance optimization

#### Introduction

This section gives a short description of how the internal volume of the system affects liquid transportation and protein purification results. For a more detailed description see *Additional literature, on page 10*.

All components in the system must in some way be connected to each other with tubing. Excess internal volume will give unnecessary peak broadening, which results in dilution of the separated proteins, and deceased resolution (purity obtained). Peak broadening is due to the flow rate in the tubing being higher towards the middle, compared with the flow rate close to the walls of the tubing. The result is that a protein peak passing through the system will become broader as it moves through the tubing, as illustrated in the picture below. On the other hand, narrow tubing cannot be run at the same flow rate as wide tubing, since the pressure generated by the liquid flow will be increased when the tubing dimension is decreased.

This means that the tubing connecting the components of the system must always be as short as possible, and the dimensions of the tubing need to be appropriate for the dimensions of the column used.

The image below illustrates peak broadening. Liquid flows faster in the middle of a tube as compared to closer to the walls. The farther a protein peak passes through a tube, the broader it becomes, as depicted in the chromatograms shown on the right.



# High resolution columns – low flow rate

The relative system contribution to peak broadening will depend on both the bead size of the chromatography resin and the column dimensions. Small beads and narrow columns result in narrow peaks (a high-resolution column), whereas large beads and wide columns result in wide peaks. The system volume can contribute significantly to the peak broadening of a narrow peak, but will contribute almost nothing to a wide peak.

For high resolution columns, the tubing inner diameter after the column should be decreased to make sure the resolution obtained in the column is kept until the Fraction collector. This is true both for the tubing from the column to the UV monitor and for the tubing from the UV monitor to the Fraction collector. The volume inside the modules in this part of the flow path also matters. Therefore, all modules that are not in use should be removed from the flow path and the UV flow cell should be exchanged to that with the smallest inner volume, which is the 5 mm flow cell.

The illustration below shows the effect of peak broadening from the UV monitor to the Fraction collector of a high resolution column where the tubing is too long and too wide. Changing to thinner and shorter tubing will reduce this effect and keep the peaks separated in the Fraction collector.



One way to minimize the volume between the column and UV monitor is to attach the column directly to the UV flow cell using a male/male fingertight connector (union 1/16"M / 1/16"M). When using this connection, use the multi-directional column clamp to hold the column.



Sample volume does not affect resolution in chromatography techniques involving adsorption of the target protein onto the column. Examples of such binding techniques are affinity chromatography (AC), ion exchange chromatography (IEX), and hydro-phobic interaction chromatography (HIC). Size exclusion chromatography (SEC), however, is a non-binding chromatography technique, and a sample zone is therefore broadened during passage through the SEC column. As a result, the sample gets diluted, and the resolution will decrease with an increasing sample volume.

#### 3.4 Performance optimization

For high resolution SEC columns, the tubing inner diameter and length from the injection valve to the column should be decreased to minimize sample dilution due to band broadening before entering the column.

The illustration below shows the different parts of the flow path that are important to consider during high resolution chromatography. The volume from the injection valve to the column (orange) is only important in non-binding techniques, such as SEC. Whereas the volume from the column to the UV detector (red) and the volume from the UV to the Fraction collector (blue) is important in all high resolution chromatography techniques.



The table below shows the recommended tubing inner diameters (i.d.) for the tubing from injection valve to Fraction collector for certain column sizes.

Column diameter	Recommended tubing i.d.
≥ 7 mm	0.5 mm (standard in ÄKTA go)
4 or 5 mm	0.25 mm
3.2 mm	0.15 mm

It is important to remember that the generated back pressure increases dramatically when the tubing inner diameter decreases. Therefore, it is important to set an appropriate pressure alarm. Moreover, high resolution columns with narrow tubing must be run at lower flow rates not to exceed the pressure limits.

#### Large columns - high flow rate

To be able to run large columns at high flow rates, the tubing from injection valve to Fraction collector needs to be changed to tubing with wider inner diameter.

Column diameter	Recommended tubing i.d.
16 mm	0.5 mm (standard in ÄKTA go)
26 mm	0.75 mm or decreased flow rate
50 mm	Not recommended for ÄKTA go

3.5 UV monitor

# 3.5 UV monitor

#### Introduction

UV monitor **U9-L** measures the UV absorbance at the fixed wavelength of 280 nm. The signal measured by the UV monitor can be used to calculate the protein concentration of a sample, when working within its linear range (up to 2000 mAU). UV absorbance signals outside the linear range are not proportional to protein concentration.

The relationship between absorbance and concentration is described by Lambert-Beer's law:

 $A = \varepsilon \times b \times c$ 

where,

A = absorbance,  $\varepsilon$  = extinction coefficient, b = cell path length, and c = concentration.

#### **UV flow cells**

The UV signal measured can be optimized according to the protein concentration of the sample used. If, for example, the protein concentration of the sample is low, the UV signal can be amplified by replacing the standard 2 mm UV flow cell with the optional 5 mm UV flow cell. See Section 5.5.4 Replace the UV flow cell, on page 181 for instructions.

UV monitor **U9-L** can be installed with one of two available flow cells.

Flow cell path length	Internal flow cell volume	Description
2 mm	30 µL	Standard UV flow cell.
5 mm	20 µL	Optional UV flow cell. Can be used to amplify the UV signal measured.

If the UV flow cell is changed, the cell path length must be adjusted. For detailed instructions, see Section 5.3.2 Calibrate the UV monitor, on page 155.

## 3.6 System wash and pump wash

#### Introduction

Contrary to the similarity in names the system wash and the pump wash are designed for different uses. The system wash is used to fill the system with a chosen solution. The pump wash is used to wash the pump and the flow path between the pump and the injection valve.

#### System wash

The system wash fills the system with the chosen buffer. This can be used for example to change buffer concentration prior to an elution step.

Due to the design of ÄKTA go, the system wash consists of two parts. In the first part, the system is washed with 15 mL of buffer at the default flow rate of 25 mL/min. The flow rate can be changed in **System Settings**. In the second part, the system is washed with a Post wash volume of buffer at the same flow rate as that used prior to the system wash. The default volume is 6 mL but can be changed in **System Settings**. If running at a low flow rate, the system wash can take a long time to be completed.

During system wash, the system state is changed to **Wash** in the **Instrument Control Panel** and in UNICORN. Any method running during a system wash is set to **Hold** until the wash is completed. After the system wash is completed, the valves return to their previous positions.

The table below shows the system wash options available for different system configurations.

System configuration	Flow path
Standard	The liquid flow path is directed to waste on the injection valve.
Column valve installed	The liquid flow path can be directed to waste on the injection valve or outlet valve. <sup>1</sup> The column valve is by-passed.
Column valve and pH valve installed	The liquid flow path can be directed to waste on the injection valve or outlet valve. <sup>1</sup> The column valve and the pH valve are by-passed.

<sup>1</sup> This can be set in *Manual instruction*  $\rightarrow$  *Pump*  $\rightarrow$  *System wash*.

**Note:** An instruction issued during system wash is not performed until the wash is completed.

- Note: A system wash cannot be performed when the system is in Hold state.
- *Note:* Pressing *Continue* during a system wash terminates the wash and continues the run.

- 3 Considerations when running the system
- 3.6 System wash and pump wash

#### **Pump wash**

The pump wash is used to wash the pump together with the flow path between the inlet valve and the injection valve. By default, the pump wash uses 65 mL of buffer at a flow rate of 65 mL/min, which is enough to remove sample from this section of the flow path.

For instructions on how to change the wash volume and flow rate, see *Wash settings*, *on page 66*.

## 3.7 Pressure alarms

#### Introduction

Pressure alarms can protect the instrument and the columns used from the pressure generated by the liquid flow. If the defined pressure limit is reached, pressure alarms stop the flow.

The pressure alarm is automatically set to 2 MPa, to provide basic column protection. However, to protect a column properly, it is recommended to set a pressure alarm adequate for the column used in each run.

To make sure the pressure is not too high, use pressure flow control. Pressure flow control regulates the flow rate to avoid overpressure and continue the run. See Section 3.8 Pressure control, on page 96 for more information.

#### **Measured pressure**

The standard configuration of ÄKTA go has one pressure monitor. To measure the highest pressure in the system, the pressure monitor is placed after the pump.

When a column is selected in systems with only one pressure sensor, the pressure alarm is automatically set according to the table below. Recommended flow rates and pressure limits are found in the column list in UNICORN.

If pre-column pressure limit	Pressure alarm setting
≥1 MPa	The delta-column pressure limit is used.
< 1 MPa	The pre-column pressure limit is used.

When using a Superloop, a Superloop pressure alarm can be set to protect it. The Superloop pressure alarm uses the pressure signal, when the Superloop is in-line. This means that there can be two alarms set on the same pressure signal – one to protect the column and one to protect the Superloop. The lowest alarm limit will trigger the alarm. To set the alarm, see Section 7.5.4 System settings - Pressure alarms, on page 235.

The Superloop pressure alarm is automatically set when using a Superloop in a predefined method. The pressure limits for each Superloop are indicated below.

Superloop volume (mL)	Pressure limit (MPa)
10	4
50	4
150	2

# Pre-column and delta-column pressure alarm

If the advanced column valve **V9-C** is installed, two additional pressure sensors are present: one before the column, and one after the column. In this case, pre-column and delta-column pressure alarms can be set. The pre-column pressure alarm protects the column hardware. The delta-column pressure alarm protects the resin and packed bed.

Pressure limits for each column are found in the column list in UNICORN. By default, pre-column and delta-column pressure alarms are automatically set when a column is selected in a method or manual run. To change these pressure alarms, see <u>Section</u> 7.5.4 System settings - Pressure alarms, on page 235.

#### Post-column pressure alarm

The post-column pressure alarm can protect the UV flow cell and the pH electrode from high pressure. If column valve **V9-C** is installed in the instrument, the default post-column pressure alarm is defined at 2 MPa. If pH valve **V9-pH** is also installed and the pH electrode is in-line, the default post-column pressure alarm is set to 0.8 MPa.

The location and description of the different types of measured pressure is presented below.



Part	Pressure type	Description
1	Pressure	Pressure measured after the pump.
2	Pre-column pressure (PreC) <sup>1</sup>	Pressure measured before the column. This is the pressure on the column hard- ware.

Part	Pressure type	Description
3	Post-column pressure (PostC) <sup>1</sup>	Pressure measured after the column.
N/A	Delta-column pressure (ΔP) <sup>1</sup>	The pressure difference between the pre-column pressure and post-column pressure. This is the pressure on the resin and packed bed.

<sup>1</sup> Pressure measured only when column valve **V9-C** is installed.

**Note:** Since the tubing between the pressure monitor and the column is short, the difference between the pressure and the pre-column pressure values is small.

3.8 Pressure control

## 3.8 Pressure control

#### Introduction

Pressure control regulates the flow rate if the pressure reaches close to the defined limit. Pressure control can be used to avoid method stops due to pressure alarms, which can happen during sample application if the viscosity of the sample is higher than the buffer.

To enable pressure control when creating a method in the **Method Editor**, select **Control to avoid Overpressure** in the **Method Settings** phase. To enable pressure control when performing a manual run, select **Manual instructions**  $\rightarrow$ **Pump**  $\rightarrow$ **Flow** in **System Control**.

#### **Pressure control parameters**

Pressure control uses PI (proportional-integral) regulation. The PI parameters have default values but can be optimized for the chromatography method in use.

To optimize the PI parameters for pressure control, change the settings manually and test the new settings with a manual run. After optimization, set the PI parameters in **System Settings**  $\rightarrow$  **Advanced**  $\rightarrow$  **Pressure control parameters**.

Parameter	Description	
Pfactor	Proportional component in PI pressure regulation. Reduces the error between the actual and the requested target pressure but may leave a permanent error.	
lfactor	Integrating component in PI pressure regulation. Elimi- nates the stationary error from the P factor but can introduce instability that may lead to oscillations in pressure and flow rate. To disable the I factor, set I = 0.	
	Note:	
	As a general guide, use a small I factor for high-pressure columns and a large I factor for low-pressure columns.	
Target value for pres- sure control	Sets the target value for PI pressure regulation as a percentage of the pressure limit.	
	The pressure limit is set in the <i>Alarm pressure</i> instruc- tion. The <i>Alarm pressure</i> used for pressure control depends on the settings in the <i>Flow</i> instruction.	
	Note:	
	If the target value is close to the pressure limit, a tran- sient pressure pulse might trigger a pressure alarm.	

The table below describes the parameters used for pressure control.

Parameter	Description
Min allowed flow rate	If the flow rate is reduced below the value set in <i>Min</i> <i>allowed flow rate</i> , the method is paused and the system is set to state <i>Alarms and errors</i> .
	<i>Min allowed flow rate</i> can be set as volumetric or as linear flow. A column type must be selected before using linear flow.

# Recommended pressure control parameters

The table below contains the recommended values for P and I parameters for different resin types.

Resin type	P factor	lfactor	Additional information
Default	8	40	N/A
Small soft resin columns (i.d. < 10 mm)	8	40	N/A
Large soft resin columns (i.d. > 10 mm)	8	300 to 600	Increase the I factor to decrease pressure ramp-up time.
Small rigid resin	8	15	Decrease the I factor to
columns (e.g., SEC columns)	20	40	avoid large fluctuations in pressure and flow rate. Alternatively, increase the P factor.

- 3 Considerations when running the system
- 3.9 Performing a run in cold environment

## 3.9 Performing a run in cold environment

When performing a run in a cold environment, pressure can increase up to 60%, compared to room temperature, due to increased liquid viscosity at low temperature.

To protect a column when performing a run in a cold environment, decrease the flow rate and set appropriate pressure limits. To decreased the flow rate automatically, select **Reduce for Cold Room** in the **Method Settings** phase, in **Method Editor**. This option reduces the flow rate to 50% of the flow rate previously set. Refer to *ÄKTA go Operating Instructions (29360951)* for more information and follow the precautions listed.

## 3.10 Conductivity temperature compensation

The conductivity of liquids is dependent on temperature. To compensate the temperature at which conductivity is measured, a **Compensation factor** can be set in **System Settings**. The **Compensation factor** has two main uses. It allows you to get a comparable conductivity signal from two similar runs at different temperatures, and makes sure that the conductivity signal does not fluctuate due to temperature changes, such as in a refrigerator.

The compensation factor is expressed as the percentage of conductivity change per degree Celsius. The user can adjust the **Compensation factor** according to the composition of the buffer used. By default, **Compensation factor** is set to 2.0%, which can be used for common salt buffers.

Follow the steps below to set the compensation factor.

#### Step Action

1 Open the **System Settings** dialog, by selecting **System** → **Settings** in the **System control** module.

#### 2 Select Conductivity → Cond temp compensation.

<ul> <li>UV</li> <li>Conductivity Alam conductivity Relative scale cond Cond temp compensation</li> <li>pH</li> <li>Pressure alarm</li> <li>Fraction collection</li> <li>Wash settings</li> <li>Watch parameters</li> <li>Advanced</li> <li>Data collection</li> </ul>	Compensation factor         [0.0 - 9.9]           2.0         ☆ %           Reference temperature         [0.0 - 40.0]           25.0         ☆ *C	
Data collection		

#### 3 Set **Compensation factor** to the desired value.

- **Note:** Do not set the conductivity temperature compensation to zero. Especially when using the instrument in refrigerators, setting the temperature compensation to zero generates a fluctuating conductivity signal.
- *Note:* When running performance tests, a compensation factor of 2.1% should be used.

# 4 Performance tests

#### About this chapter

Performance tests are used to test the function of ÄKTA go after installation or replacement of individual modules. Performance tests can also be used to check the condition of the system if unexpected results are obtained or after a prolonged stop.

This chapter describes the available performance tests for ÄKTA go. It contains information about when to run performance tests, detailed descriptions of the tests, and how to troubleshoot failed performance tests.

For instructions on how to run performance tests, refer to ÄKTA go Operating Instructions (29360951). Detailed instructions for each test are provided in the performance test method notes in UNICORN.

#### In this chapter

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4.2	Description of performance tests	102
4.3	Failed performance tests	111

## 4.1 When to run performance tests

#### Schedule

Performance tests can be run at any time, as required. The following table provides recommendations for when to run performance tests.

Occasion	Test
After system installa- tion	Run the <b>System test</b> and <b>Mixer test</b> . If you have installed additional modules that have performance tests, run the corresponding tests as well.
After module installa- tion	Run the performance test applicable to the installed module. $^{\rm 1}$
If a problem is suspected	Run the performance test applicable to the module or modules causing the problem.
After a prolonged stop	Run the <b>System test</b> and <b>Mixer test</b> . If you have installed additional modules that have performance tests, run the corresponding tests as well.
If the <b>System test</b> fails due to the pump.	Run the <b>System test</b> or the <b>Pump test</b> after trouble- shooting.

<sup>1</sup> Not all modules have an applicable performance test.

# Considerations before running performance tests

Before running a performance test, the following aspects should be considered:

- Disconnect all columns from the flow path. Columns should not be used when running a performance test.
- Make sure that the correct path length is set for the UV flow cell. The correct path length is set by default, but has to be set if the flow cell is changed to a 5 mm flow cell. See Section 5.3.2 Calibrate the UV monitor, on page 155.
- Set the temperature compensation factor to 2.1%, which is suitable for a solution of 1 M NaCl 1% acetone. See Section 3.10 Conductivity temperature compensation, on page 99 for instructions.

# 4.2 Description of performance tests

#### Introduction

This section provides a detailed description of performance tests.

All performance tests generate a chromatogram. The **System test**, **Mixer test**, **Pump test**, and **Column valve V9-C test** generate a test report. The **Fraction collector F9-R and F9-T test** is evaluated visually.

#### In this section

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#### 4.2.1 Test result and report

The performance test data are displayed in **System Control** as the test progresses. When completed, the result can be found in the **Evaluation** module. Test results are saved in the user home folder. A different destination folder can be set in **Start Protocol**.

A test report is also generated specifying whether a performance test has passed or failed. This report can be found in the **Method Notes** panel when selecting the test in the **System Performance Test** tab in the **System Control** module. By default the file name is the name of the test. A different name can be entered in **Start Protocol**. The test result is stated at the top of the report. If the test was failed refer to respective test in *Section 4.3 Failed performance tests, on page 111*.

The example below shows the report from a *Mixer test*.

Mixer test.txt - Notepad		-		×
<u>F</u> ile <u>E</u> dit F <u>o</u> rmat <u>V</u> iew <u>H</u> elp				
!				~
! Test Report Summary !				
! The test passed !				
!=====!				
11				
 I Test Information				
! Mixer Test !				
! System name: ÄKTA go !				
! Version: 7.4.0.800 !				
! Time: 2019-08-15 15:58:15 +01:00 !				
! User: System !				
! Result: Mixer test !				
!!				
Puplog Event Test Posult			!	
I Eluctuation on conductivity less than	1 mS/cm and t	the test pass	ed. I	
			!	
			!	
! Runlog Event Test Result			1	
			!	
Pressure check passed. The pressure wa	as petween 0.2	L and 0.4 MPa	. !	
!			1	
<				>
	Windows (CRLE)	In 1 Col 1	100%	
	WINDOWS (CITEF)	Lift, Corr	10076	

4 Performance tests4.2 Description of performance tests4.2.2 System test

### 4.2.2 System test

The **System test** is used to verify the functionality of the inlet valve, pump, pressure monitor, UV monitor, conductivity monitor, and flow restrictor.

The following chromatogram shows an example of a **System test** that has passed.



The main stages of the **System test** indicated in the chromatogram are described below.

Stage	Test	Description
1	UV signal before auto zero	Determines correct mounting of the UV flow cell
2	UV noise test and absolute deviation from zero	Tests the function of the UV monitor
3	Gradient test	Tests the pump, UV monitor, and inlet valve, by measuring the UV signal during linear gradient formation.
4	UV absorbance test and Conductivity test at 100% B	• Tests that the correct path length is set for the UV flow cell and tests the function of inlet valve port <b>B</b> , by comparing the UV signal with a theoretical value.
		• Tests the calibration and function of the conductivity monitor, by comparing the conductivity signal with a theoretical value.

Stage	Test	Description
5a-5c	Step response test and Conductivity test at 50%	• Tests the pump, UV monitor, and inlet valve port <b>B</b> at the 95% (5a), 50% (5b), and 5% (5c) steps, by meas- uring the UV signal.
		• Tests the conductivity monitor and inlet valve at the 50% step (5b), by measuring the conductivity signal.
6	Peaktest	Tests the inlet valve by comparing the conductivity curves from inlet valve ports <b>C</b> and <b>Sample</b> .
7a-7d	Pump pulsation test	Checks whether air has entered the flow path, by meas- uring the pressure signal when different inlets are in use: <b>A</b> inlet (7a), <b>B</b> inlet (7b), <b>C</b> inlet (7c), and <b>Sample</b> inlet (7d).
8	Pressure check at 25 mL/min	Tests flow restrictor function, pressure monitor calibra- tion, and system tubing, by applying a 25 mL/min flow rate throughout the system.
9	Pressure check at 1 mL/min	Tests flow restrictor function.

The following image shows an amplified view of the last section of the chromatogram above.



4 Performance tests4.2 Description of performance tests4.2.3 Mixer test

### 4.2.3 Mixer test

The *Mixer test* is used to verify the functionality of the mixer in combination with the inlet valve and the pump. The mixer performance test checks pressure and conductivity fluctuations, during gradient formation. A functional mixer maintains a stable conductivity signal.

The illustration below shows a chromatogram from a *Mixer test* that has passed.



### 4.2.4 Pumptest

The **Pump test** verifies the functionality of the pump by checking pressure stability. This test can be run after troubleshooting of the pump.

The illustration below shows a chromatogram of a passed **Pump test**.



4 Performance tests4.2 Description of performance tests4.2.5 Column valve **V9-C** test

### 4.2.5 Column valve V9-C test

The **Column valve V9-C test** checks the pressure sensors in column valve **V9-C**. Functional pressure sensors detect pressure changes when the column valve rotates and the liquid flow passes through the **Ref 1** tubing mounted on the column valve.

Note: The Ref 1 tubing is supplied with column valve V9-C.

The illustration below shows a chromatogram of a passed Column valve V9-C test.



#### Column valve V9-C test
# 4.2.6 Fraction collector F9-T test

The *Fraction collector F9-T test* checks that the fraction collector works as required, by testing correct liquid dispensing into wells and liquid spillage.

The table below shows the volumes dispensed in the corresponding wells. Two 96 deep well plates are used in the *Fraction collector F9-T test*.

Well	Volume
1.A.1	Pre-fractionation volume
1.A.2, 1A.3, 1.A.4	2 mL
2.H.12, 2.H.11, 2.H.10	1 mL
1.A.5, 2.G.11, 2.G.12, 2.H.9	Small volume, no need to check

The illustration below shows a chromatogram of a passed *Fraction collector F9-T test*. The fractionation marks at the bottom of the chromatogram correspond to the filled wells.



**Note:** The fractionation marks at the bottom of the chromatogram correspond to the filled tubes.

4 Performance tests

4.2 Description of performance tests

4.2.7 Fraction collector F9-R test

# 4.2.7 Fraction collector F9-R test

The **Fraction collector F9-R test** checks that the Fraction collector works as required, by testing correct liquid dispensing into collection tubes and liquid spillage. The table below shows the volumes dispensed and the corresponding tubes.

Tube number	Volume
1	Pre-fractionation volume
2-4	2 mL
5–6	1 mL

The illustration below shows a chromatogram of a passed *Fraction collector F9-R test*.

#### Fraction collector F9-R test



**Note:** The fractionation marks at the bottom of the chromatogram correspond to the filled tubes.

# 4.3 Failed performance tests

#### Introduction

If a performance test fails, see the corresponding section to check and correct the possible causes of the failure. If a failed performance test cannot be corrected by performing the suggested actions or the test indicates a type of malfunction not listed below, the module might have to be repaired or replaced. Contact your local Cytiva representative for this.

#### In this section

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# 4.3.1 System test failure

#### Introduction

A failed **System test** indicates a malfunction of ÄKTA go. Review the test report to check which part of the test failed and check the corresponding section for possible causes of the failure. After performing the suggested corrective actions, verify that the issue is fixed by rerunning the **System test**.

**Note:** It is important that the correct solution concentrations (1.0% (v/v) acetone and 1.0 M NaCl) are used in the **System test** and that the solutions are thoroughly mixed.

### UV signal before auto zero failed

Test outcome	Cause	Action
High UV signal	UV flow cell not properly attached to the UV monitor	Make sure that the UV flow cell is prop- erly attached to the UV monitor.
	Incorrect UV cell path length set in UNICORN	See Section 5.3.2 Calibrate the UV monitor, on page 155.

The chromatogram below shows an example of a failed system performance test caused by a UV flow cell not properly attached to the UV monitor.



# Pump pulsation test failed

Test outcome	Cause	Action
Unstable pres- sure	Air in the pump or faulty pump	Make sure that the tubing is correctly connected to the inlet valve and connectors are tightened. Prime and purge the pump, according to the <i>Operating Instructions</i> , before starting the test.
		If air persists in the pump, run the system with 100% methanol, to remove it.
		If the problem persists, change the pump piston seals. See <i>Replace piston</i> , <i>piston seal</i> , <i>and rinse membrane</i> , <i>on</i> <i>page 172</i> .

The chromatogram below shows an example of a failed **System test** due to air in the pump.



# UV noise test failed

Test outcome	Cause	Action
High UV signal noise	Air in the UV flow cell or clogged UV flow cell	Clean the UV flow cell. See <i>Clean the</i> UV flow cell, on page 140.

#### 4 Performance tests

4.3 Failed performance tests

4.3.1 System test failure

Test outcome	Cause	Action
	Air in the UV flow cell due to unin- stalled or faulty flow restrictor	Add or replace the flow restrictor. See <i>Instruction, on page 183</i> .
	Contaminated buffers	Check the buffers.
	Faulty electronics in the UV monitor	Replace the UV monitor.

### **Gradient test failed**

Test outcome	Cause	Action
Non-linear UV signal	Air in the pump or faulty pump	Make sure that the tubing is correctly connected to the inlet valve and connectors are tightened. Prime and purge the pump, according to the <i>Operating Instructions</i> , before starting the test.
		If air persists in the pump, run the system with 100% methanol, to remove it.
		If the problem persists, change the pump piston seals. See <i>Replace piston</i> , <i>piston seal</i> , <i>and rinse membrane</i> , <i>on</i> <i>page 172</i> .
	Air in the UV flow cell due to unin- stalled or faulty flow restrictor	Add or replace the flow restrictor. See <i>Instruction, on page 183</i> .
	Damaged pump piston seals	Replace pump piston seals. See Replace piston, piston seal, and rinse membrane, on page 172.
	Faulty UV monitor	Clean the UV flow cell following the instructions in <i>Clean the UV flow cell,</i> <i>on page 140</i> . If the problem persists contact service to repair or replace the UV monitor.

The chromatogram below shows an example of a failed  ${\it System test}$  caused by air in tubing  ${\it B}.$ 

4 Performance tests 4.3 Failed performance tests 4.3.1 System test failure



### Absolute deviation from zero failed

Test outcome	Cause	Action
Drifting UV signal baseline.	Air in the UV flow cell or clogged UV flow cell	Clean the UV flow cell. See <i>Clean the</i> <i>UV flow cell, on page 140.</i> If the problem persists, contact service.
Spikes or steep decreases in the UV signal.	Air in the system	Prime buffer inlets and purge the pump according to the <i>Operating Instructions</i> .
	Air in the UV flow cell due to unin- stalled or faulty flow restrictor	Add or replace the flow restrictor. See <i>Instruction, on page 183</i> .

The chromatogram below shows an example of a **System test** failed due to UV signal deviating from zero.

#### 4 Performance tests

4.3 Failed performance tests

4.3.1 System test failure

#### System test



# Step response test failed

Test outcome	Cause	Action
All values failed	Air in the pump or faulty pump	Prime buffer inlets and purge the pump, according to the <i>Operating Instructions</i> , before starting the test.
		Clean the UV flow cell. See <i>Clean the UV flow cell, on page 140.</i>
		If air persists in the pump, run the system with 100% methanol, to remove it.
		If the pump is faulty, see <i>Instruction, on page 178</i> .
Failed values at 5%	Damaged inlet valve <b>K9</b> or loose connector in the <b>B</b> inlet	Check tubing connections to the inlet valve or replace the inlet valve. See
Failed values at 50%	Damaged inlet valve <b>K9</b> or loose connector in the <b>A</b> or <b>B</b> inlets	Section 5.5.1 Replace AKTA go modules, on page 177.
Failed values at 95%	Damaged inlet valve <b>K9</b> or loose connector in the <b>A</b> inlet	

#### UV absorbance test failed

Test outcome	Cause	Action
High UV signal amplitude devia- tion.	Incorrectly prepared acetone solution	Make sure that the used acetone solu- tion is 1.0% (v/v) and that no liquid has evaporated.
	Incorrect UV cell path length set in UNICORN	Set the UV cell path length in UNICORN. See Section 5.3.2 Calibrate the UV monitor, on page 155 for instructions.

The chromatogram below shows an example of a failed **System test** due to incorrectly set UV cell path length. In this example, the cell path length has been set to 2 mm in the software, but a 5 mm UV cell is in use on the system.



# Conductivity test at 100% failed or conductivity test at 50% failed

Test outcome	Cause	Action
High conductivity signal amplitude deviation.	Incorrectly prepared NaCl solution	Make sure that the concentration of the NaCl solution is 1.0 M.
	Incorrect calibration of the conduc- tivity monitor	See Section 5.3.3 Calibrate the conductivity monitor, on page 159.

#### 4 Performance tests

4.3 Failed performance tests

4.3.1 System test failure

Test outcome	Cause	Action
	Faulty or dirty conductivity monitor	Clean the conductivity flow cell following the instructions in Section 5.2.5 Maintenance of the conductivity flow cell, on page 142. If the problem persists contact service to repair or replace the conductivity monitor.
	Incorrect <b>Cond temp compensation</b> factor value	Make sure the conductivity tempera- ture compensation factor is turned on. Use 2.1% when running performance tests.
	Damaged inlet valve <b>K9</b> or loose connector in the <b>B</b> inlet	Check tubing connections to the inlet valve or replace the inlet valve. See Section 5.5.1 Replace ÄKTA go modules, on page 177

The chromatogram below shows an example of a failed **System test** due to incorrect calibration of the conductivity monitor.



#### **Peak test failed**

Test outcome	Cause	Action
Conductivity peaks not proportional	Air in the pump or faulty pump	Prime buffer inlets and purge the pump, according to the <i>Operating</i> <i>Instructions</i> , before starting the test.
D c ir		Clean the UV flow cell. See <i>Clean the</i> UV flow cell, on page 140.
		If air persists in the pump, run the system with 100% methanol, to remove it.
		If the pump is faulty, see <i>Instruction, on</i> page 178.
	Damaged inlet valve <b>K9</b> or loose connectors on the <b>C</b> inlet or <b>Sample</b> inlet	Check tubing connections to the inlet valve or replace the inlet valve. See Section 5.5.1 Replace ÄKTA go modules, on page 177.

The chromatogram below shows an example of a failed **System test** due to a loose sample tubing connector.



4 Performance tests4.3 Failed performance tests4.3.1 System test failure

# Pressure check at 25 mL/min flow rate failed

Test outcome	Cause	Action
High pressure	Folded, twisted, or blocked tubing	Check the tubing.
	Incorrect calibration of the pressure sensors	Calibrate the pressure sensors. See Section 5.3.1 Calibrate the pressure sensors, on page 153.
	High back pressure from the flow restrictor	Clean or adjust the flow restrictor. If the problem persists, replace the flow restrictor. See Section 5.2.6 Mainte- nance of the flow restrictor, on page 144.

The chromatogram below shows an example of a failed **System test** due to high back pressure from the flow restrictor.



#### Pressure check at 1 mL/min flow rate failed

Test outcome	Cause	Action
Pressure outside of range	Pressure outside Low back pressure from the flow restrictor	Clean or adjust the flow restrictor. If the problem persists, replace the flow restrictor. See Section 5.2.6 Mainte- nance of the flow restrictor, on page 144.
	Leakage in the flow path	Make sure that all tubing are properly attached.

# 4.3.2 Mixer test failure

### Introduction

A failed *Mixer test* indicates a malfunction of the mixer. Review the test report to check which part of the test failed and check the corresponding section for possible causes of the failure. After performing the suggested corrective actions, verify that the issue is fixed by rerunning the *Mixer test*.

**Note:** It is important that the correct solution concentrations (1.0% (v/v) acetone and 1.0 M NaCl) are used in the **Mixer test** and that the solutions are thoroughly mixed.

### **Conductivity fluctuation test failed**

Testoutcome	Cause	Action
High conductivity signal fluctuation	Clogged mixer	Clean the mixer and install the online filter. If the online filter is already installed, replace the filter. See Section 5.2.3 Maintenance of the mixer, on page 137 and Section 5.2.2 Exchange the online filter, on page 136.
	No membrane in the mixer	Make sure that a membrane is mounted in the mixer. See Section 5.2.3 Maintenance of the mixer, on page 137.

The chromatogram below shows an example of a failed *Mixer test* due to absence of membrane in the mixer.



# **Pressure check failed**

Test outcome	Cause	Action
High pressure	Incorrect calibration of the pressure sensors	Calibrate the pressure sensors. See Section 5.3.1 Calibrate the pressure sensors, on page 153.
	Folded, twisted, or blocked tubing	Check the tubing and replace if neces- sary.
	Clogged mixer	Clean the mixer and install the online filter. If the online filter is already installed, replace the filter. See Section 5.2.3 Maintenance of the mixer, on page 137 and Section 5.2.2 Exchange the online filter, on page 136.
	Clogged online filter	Replace the online filter. See Section 5.2.2 Exchange the online filter, on page 136.

4 Performance tests4.3 Failed performance tests4.3.3 Pump test failure

# 4.3.3 Pump test failure

A failed *Pump test* indicates a malfunction of the pump. Check the table below for possible causes of the failure. After performing the suggested corrective actions, verify that the issue is fixed by rerunning the *Pump test*.

Testoutcome	Cause	Action
High pressure and/or increased pressure fluctua- tion	Erratic pump pressure	Refer to Section 5.4.1 Maintenance of the pump head check valves, on page 166 for cleaning or replacing the pump head check valves. If the problem persists, refer to Instruction, on page 178 for pump replacement instruc- tions.
	Folded, twisted, or blocked tubing	Check the tubing and replace if neces- sary.
	Incorrect calibration of the pressure sensors	Calibrate the pressure sensors. See Section 5.3.1 Calibrate the pressure sensors, on page 153.
	High back pressure from the flow restrictor	Clean or adjust the flow restrictor. If the problem persists, replace the flow restrictor. See Section 5.2.6 Mainte- nance of the flow restrictor, on page 144.
Unstable pres- sure	Air in the pump or faulty pump	Make sure that the tubing is correctly connected to the inlet valve and connectors are tightened. Prime and purge the pump, according to the <i>Operating Instructions</i> , before starting the test.
		If air persists in the pump, run the system with 100% methanol, to remove it.
		If the problem persists, change the pump piston seals. See <i>Replace piston</i> , <i>piston seal</i> , <i>and rinse membrane</i> , <i>on</i> <i>page</i> 172.

The chromatogram below shows an example of a failed *Pump test* due to high back pressure from the flow restrictor.

4 Performance tests4.3 Failed performance tests4.3.3 Pump test failure



# 4.3.4 Column valve V9-C test failure

#### Introduction

A failed **Column valve V9-C test** indicates a malfunction of column valve **V9-C**. Review the test report to check which part of the test failed and check the corresponding section for possible causes of the failure. After performing the suggested corrective actions, verify that the issue is fixed by rerunning the **Column valve V9-C test**.

# Failed pre-, post-, and delta-column pressure tests

Testoutcome	Cause	Action
Below minimum or no pressure change	Incorrect calibration of the integrated pressure sensors	Calibrate the integrated pressure sensors. See Section 5.3.1 Calibrate the pressure sensors, on page 153.
	Incorrect system preparation for the performance test	Make sure that the <b>Ref1</b> reference tubing is connected between the column valve ports <b>1A</b> and <b>1B</b> .
		Note:
		If a pH monitor is connected, make sure the flow restrictor in-line.
	Faulty column valve	Replace the column valve following the instructions in Section 5.5.1 Replace ÄKTA go modules, on page 177. For repair of the column valve contact service.

If a faulty column valve cannot rotate to the correct positions, no pressure is generated. The chromatogram below shows an example of a failed **Column valve V9-C test** due to a faulty column valve.

4 Performance tests 4.3 Failed performance tests 4.3.4 Column valve **V9-C** test failure





#### Failed delta-column pressure test

Test outcome	Cause	Action	
Below minimum or no pressure change	Incorrect calibration of the integrated pressure sensors	Calibrate the integrated pressure sensors. See Section 5.3.1 Calibrate the pressure sensors, on page 153.	
	Incorrect system preparation for the performance test	Make sure that the reference tubing <b>Ref 1</b> is connected between the column valve ports <b>1A</b> and <b>1B</b> .	
		Note:	
		If a pH monitor is connected, make sure the flow restrictor in-line.	

The chromatogram below shows an example of a failed **Column valve V9-C test** due to wrong **Ref 1** tubing connection in port **1B**.

#### 4 Performance tests

4.3 Failed performance tests

4.3.4 Column valve V9-C test failure

#### Column valve V9-C test



# 4.3.5 Fraction collector F9-T test failure

A failed *Fraction collector F9-T test* indicates a malfunction of the Fraction collector. Visually inspect the system and the resulting chromatogram to understand the causes for test failure. Check the table below for possible causes of the failure. After performing the suggested corrective actions, verify that the issue is fixed by rerunning the *Fraction collector F9-T test*.

Test outcome	Cause	Action
Unstable pressure and incorrect volumes dispensed in the wells.	Air in the pump or faulty pump.	Make sure that the tubing is correctly connected to the inlet valve and connectors are tightened. Prime and purge the pump, according to the ÄKTA go Operating Instructions (29360951), before starting the test.
		If air persists in the pump, run the system with 100% methanol, to remove it.
		If the problem persists, change the pump piston seals. See <i>Replace piston</i> , <i>piston seal</i> , <i>and rinse membrane</i> , <i>on</i> <i>page 172</i> .
Incorrect volume dispensed in 1.A.1.	<b>Delay volume</b> incorrectly set.	Set correct <b>Delay volume</b> in <b>System</b> settings.
Liquid dispersed in the wrong well/	Calibrate Fraction collector.	Contact Service personnel for help with calibration.
outside of well.	Deep well plate incorrectly posi- tioned in the frac- tion collector.	Repeat the test with a properly posi- tioned deep well plate.
Spillage between wells	Drop sync func- tion is inactive.	Set the drop sync function to <b>Auto</b> or <b>On</b> in <b>System Settings</b> .

4 Performance tests4.3 Failed performance tests4.3.6 Fraction collector F9-R test failure

# 4.3.6 Fraction collector F9-R test failure

A failed *Fraction collector F9-R test* indicates a malfunction of the Fraction collector. Visually inspect the system and the resulting chromatogram to understand the causes for test failure. Check the table below for possible causes of the failure. After performing the suggested corrective actions, verify that the issue is fixed by rerunning the *Fraction collector F9-R test*.

Testoutcome	Cause	Action
Unstable pressure and incorrect volumes dispensed in the collection tubes.	Air in the pump or faulty pump	Make sure that the tubing is correctly connected to the inlet valve and connectors are tightened. Prime and purge the pump, according to the ÄKTA go Operating Instructions (29360951), before starting the test.
		If air persists in the pump, run the system with 100% methanol, to remove it.
		If the problem persists, change the pump piston seals. See <i>Replace piston</i> , <i>piston seal</i> , <i>and rinse membrane</i> , <i>on</i> <i>page 172</i> .
Liquid dispensed in the wrong collection tubes.	Incorrect positioning of the fractionation arm	Make sure that the fractionation arm is positioned above tube number one before starting the test.
Spillage between tubes.	Drop sync function inactive	Enable Drop sync in <b>System Settings</b> . See Section 7.5.7 System settings - Fraction collection F9-R, on page 242

# 5 Maintenance and service

#### About this chapter

This chapter describes the maintenance program for ÄKTA go and provides instructions for maintenance, calibration, basic service, and replacement of modules and components.

### In this chapter

Section		See page
5.1	Maintenance schedule	132
5.2	Maintenance procedures	134
5.3	Calibration of monitors and sensors	152
5.4	Service	165
5.5	Replacement of modules and components	176
5.6	Maintenance Manager	187

#### 5 Maintenance and service

5.1 Maintenance schedule

# 5.1 Maintenance schedule

#### Introduction

This section covers the maintenance procedures to be performed by the user of  $\ddot{\mathsf{A}}\mathsf{KTA}$  go, as outlined below.

#### **Periodic maintenance**

The user should perform the following periodic maintenance.

Interval	Maintenance action	Instructions
Daily or before each run	Calibrate the pH monitor	See Section 5.3.5 Calibrate the pH monitor, on page 163.
Weekly	Change pump rinsing solu- tion	Change the pump rinsing solution daily if aqueous buffers are used, or weekly if 20% ethanol is used. For instructions refer to ÄKTA go Operating Instructions (29360951)
	Calibrate the pressure sensors	See Section 5.3.1 Calibrate the pressure sensors, on page 153.
	Clean the Fraction collector drop sync sensor	See Section 5.2.8 Maintenance and cleaning of Fraction collector F9-T, on page 149 and Section 5.2.9 Maintenance and cleaning of Fraction collector F9-R, on page 151.
Monthly	Check the flow restrictor	See Section 5.2.6 Maintenance of the flow restrictor, on page 144.
Every 6 months	Clean the UV flow cell	See Clean the UV flow cell, on page 140.
	Replace the pH electrode	See Replace the pH electrode, on page 147.

#### Maintenance when required

The following maintenance should be performed when required.

Maintenance action	Instructions
Clean the instrument surfaces	Refer to ÄKTA go Operating Instructions (29360951)
Run <b>System CIP</b> (System cleaning-in-place)	Refer to ÄKTA go Operating Instructions (29360951)

#### 5 Maintenance and service 5.1 Maintenance schedule

Maintenance action	Instructions
Clean and store the pH electrode	See Section 5.2.7 Maintenance of the pH valve and electrode, on page 145.
Clean the conductivity flow cell	See Section 5.2.5 Maintenance of the conduc- tivity flow cell, on page 142
Calibrate the conductivity monitor	See Section 5.3.3 Calibrate the conductivity monitor, on page 159
Calibrate the temperature sensor	See Section 5.3.4 Calibrate the temperature sensor, on page 162
Reset the pressure sensors	See Section 5.3.1 Calibrate the pressure sensors, on page 153
Replace tubing and connectors	See Section 5.5.6 Replace tubing and connectors, on page 184
Replace the UV flow cell	See Instruction, on page 181
Replace pump rinsing system tubing	Refer to ÄKTA go Operating Instructions (29360951)
Replace main fuses	Refer to ÄKTA go Operating Instructions (29360951)

5.2 Maintenance procedures

# 5.2 Maintenance procedures

#### **About this section**

This section gives instructions for maintenance of the modules and components in the  $\ddot{\mathsf{A}}\mathsf{KTA}$  go instrument.

# In this section

Secti	Section See pa	
5.2.1	Maintenance of the pump	135
5.2.2	Exchange the online filter	136
5.2.3	Maintenance of the mixer	137
5.2.4	Maintenance of the UV flow cell	140
5.2.5	Maintenance of the conductivity flow cell	142
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5.2.7	Maintenance of the pH valve and electrode	145
5.2.8	Maintenance and cleaning of Fraction collector F9-T	149
5.2.9	Maintenance and cleaning of Fraction collector F9-R	151

5 Maintenance and service5.2 Maintenance procedures5.2.1 Maintenance of the pump

# 5.2.1 Maintenance of the pump

#### **Maintenance interval**

Clean the pump when required. During the first months of use it is normal that excess oil leaks out of the drain hole below the pump. The function of the pump is not in any way affected by this.

#### **Required material**

- Cloth
- Mild cleaning agent or 20% ethanol

#### **Clean the pump**

Follow the steps below to clean the pump externally.

Step	Action
1	Switch off the instrument.
2	Wipe off the excess oil from the pump head with a damp cloth. Wipe off stains using a mild cleaning agent or 20% ethanol.
3	Let the pump dry completely before using the instrument.

5 Maintenance and service

5.2 Maintenance procedures

5.2.2 Exchange the online filter

# 5.2.2 Exchange the online filter

#### **Maintenance interval**

Exchange the online filter every week, or when required, for example when the filter becomes clogged.

#### **Required material**

- Filter 10PP
- Forceps
- Gloves

#### Instruction

Follow the steps below to exchange the online filter.

*Tip:* Use forceps and gloves during the replacement procedure to avoid contamination.

#### Step Action

1 Unscrew the nut from the online filter.



2 Remove the old filter (1) using forceps. Replace the support net (2) if damaged.



- 3 Mount the new filter. Carefully push the filter onto the holder (3).
- 4 Refit the nut and tighten the online filter by hand.

5 Maintenance and service5.2 Maintenance procedures5.2.3 Maintenance of the mixer

## 5.2.3 Maintenance of the mixer

#### **Maintenance interval**

Clean the mixer membrane when the mixer performance test fails or if the pressure in the system is higher than expected. Replace the mixer membrane if the problem persists.

Replace the O-rings inside the mixer if they are damaged.

#### **Required material**

- Mixer O-rings and membrane
- 20% ethanol
- Distilled water
- Ultrasonic bath

# Clean or replace the mixer membrane and O-ring

Follow the steps below to replace the O-rings and membrane inside the mixer.

Tip:	Use gloves during the replacement procedure to avoid contaminating the
	mixer components.

#### Step Action

- 1 Disconnect all tubing to the mixer.
- 2 Unscrew the top section of the mixer.



- 5 Maintenance and service
- 5.2 Maintenance procedures
- 5.2.3 Maintenance of the mixer



5

Clean the membrane (1) according to the solutions used:

- If using biological agents, immerse the membrane completely in 20% ethanol and place it in an ultrasonic bath for a few minutes. Repeat the ultrasonic bath with distilled water.
- If using only salt buffers, rinse the membrane with distilled water.
- 6 Remove the O-ring (2) from the bottom section.
- 7 Wet the new O-rings with 20% ethanol and fit them into position.

#### Step Action

8 To reassemble the mixer components, hold the top section of the mixer upside-down and place the membrane on top of the section. Fit the membrane indentations to the protrusions on the top section.



Place the bottom section of the mixer on top of the membrane. Make sure that the top and bottom sections are aligned.



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9

Screw the metal section onto the inner components of the mixer and connect the tubing.



Note:

Make sure to assemble the mixer correctly, following the instructions above. Incorrect assembly of the mixer can cause leakage. 5 Maintenance and service

5.2 Maintenance procedures

5.2.4 Maintenance of the UV flow cell

# 5.2.4 Maintenance of 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**

- Luer connector
- Syringe, 25-30 mL
- 10% surfactant detergent solution (e.g., Decon<sup>™</sup> 90, Deconex<sup>™</sup> 11, or RBS<sup>™</sup> 25)
- Distilled water

#### Clean the UV flow cell

Follow the steps below to clean the UV flow cell.

Step	Action
1	Disconnect all tubing from the UV monitor.

2 Replace the top fingertight connector with a Luer connector, and connect a piece of waste tubing to the bottom of the UV monitor. Insert the waste tubing into a waste container.



#### Step Action

3 Fill a syringe with distilled water, and connect the syringe to the Luer connector.



- 4 Inject the distilled water through the UV flow cell carefully. Disconnect the syringe.
- 5 Fill a syringe with a 10% surfactant detergent solution.

#### Tip:

Heat the 10% surfactant detergent solution to 40°C to increase the cleaning effect.

- 6 Connect the syringe to the Luer connector and inject the detergent solution through the UV flow cell. Repeat steps 5 and 6 about five times.
- 7 Disconnect the syringe and leave the detergent solution in the flow cell for at least 20 minutes.
- 8 Fill a syringe with distilled water. Connect the syringe to the Luer connector.
- 9 Inject the distilled water into the UV flow cell to rinse the flow cell. Disconnect the syringe.
- 10 Disconnect the Luer connector from the top of the UV monitor and the waste tubing from the bottom of the UV monitor. Reconnect the tubing to the UV monitor.

5 Maintenance and service

5.2 Maintenance procedures

5.2.5 Maintenance of the conductivity flow cell

# 5.2.5 Maintenance of the conductivity flow cell

#### **Maintenance interval**

Clean the conductivity flow cell when required.

#### **Required material**

- Luer connector
- Waste container
- Syringe, 25-30 mL
- 1 M NaOH
- Distilled water

**Note:** Use protective gloves when handling 1 M NaOH.

#### Clean the conductivity flow cell

Follow the steps below to clean the flow cell of the conductivity monitor.

Step	Action
1	Disconnect all tubing from the conductivity monitor.
2	Replace the top fingertight connector with a Luer connector, and connect a

2 Replace the top fingertight connector with a Luer connector, and connect a piece of waste tubing to the bottom of the conductivity monitor. Insert the waste tubing into a waste container.



#### Step Action

3 Fill a syringe with distilled water, and connect the syringe to the Luer connector



- 4 Inject distilled water through the conductivity flow cell carefully. Disconnect the syringe.
- 5 Fill a syringe with 1 M NaOH, connect the syringe to the Luer connector, and inject the solution through the conductivity flow cell.
- 6 Repeat step 5 about five times.
- 7 Leave the liquid in the flow cell for at least 15 minutes.
- 8 Fill a syringe with distilled water. Connect the syringe to the Luer connector.
- 9 Inject the distilled water into the conductivity flow cell to rinse the flow cell. Disconnect the syringe.
- 10 Disconnect the Luer connector from the top of the conductivity monitor and the waste tubing from the bottom. Reconnect the fingertight connectors with the respective tubing.

5 Maintenance and service

5.2 Maintenance procedures

5.2.6 Maintenance of the flow restrictor

# 5.2.6 Maintenance of the flow restrictor

#### **Maintenance interval**

Check the back pressure of the flow restrictor every month.

Clean the flow restrictor when required, for example if there is high back pressure from the flow restrictor, the pressure is unstable, or the pressure is close to zero.

#### Location of the flow restrictor

The recommended positions for the flow restrictor are the following:

• Connected to the conductivity monitor

or

• If a pH valve is installed, connected to the pH valve.

#### **Check the flow restrictor**

Follow the steps below to check the back pressure of the flow restrictor:

Step	Action
1	Set the flow restrictor in-line and start a flow of 2.5 mL/min. Take note of the pressure reading.
2	If the flow restrictor is connected to the conductivity monitor, remove the restrictor and connect the tubing using the 1/16"F / 1/16"F union connector. If a pH valve is installed, change the position to by-pass the flow restrictor.
3	Start a flow of 2.5 mL/min and take note of the pressure reading.
4	Check that the pressure difference is within 0.2 $\pm$ 0.05 MPa. If this is not the case, replace the flow restrictor.

#### **Clean the flow restrictor**

The flow restrictor is cleaned during a System CIP. If the flow restrictor is installed in a pH valve, select **pH valve** in the System CIP phase properties dialog, and perform a System CIP according to the instructions. Refer to the *Operating Instructions* for more information.

*Note:* If required, remove the flow restrictor from the instrument and use an ultrasonic bath for cleaning.
# 5.2.7 Maintenance of the pH valve and electrode

# Maintenance interval

Clean the pH electrode and the pH valve when required. The pH electrode has a limited longevity and should be replaced every six months or when the response time is slow. After cleaning or replacement, recalibrate the pH monitor, see Section 5.3.5 Calibrate the pH monitor, on page 163.

Store the pH electrode when pH monitoring is not used.

**Note:** The pH electrode and part of the valve, including the injection port, are not cleaned during a System CIP. These parts have to be cleaned manually as described below.

# **Required material**

- pH electrode
- Distilled water
- Standard buffer, pH 4
- Syringe, 25 to 30 mL
- 0.1 M HCl and 0.1 M NaOH (for cleaning of salt deposits)
- Liquid detergent (for cleaning of oil and grease)
- 1% pepsin solution in 0.1 M HCl and 1 M KNO<sub>3</sub> (for cleaning protein deposits)
- 1M NaOH (for cleaning the pH valve)

# pH electrode cleaning procedures

The pH electrode can be cleaned installed in the pH valve or removed from the pH valve. Instructions are provided in the following sections.

Clean the pH electrode following the procedures below. If these methods fail to improve the electrode, replace the electrode. See *Replace the pH electrode, on page 147*.

Required cleaning	Procedure
Salt deposits	Wash the electrode sequentially with 0.1 M HCl, 0.1 M NaOH, and 0.1 M HCl. Leave the electrode immersed for 5 minutes in each solution and rinse with distilled water between each solution.
Oil and grease films	Wash the electrode tip in liquid detergent and water. Use organic solvents if known to dissolve the specific films, and rinse with distilled water.
Protein deposits	Wash the electrode with a solution of 1% pepsin in 0.1 M HCl for five minutes, followed by thorough rinsing with distilled water.

5.2 Maintenance procedures

5.2.7 Maintenance of the pH valve and electrode

# Clean the pH electrode installed in the pH valve

Follow the steps below to clean the pH electrode installed in the pH valve. The calibration function is used to switch the position of the pH valve. However, no calibration is performed.

### Step Action

- 1 Use the calibration function to switch the position of the pH valve:
  - a. Open the Calibration dialog from the System Control module.
  - b. Select *pH* in *Monitor to calibrate*.
  - c. Click Prepare for calibration.
- Fill a syringe with approximately 10 mL of the chosen cleaning solution.
   Connect the syringe to the pH valve port **Cal**. Inject the liquid and wait for 5 minutes. Disconnect the syringe.



- 3 If several cleaning solutions are to be used, repeat step 4 with distilled water and then with the next solution.
- 4 As the last step in the cleaning procedure, rinse the pH electrode with distilled water. Connect a syringe with distilled water to port **Cal**, inject the water, and disconnect the syringe.
- 5 In the *Calibration* dialog, press the *Close* button.

Result:

The pH valve switches back to the default position and the **Calibration** dialog closes. No calibration is performed.

# Clean the pH electrode removed from the pH valve

If the pH electrode has been removed from the pH valve, wash the electrode tip by immersion in the solutions recommended for the required cleaning.

If the removal of protein deposits has failed following the procedure in *pH electrode cleaning procedures, on page 145,* clean the pH electrode with a 1 M KNO<sub>3</sub> solution. Heat the solution to 60°C to 80°C and immerse the electrode tip in the solution. Allow the electrode to cool while immersed in the KNO<sub>3</sub> solution before use.

**Note:** This procedure can only be performed when the pH electrode is <u>not</u> installed in the pH valve.

# **Clean the pH valve**

To clean the pH valve, follow the procedure used to clean the pH electrode installed in the valve. Use a 1 M NaOH solution for cleaning the pH valve.

**Note:** Replace the pH electrode with the dummy electrode before cleaning the pH valve.

# 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 of the used pH electrode from the connection on the front of the pH valve.	
2	Unscrew the nut of the pH electrode by hand, and pull the used electrode away.	
3	Unpack the new pH electrode. Remove the cover from the tip of the new pH electrode. Make sure that the electrode is not broken or dry.	
4	Prior to first use of the electrode, immerse the glass tip in distilled water for 30 minutes and then in a standard buffer, pH 4, for 30 minutes.	
5	Carefully insert the new pH electrode into the pH flow cell. Tighten the nut by hand to secure the electrode.	
6	Connect the pH electrode cable of the new electrode to the connection on the front of the pH valve.	
7	Calibrate the new pH electrode, see Section 5.3.5 Calibrate the pH monitor, on page 163.	

- 5 Maintenance and service
- 5.2 Maintenance procedures
- 5.2.7 Maintenance of the pH valve and electrode

# Storage of the pH electrode

The pH electrode can be stored in storage solution inside the pH flow cell. If pH monitoring is not used for a week or longer, inject new storage solution into the pH flow cell or replace the pH electrode with the dummy electrode that was installed in the pH valve on delivery.

Follow the steps below to store the pH electrode in the pH flow cell.

Step	Action
1	Use the calibration function to switch the position of the pH valve:
	a. Open the Calibration dialog from the System Control module.

- **b.** Select *pH* in *Monitor to calibrate*.
- c. Click Prepare for calibration.
- 2 Fill a syringe with approximately 10 mL of the storage solution. Connect the syringe to the pH valve port **Cal**, and inject the storage solution.



3 Click Close.

# 5.2.8 Maintenance and cleaning of Fraction collector F9-T

# **Maintenance interval**

Clean the Fraction collector externally when necessary. Do not allow spilled liquid to dry on the Fraction collector.

# **Required material**

The following materials are required:

- Cloth
- Water, mild cleaning agent or 20% ethanol.

# Instruction

Follow the steps below to clean the fraction collector externally.

Step	Action
1	Check that no run is in progress.
2	Turn off the ÄKTA go instrument.
3	Remove the tube rack by pressing the wedge and pulling the rack out, discard any remaining tubes and wash the tube rack with mild cleaning agent or 20% ethanol.



Wipe the surface of the Fraction collector with a damp cloth. Wipe off stains using a mild cleaning agent or 20% ethanol. Remember to wipe all sides, including the underside of the fractionation arm. Wipe off any excess of liquid.

4

- 5 Maintenance and service
- 5.2 Maintenance procedures
- 5.2.8 Maintenance and cleaning of Fraction collector F9-T





- 6 Insert the nozzle into the fractionation arm.
- 7 When the tube rack is dry, add a 15 mL tube and put it back into position.
- 8 Let the Fraction collector dry completely before restart.

# 5.2.9 Maintenance and cleaning of Fraction collector F9-R

# Maintenance interval

Clean the Fraction collector when required, for example in case of liquid spill.

# **Required material**

- Water or 20% ethanol
- Cloth

# Clean the Fraction collector externally

Follow the steps below to clean the Fraction collector F9-R externally and the drop sync sensor.

Step	Action
1	Switch off the instrument.
2	Wipe the surface with a damp cloth. Remove stains using a mild cleaning agent or 20% ethanol. Wipe off any excess.
2	Clean the drep superconcer leasted under the delivery arm with a domp

3 Clean the drop sync sensor located under the delivery arm with a damp cloth.



4

Let the Fraction collector dry completely before restart.

# 5.3 Calibration of monitors and sensors

# Introduction

This section provides instructions for calibration of the instrument monitors and sensors. The calibration is performed using the **Calibration** dialog in the **System Control** module in UNICORN.

# In this section

Section		See page
5.3.1	Calibrate the pressure sensors	153
5.3.2	Calibrate the UV monitor	155
5.3.3	Calibrate the conductivity monitor	159
5.3.4	Calibrate the temperature sensor	162
5.3.5	Calibrate the pH monitor	163

# 5.3.1 Calibrate the pressure sensors

# Introduction

ÄKTA go can have up to three pressure sensors:

- One pressure sensor in the pressure monitor, that measures the pressure of the system.
- Two column pressure sensors in column valve **V9-C**, that measure pre-column pressure and post-column pressure.

# **Maintenance interval**

Check the pressure sensors every week, or when the ambient temperature has changed by more than  $\pm$  5°C.

# Instruction

Follow the steps below to check and calibrate the pressure sensors. The procedure is the same for each sensor.

Step	Action
1	Disconnect the relevant tubing to the pressure sensor being checked. See <i>Pressure sensor tubing, on page 153.</i>
2	In the <b>System Control</b> module, select <b>System →Calibrate</b> to open the <b>Calibration</b> dialog.
3	Select the pressure sensor to calibrate from the <i>Monitor to calibrate</i> drop- down list and check the pressure value(s) displayed in <i>Current value</i> .
4	If the pressure reading is outside the range $\pm$ 0.02 MPa, press <b>Reset Pressure</b> .

### **Pressure sensor tubing**

The table below shows the tubing to disconnect when checking and calibrating the pressure sensors.

5.3 Calibration of monitors and sensors

5.3.1 Calibrate the pressure sensors

Pressure sensor	Tubing to disconnect
System pressure sensor	Tubing between the pressure monitor and the mixer.
Pre-column pressure sensor	Tubing in column valve <b>V9-C</b> port <b>In</b>
Post-column pressure sensor	Tubing in column valve <b>V9-C</b> port <b>Out</b>

# 5.3.2 Calibrate the UV monitor

# Introduction

The path length in the UV flow cell might differ from the nominal length, which leads to incorrect results in the calculation of protein concentration in the eluate. To achieve normalized absorbance, the path length in the UV flow cell must be calibrated. The calibration procedure is described below.

# **Maintenance interval**

Calibrate the UV monitor when a new UV flow cell is installed, to obtain the real UV cell path length.

# **Required material**

- UV Test accessories (product code 29293950)
- Waste beaker
- UV Test Kit, 1 and 2 mm or UV Test Kit, 5 and 10 mm
- UV Test Calculation protocol
- UV Test Kit Instructions

# Calibration test kit and calculation protocol

To calibrate the UV monitor, a calibration kit and a calculation protocol are required. The calibration kit contains test solutions which should be used according to the flow cell path length.

UV flow cell path length	Calibration kit
2 mm	UV Test Kit, 1 and 2 mm (Product Code 29276997)
5 mm	UV Test Kit, 5 and 10 mm (Product Code 29276998)

The calculation protocol and UV test kit instructions can be downloaded from *cytiva.com*. Search for the product code of the suitable calibration kit as indicated above, and click on **Related documents** to access the documents.

# **UV** calibration setup

For convenience, use the tubing and fittings included in the UV test accessories. The illustration below shows the setup. If possible, fit the syringe directly to the flow cell as shown in illustration A. If not, connect the syringe as shown in illustration B.

5.3 Calibration of monitors and sensors

5.3.2 Calibrate the UV monitor

### *Tip:* Adapt tubing length to minimize the use of test solution.



Part	Function	Note
1	Syringe, 3 mL	Part of UV Test Kit
2	Female Luer-Male Luer connector	Part of UV test accessories
3	UV flow cell	N/A
4	Fingertight HPLC	Part of UV test accessories
5	EFTE tubing, i.d. 1 mm, o.d. 1/16	Part of UV test accessories
6	Waste beaker	Supplied locally
7	Union 1/16"F / 1/16"F	Part of UV test accessories

The UV test kit is used to calibrate many different UV monitors. The table below shows the concentration of iron(III)-sulphate that should be used for the respective UV flow cells available for ÄKTA go.

Concentration of iron(III)-sulphate in 0.1 M sulphuric acid (mg/L)	Concentration of iron(III)-sulphate in 0.1 M sulphuric acid (mg/L)
2 mm flow cell path length	5 mm flow cell path length
0	0
117	55
234	117
570	234
1420	570

# Instruction

Follow the steps below to calibrate UV monitor **U9-L**.

Step	Action
1	See the attached Certificate of Analysis (CoA) for the concentration of Fe(III)-SULF (mg/L) and the reference absorbance values (AU/cm).
	<b>Note:</b> The values for the test solutions stated in the CoA are only valid at room temperature.
2	Enter the concentrations of the solutions (lower concentrations first) in the column <b>UV Test Kit, Concentration Fe(III)SULF (mg/L)</b> in the UV Test Kit Calculation Protocol.
3	Enter the reference absorbance values into the column <b>UV Test kit, Certifi- cate Absorbance (AU/cm)</b> in the UV Test Kit Calculation Protocol.
4	Fill one of the supplied syringes with the first solution (0 mg/L). Make sure that there are no air bubbles in the syringe.
5	Fit the syringe in the connector and inject ~ 2 mL of the solution to rinse the flow cell.
6	Refill the syringe and repeat the injection. DO NOT remove the syringe.
7	Wait until the monitored absorbance value has stabilized.
8	In <b>Process Picture</b> , select the UV monitor and perform an <b>Auto Zero UV</b> for the first solution (0 mg/L).
9	Remove the syringe.
10	Fill a new syringe with the next solution, in increasing concentration order. Make sure that there are no air bubbles in the syringe.
11	Repeat steps 5 to 7 with the remaining test solutions in increasing concen- tration order according to UV test solution tables.
	<b>Note:</b> Use a new syringe for each solution.
12	Wait for a stable absorbance value after each injection. Enter the values into the column <b>UV Monitor, Measured Absorbance (AU)</b> in the UV Test Kit Calculation Protocol.
	<i>Note:</i> The values should be converted from mAU to AU.

5.3 Calibration of monitors and sensors

5.3.2 Calibrate the UV monitor

Step	Action
13	In the UV Test Kit Calculation Protocol, read the <b>Real cell length (in mm)</b> and <b>Linearity Deviation</b> when all absorbance values have been entered in the table. The values are shown at the bottom of the table.
	<b>Note:</b> The regression coefficient R2 should be larger than 0.999. If not, refer to the instructions for the flow cell.

# Adjust the UV cell path length

Follow the steps below to define the **UV cell path length** in UNICORN. The flow cell path length must be adjusted when the flow cell has been replaced or calibrated.

Step	Action
1	Open the <b>Calibration</b> dialog from the <b>System Control</b> module, by selecting <b>System →Calibrate</b> .
2	Select <b>UV cell path length</b> from the <b>Monitor to calibrate</b> drop-down list.
3	Enter the nominal flow cell path length in the <b>Nominal length</b> input field and click <b>Set</b> .
4	Enter the flow cell path length in the <b>Real length</b> input field:
	a. If a calibration has been performed, enter the calculated flow cell path length, obtained from the calibration procedure.
	<b>b.</b> If no calibration has been performed, enter the nominal flow cell path length.
	Click <b>Set</b> .
	Result:
	The UV flow cell path length has been adjusted.

# 5.3.3 Calibrate the conductivity monitor

# Introduction

Two types of calibration of the conductivity monitor can be performed:

- **Conductivity monitor factory calibration**: Restores the conductivity flow cell constant to the factory default value. Perform this calibration to override an incorrect user calibration.
- **Conductivity monitor user calibration**: The user calibrates the conductivity flow cell constant. Perform calibration after cleaning, when the signal is unstable, or when you suspect that the calibration is incorrect.

# **Required material**

- Distilled water
- 1.00 M NaCl
  - or
- Certified conductivity standard solution

# Instruction

To perform a factory calibration of the conductivity monitor, select **Conductivity monitor - factory calibration** in the **Calibration** dialog and click **Restore**.

Follow the steps below to perform a user calibration of the conductivity flow cell constant.

Step	Action
1	Make sure that the instrument has been switched on for at least one hour.
2	Open the <b>System Settings</b> dialog by selecting <b>System →Settings</b> from the <b>System Control</b> module.

### 3 Set the **Compensation factor** to 0%.

ystem Settings - AKTA go		×
Instructions: UV Conductivity Alarm conductivity Relative scale cond Cond temp compensation PH Pressure alarm Graction collection Wash settings Watch parameters Advanced Data collection	Parameters for Cond temp compensation Compensation factor (0.0 - 9.9) 000 ☆ % Reference temperature (0.0 - 40.0) 25.0 ☆ °C	
0	Set Parameters To Strategy Default Values OK Cancel	əl

5.3 Calibration of monitors and sensors

5.3.3 Calibrate the conductivity monitor

Step	Action
4	Wash the whole flow path and fill it with distilled water using a suitable inlet and the pump, until the conductivity value reaches 0.00 mS/cm.
5	Prime and purge the inlet used in step 4 with the 1.00 M NaCl calibration solution. Fill the conductivity cell with the calibration solution at a 1 mL/min flow rate. Pump in at least 15 mL of the calibration solution and wait until the conductivity signal and the temperature have stabilized before continuing the calibration.
6	Open the <b>Calibration</b> dialog from the <b>System Control</b> module, by selecting <b>System →Calibrate</b> .
7	Select <b>Conductivity monitor - user calibration</b> from the <b>Monitor to cali- brate</b> drop-down list.
8	Enter the theoretical conductivity value at the current conductivity temper- ature in the <b>Enter theoretical conductivity value</b> input field.
	<ul> <li>If a certified conductivity standard solution is used, use the supplied theoretical conductivity value.</li> </ul>
	<b>b.</b> If a manually prepared 1.00 M NaCl calibration solution is used, see <i>Graph for conductivity value, on page 160.</i>
	Тір:
	The temperature is available in the <b>Run Data</b> field.
9	Click <b>Calibrate</b> .
	Result:
	The new conductivity cell constant is displayed in the <b>Conductivity cell 1 constant/cm</b> box. The new constant should normally be $40 \pm 10$ cm <sup>-1</sup> . The date and time for the calibration are also displayed.
10	In the <b>System Control</b> toolbar, click the <b>End</b> icon to end the run.
11	In the <b>System Settings</b> dialog, select <b>Conductivity</b> → <b>Cond temp</b> <b>compensation</b> and set the <b>Compensation factor</b> back to the desired value. Click <b>OK</b> .

# Graph for conductivity value

The graph below shows the conductivity value at the current temperature when  $1.00\,\text{M}$  NaCl calibration solution is used.



Conductivity of 1.00 M NaCl at 20–30°C

5.3 Calibration of monitors and sensors

5.3.4 Calibrate the temperature sensor

# 5.3.4 Calibrate the temperature sensor

# Introduction

The temperature sensor is located in the conductivity monitor, and monitors the temperature to give accurate conductivity measurements.

# **Maintenance interval**

Calibrate the temperature sensor when required.

# **Required material**

- External temperature sensor
- Distilled water

or

Buffer

# Instruction

Follow the steps below to calibrate the temperature sensor.

Step	Action
1	Open the <b>Calibration</b> dialog by selecting <b>System →Calibrate</b> , in the <b>System Control</b> module.
2	Select <b>Temperature calibration</b> and follow the instructions in the <b>Cali-</b> <b>bration</b> dialog.

# 5.3.5 Calibrate the pH monitor

# **Maintenance interval**

Calibrate the pH monitor once a day, when the pH electrode has been replaced, or if the ambient temperature has changed by more than  $\pm$  5°C.

# **Required material**

- Syringe
- Luer connector
- pH calibration buffers

**Note:** Use two pH calibration buffers with a difference of at least one pH unit. Preferably use a pH standard buffer, pH 4 or pH 7, as the first calibration point, and a pH standard buffer close to the lowest or highest pH needed to measure as the second point. Allow the buffers to reach to ambient temperature before use.

# Instruction



Follow the steps below to calibrate the pH monitor.

Step	Action
1	Attach a Luer connector to the pH valve port <b>Cal</b>
2	Open the <b>Calibration</b> dialog from the <b>System Control</b> module, by selecting <b>System →Calibrate</b> .
3	Select <b>pH</b> from the <b>Monitor to calibrate</b> drop-down list and click the <b>Prepare for calibration</b> button.
	Result:
	The pH valve switches to the calibration position.
4	Enter the pH of the first pH standard buffer in the <b>pH for buffer 1</b> field.

5.3 Calibration of monitors and sensors

5.3.5 Calibrate the pH monitor

# Step Action 5 Fill a syringe with approximately 10 mL of the first pH standard buffer.

Connect the syringe to the Luer connector in port **Cal** and inject the buffer.



- 6 When the **Current value** is stable, click the **Calibrate** button.
- 7 Using a new syringe, wash the pH flow cell by injecting water into port **Cal**.
- 8 Enter the pH of the second pH standard buffer in the **pH for buffer 2** field.

9 Repeat steps 5-7 using the second pH standard buffer. *Result:* 

The calibration date and time are displayed in the dialog, along with values for **Calibrated electrode slope** and **Asymmetry potential at pH 7**.

10 Check that **Calibrated electrode slope**  $\ge$  80% and **Asymmetry potential** at pH 7 is within the  $\pm$  60 mV range.

### Note:

If the values are not within the specified range, repeat the calibration procedure. If the problem persists, replace the electrode as indicated in *Replace the pH electrode, on page 147.* 

11 Click the **Close** button to switch the pH valve back to the default position and to close the **Calibration** dialog.

# 5.4 Service

# Introduction

This section describes basic service procedures that can by performed by an experienced user. It contains instructions on how to repair modules or replace parts of modules to repair them.

# In this section

Section		See page
5.4.1	Maintenance of the pump head check valves	166
5.4.2	Maintenance of the pump pistons and piston seals	171

5 Maintenance and service5.4 Service5.4.1 Maintenance of the pump head check valves

# 5.4.1 Maintenance of the pump head check valves

# Maintenance interval

Replace or clean a check valve when required, for example if the check valve is clogged or damaged.

# **Required material**

- Adjustable wrench
- 20% ethanol
- Distilled water
- Ultrasonic bath
- Check valve kit

# Clean the pump head check valves

Follow the steps below to remove and clean the pump head check valves.



# WARNING

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

Step	Action
1	Switch off the instrument and disconnect all tubing from the pump head.
2	Remove the upper and lower check valves from the pump head, following the instructions in <i>Replace the pump head check valves, on page 167</i> .
3	Clean the check valves according to the solutions used:
	• If using biological agents, immerse the check valves completely in meth- anol and place them in an ultrasonic bath for a few minutes. Repeat the ultrasonic bath with distilled water.
	<ul> <li>If using only salt buffers, rinse the valves with distilled water.</li> </ul>
4	Refit the check valves in the pump head.
5	Refit the inlet manifold and reconnect the tubing to the pump head.

# Replace the pump head check valves



# NOTICE

Handle the check valves with care when they have been removed from the pump heads, to prevent loss of any internal components.

Follow the steps to replace the check valves of a pump.

Step	Action
1	Switch off the instrument.
2	Disconnect the tubing from the check valves, the pump inlet tubing, and the tubing of the pump rinsing system.
3	Unscrew the purge valve by turning it counter-clockwise, and lift off the ring.



### 5.4 Service

5.4.1 Maintenance of the pump head check valves

Step Action

4

Unscrew the plastic nut of the upper check valve using an adjustable wrench (1), and lift off the upper check valve (2).





- 5 Replace the upper check valve with a new one.
- 6 Tighten the nut until fully finger-tight. Use an adjustable wrench to further tighten it.

### Note:

Do not over tighten the nut, as doing so might cause it to break.

- 7 Place the ring onto the new upper check valve, and screw the purge valve.
- 8 Unscrew the two white plastic screws located below each pump head. Pull the plastic connectors to the sides to release the inlet manifold.



#### Step Action

9 Unscrew the lower check valve using an adjustable wrench, and remove it from the pump head.



- 10 Replace the lower check valve with a new one.
- 11 Tighten the nut until fully finger-tight. Use an adjustable wrench to further tighten it.

### Note:

Do not over tighten the nut, as doing so might cause it to break.

12 Refit the inlet manifold and reconnect the tubing to the pump.

# **Replace O-ring**

Follow the steps below to the replace the O-ring located in the purge valve.

Step	Action
1	Unscrew the purge valve of the pump head and remove the O-ring from the
	purge valve.

### 5.4 Service

5.4.1 Maintenance of the pump head check valves

Step	Action
2	Wet a new O-ring with lubricant and fit it to the purge valve.



### Note:

Always use Lubricant 56686700 when exchanging the O-ring 3 x 1 mm.

3 Screw the purge valve back into the pump head.

# 5.4.2 Maintenance of the pump pistons and piston seals

# **Maintenance interval**

Replace or clean the pistons, piston seals, rinse membranes, or O-rings of the pump if they are damaged. After replacement, perform a run to break in the new piston seals.

**Note:** Always replace the O-rings, piston seals, and pump membrane housing of both pump heads at the same time.



### NOTICE

**Advanced maintenance.** Read the instruction carefully before disassembly of the pump head.

# **Required material**

- Torx T20 screwdriver
- Allen key
- Ultrasonic bath
- Ethanol, 20%
- Tubing giving a back pressure of 2 to 4 MPa
- P9-S Seal kit, 65 mL

# Illustration

The illustration below shows the parts of the pump head in pump P9-S.



### 5.4 Service

5.4.2 Maintenance of the pump pistons and piston seals

Part	Description	Part	Description
1	Return spring	8	Pump head
2	Piston	9	Lower check valve
3	Star screws	10	Upper check valve
4	Drain plate	11	O-ring
5	Rinse membrane	12	Purge valve
6	Rinse chamber	13	Allen screws
7	Piston seal		

# Replace piston, piston seal, and rinse membrane

Step	Action
1	Disconnect all tubing from the pump.
2	Unscrew the two white plastic screws located below each pump head by hand. Pull the plastic connectors to the sides to release the inlet manifold.



### Step Action

3 Unscrew one of the Allen screws from the front section of the pump head, using an Allen key. Unscrew the second screw, while pushing firmly on the front section of the pump head to compensate for the pressure of the piston return spring.



- 4 Place the front section of the pump head face down on the bench. Pull out the piston together with the return spring.
- 5 Inspect the piston and return spring for signs of damage. If damaged, discard the piston and return spring and use a new piston and return spring when assembling the pump head.
- 6 Unscrew the two screws of the drain plate using a Torx T20 screwdriver. Remove the drain plate.



7

Remove the rinse membrane using the piston.



8

Remove the rinse chamber and discard the piston seal.

### 5.4 Service

5.4.2 Maintenance of the pump pistons and piston seals

Step	Action
9	Clean the pump head, rinse chamber, and drain plate in an ultrasonic bath. If there are particles on any surfaces, the check valves should be removed and cleaned separately. See <i>Clean the pump head check valves, on page 166</i>
10	Wet a new piston seal with 20% ethanol. Place the new piston seal in the hole in the front section of the pump head and press it into position.
11	Place the rinse chamber onto the pump head, with the conical depression in the rinse chamber facing outwards. Fit the new rinse membrane with the conical depression inwards.

- 12 Place the drain plate on top of the assembly. Tighten the screws with a Torx T20 screwdriver.
- 13 Wet the new piston with 20% ethanol and insert it into the return spring. Insert the piston into the pump head gently and press it firmly downwards into the piston seal.

### Note:

Do not push the piston at an angle to the pump head. Once inserted into the pump head, do not twist the piston.

14 Mount the pump head onto the instrument. Push firmly on the front section of the pump head and tighten the screws with an Allen key.

# Break in the new pump piston seal

Follow the steps below to break in the new pump piston seal in the pump.

Step	Action
1	Fill a buffer bottle with at least 50 mL of 20% ethanol in water. Immerse the inlet tubing in the buffer vessel. Place the buffer vessel on the top tray.
2	Prime the inlets and purge the pump heads. Refer to the <i>ÄKTA go Operating Instructions (29360951)</i> for instructions.

Step	Action
3	Connect tubing that gives a back pressure of 2 to 4 MPa at 25 mL/min, between the injection valve and the UV monitor.
4	Immerse the waste tubing in the buffer vessel to recirculate the liquid.
5	Set the system flow rate to 25 mL/min.
6	Run the flow for 2 hours.
7	Discard the used ethanol.

# 5.5 Replacement of modules and components

# About this section

This section gives instructions for replacing the modules and components in the  $\ddot{\mathsf{A}}\mathsf{KTA}$  go instrument.

# In this section

Section		See page
5.5.1	Replace ÄKTA go modules	177
5.5.2	Replace the pump	178
5.5.3	Replace the pressure monitor	179
5.5.4	Replace the UV flow cell	181
5.5.5	Replace the flow restrictor	183
5.5.6	Replace tubing and connectors	184
5.5.7	Replace the inlet filters	186

# 5.5.1 Replace ÄKTA go modules

The procedure to replace or install a module is similar for most modules. To replace modules in the ÄKTA go instrument, refer to *Install optional modules, on page 28* for detailed instructions.

 $5.5 \ \ {\rm Replacement} \ of modules \ {\rm and} \ {\rm components}$ 

5.5.2 Replace the pump

# 5.5.2 Replace the pump

# **Maintenance interval**

Replace the pump if damaged or if replacement of the pump head check valves and the pump piston seals is not sufficient to fix the pump.

# **Required material**

• Torx<sup>™</sup> T20 screwdriver

# Instruction

Follow the steps below to replace pump P9-S.

Step	Action
1	Disconnect power from the instrument by using the instrument power button.
2	Disconnect the tubing from the check valves, the pump inlet tubing, and the tubing of the pump rinsing system.
3	Loosen the screw with a Torx T20 screwdriver.
4	Remove the pump module and disconnect the cable from the back of the module.
5	Connect the cable to the new pump, insert the pump into the chassis, and fasten the screw with a Torx T20 screwdriver.
6	Connect the tubing to the pump.

# 5.5.3 Replace the pressure monitor

# **Maintenance interval**

Replace the pressure monitor when required.

# **Required material**

- Pressure monitor **R9-1n**
- Torx T20 screwdriver

# Instruction

Follow the steps below to replace the pressure monitor.

**Note:** A pressure monitor must always be installed in ÄKTA systems to protect the instrument from damage caused by high pressure.

Step	Action
1	Disconnect power from the instrument by using the instrument power button.
2	Loosen the tubing connectors and remove the tubing from the pressure monitor.

3 Loosen the top and bottom screws in the pressure monitor with a Torx T20 screwdriver.





5.5 Replacement of modules and components

5.5.3 Replace the pressure monitor

4

### Step Action

Remove the pressure monitor and disconnect the cable at the back.



- 5 Connect the cable to the new pressure monitor and fasten both screws with a Torx T20 screwdriver.
- **Note:** When the pressure monitor is replaced, the pressure monitor should be calibrated in UNICORN. See Section 5.3.1 Calibrate the pressure sensors, for instructions.
# 5.5.4 Replace the UV flow cell

#### **Maintenance interval**

Replace the UV flow cell when it is desired to use a flow cell with a different path length, or if the flow cell is damaged.

#### **Required material**

• UV flow cell

#### Instruction



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.
- 3 Unscrew the wheel at the bottom of the UV monitor and press the wheel upwards to release the flow cell.



5 Maintenance and service

5.5 Replacement of modules and components

5.5.4 Replace the UV flow cell

#### Step Action

4

Pull the flow cell upwards out of the monitor. Hold the flow cell by the top part with the O-ring. Do not touch the optical surfaces of the flow cell.



#### Note:

Make sure that the flow cell does not come into contact with any liquid, and that no liquid enters the monitor.

- 5 Insert a new flow cell into the monitor.
- 6 Tighten the wheel firmly.
- 7 Connect the tubing to the new flow cell.
- 8 Switch on the instrument.
- 9 If a cell with a different path length is used, adjust the UV flow cell path length in the **Calibrate** dialog, in **System Control**.

#### Note:

If a real cell path length is needed, calibrate the new UV flow cell path length instead of using the nominal value.

After replacing the UV flow cell, run a system performance test to check that the flow cell is working properly. Refer to *ÄKTA go Operating Instructions (29360951)* for instructions.

# 5.5.5 Replace the flow restrictor

#### **Maintenance interval**

Replace the flow restrictor when required, for example if there is high back pressure from the flow restrictor, the pressure is unstable, or the pressure is close to zero.

#### **Required material**

• Flow restrictor **FR-902** 

#### Instruction

Follow the steps below to replace the flow restrictor.

Step	Action	
1	Disconnect the tubing from the used flow restrictor, and discard the flow restrictor.	
2	Connect the tubing to the new flow restrictor:	
	<ul> <li>a. If connecting the flow restrictor to the conductivity monitor, connect the flow restrictor port IN to the outlet of the conductivity monitor, and connect the flow restrictor port OUT to the outlet valve port In.</li> </ul>	
	b. If connecting the flow restrictor to the pH valve, connect the flow restrictor port IN to the pH valve port ToR (To Restrictor), and connect the flow restrictor port OUT to the pH valve port FrR (From Restrictor).	
3	Check the back-pressure of the new flow restrictor, following the instruc- tions above.	

After replacing the flow restrictor, run a system performance test to check that the flow restrictor is working properly. Refer to the *Operating Instructions* for instructions.

5 Maintenance and service

5.5 Replacement of modules and components

5.5.6 Replace tubing and connectors

# 5.5.6 Replace tubing and connectors

#### **Maintenance interval**

Replace tubing and connectors when required, for example if a tubing is clogged or kinked disturbing the system flow.

### **Required material**

- Tubing and connectors
- Tubing cutter
- Fingertight wrench

#### Instruction

Follow the steps below to replace tubing and connectors.

Step	Action
1	Unscrew the connectors, and disconnect the tubing.
2	If the tubing has labels, remove the labels to be used with the new tubing later.

#### Step Action

3

Cut the new tubing to the same length as the old tubing. Use a tubing cutter to get a straight angle cut.





#### CAUTION

**Cut injuries.** The tubing cutter is very sharp and must be handled with care to avoid injuries.

#### Note:

When replacing system tubing, use the original inner diameter and length to ensure that the correct internal volumes are maintained. Inlet and outlet tubing may be shortened if required.

- 4 Put the old labels on the new tubing.
- 5 Mount the new connectors on the tubing.
  - For fingertight connectors, slide the connector onto the tubing.
  - For tubing connectors 1/8", slide the connector onto the tubing. Slide the ferrule onto the tubing with the thick end towards the end of the tubing.
- 6 Insert the tubing with connector into the port. Make sure to insert the tubing all the way into the bottom of the port.
- 7 Tighten the connector fully. For areas difficult to access, use the fingertight wrench included in the accessory kit.

5 Maintenance and service5.5 Replacement of modules and components5.5.7 Replace the inlet filters

# 5.5.7 Replace the inlet filters

#### **Maintenance interval**

Replace the inlet filters when required, for example when the filters are clogged.

# **Required material**

• Inlet filter set

# Instruction

Follow the steps below to replace the inlet filter and support net from inlet tubing.

Step	Action
1	Pull off the inlet filter and the support net from the inlet filter holder.
2	Fit the new support net and inlet filter, and press the filter into position.

# 5.6 Maintenance Manager

#### Introduction

**Maintenance Manager** in UNICORN allows the user to display general information about the system and its modules, as well as operational statistics of the modules. There are predefined maintenance notifications of the system and its modules based on calendar periods of system use and on operational statistics of the modules. To enable predefined maintenance notifications refer to UNICORN help.

Additional maintenance notifications can also be added for the system. See *Add a new system notification, on page 188,* for instructions.

#### **Open Maintenance Manager**

Access *Maintenance Manager* from the *System Control* module, in UNICORN. Go to the *System* menu and select *Maintenance Manager* to open the *Maintenance Manager* dialog.

# View general information and statistics

In the left pane of the *Maintenance Manager* dialog box, select the system of interest to view general system information and information for a module of interest. When modules are selected, operational statistics are also displayed.

Maintenance Manager - ÄKTA go			×
⊕ ∰r ÄkTA go           ⊕ ∰rection valve (V3-s)           ⊕ ∯rection valve (V3-s)           ⊕ ∯rection valve (V3-s)           ⊕ Årelte valve (V3-s)	<ul> <li>Injection valve (V9-J)</li> <li>General</li> <li>Name</li> <li>Serial Number</li> </ul>	Value P2.02 P3.02	
System pressure monitor (R9)     Conductivity monitor (C9)     UV monitor (U9-1)     PH monitor (H9)     PH monitor (H9)     PH monitor (F9-R)     PH monitor (F9-R)     PH valve (K9)     Sate     Gate	Sonware Version Hardware Version Statistics	28920910	
	Name	Value	
	Serial Number	P2 02	
	Software Version	V1.03.33 28920910	
	No of valve turns	2829	
	Accumulated power on time	6490 hours	
	Number of power ons	578	
New System Notification     Delete	Print	Close	

5.6 Maintenance Manager

### Add a new system notification

1

2

The user can add new system notifications to the list of system events. Follow the steps below to add a new system notification.

#### Step Action

In the Maintenance Manager dialog box, click New System Notification.

ÄKTA go     Scheduled maintenance     NewNet#ination	NewNotification
	Penelle Event Name: NewNotification Peneurs august 12 month(a)
B Inlet valve (V9-lm)     Sample inlet valve (V9-lm)     System pump A (P9 A)     System pressure monitor (P9)     Conductivity monitor (C9)     On (V1 monitor (U9-L))	Next notification at: 2020-07-09 Restart
	The System/Module has reached its service interval limit and requires maintenance. Please cont your GE service representative.
	Default Cancel Arr

#### In the **NewNotification** pane:

- a. Enter a name for the new notification.
- **b.** Select a time interval after which the new notification is issued.
- c. If desired, write a message to be shown in the maintenance notification.
- 3 Click *Apply* to save the changes and apply the notification settings.

# 6 Troubleshooting

## About this chapter

This chapter describes how to troubleshoot ÄKTA go in the case of a system malfunction. Possible causes and corrective actions are described in the following sections.

# In this chapter

Section		See page
6.1	Hardware issues	190
6.2	Software issues	203
6.3	Application issues	204

# 6.1 Hardware issues

## Introduction

If malfunction of a module is suspected, check the following sections for possible causes and corrective actions for the issue. If there is a malfunction of the system but the issue or module affected is unknown, run a suitable performance test as the first step in troubleshooting the system. For instructions on how to run performance tests refer to *ÄKTA go Operating Instructions (29360951)*. For a detailed description of the performance tests, see *Chapter 4 Performance tests, on page 100*.

# **Power supply**

Problem	Possible cause	Action
Instrument does not turn on	Power cord not connected	Connect the power cord to the wall outlet and to the electrical inlet on the instrument.
	No electric current in the wall outlet	Make sure that there is electric current in the wall outlet.
	Broken fuse in the instrument	Replace the fuse. Refer to ÄKTA go Operating Instructions (29360951).
	Overheated instru- ment	Switch off the instrument and wait until the temperature has decreased before restarting it. If the problem persists, generate a System error report and contact Service.

# **High internal temperature**

Problem	Possible cause	Action
High internal temper- ature	Insufficient instru- ment ventilation	Make sure that the air ventilation on the back of the instrument is not covered. There should be at least 10 cm clearance at the back of the instru- ment to allow adequate air circulation.
	Hot surroundings	Decrease the room temperature. Maximum operating temperature is 35°C.
	Faulty fan	Contact Service.

# Instrument communication

Problem	Possible cause	Action
The instrument is not detected by UNICORN	Disconnected network cable between the instrument and the control computer	Connect the cable.
The system could not be connected to UNICORN	UNICORN instrument server has shut down	Restart the control computer.
The UNICORN client has lost connection to the instrument server	Temporary overload of the processor	Restart the UNICORN client to regain control.
Multiple error messages in UNICORN: <b>One or</b>	A cable between a module and the instru- ment is not connected	Check all modules and connections.
more module(s) is not found by the instrument	A UniNet-9 connector is not plugged	Check that all UniNet-9 connectors that are not in use are plugged with gray jumpers.
	Incorrect Node ID for one module	Change the Node ID. See Section 7.12 Node ID, on page 280.
Module not found by the instrument or warning message in UNICORN: (Warning) Two instrument modules have the same Node ID	Two or several modules have the same Node ID	Change the Node ID. See Section 7.12 Node ID, on page 280.
Warning message in UNICORN: Instru- ment module is missing	The module is not functioning properly	In the displayed dialog in UNICORN, select the option <b>Restart the system only</b> and click <b>OK</b> . If the problem persists, contact Service.
Warning messages in UNICORN: <b>(Warning)</b> Gate (12): Internal instrument error	Incorrect Node ID for one module	Change the Node ID. See Section 7.12 Node ID, on page 280.
Error message from a module: <b>Internal</b> instrument error	Various causes	Restart the instrument. If the problem persists, generate a System error report, and contact Service.

6.1 Hardware issues

# Leakage

Problem	Possible cause	Action
Leakage around a connector	Connector not tight- ened	Tighten the connector. If necessary, replace the connector.
	Worn connector	Unscrew the connector to check it. If necessary, replace the connector.
	Crystallized material around the connector	Unscrew to clean the connector. If necessary, replace the connector.

# **High pressure**

Problem	Possible cause	Action
Pressure alarm or higher pressure than expected	Pressure increase due to viscosity (e.g. samples or run in a cold environment)	Lower the flow rate or activate <b>Pressure</b> <b>Control</b> .
	Pressure alarm incor- rectly set	Check the pressure alarm setting in <b>System Settings</b> .
	Clogged online filter	Replace the online filter. See Section 5.2.2 Exchange the online filter, on page 136.
	Tubing i.d. changed	The back pressure for a 0.25 mm i.d. tubing is 16 times higher than that for a 0.50 mm i.d. tubing used with the same running conditions.
	Blocked flow path	Remove obstructions to the flow path. For example, remove stop plugs and replace constricted tubing.
High pressure alarm when pressure control is activated	Inappropriate pres- sure control param- eter selected	See Section 3.8 Pressure control, on page 96.

# Inlet valve K9

Problem	Possible cause	Action
Air in tubing after the inlet valve	Connectors not tight- ened	Tighten the connectors. If necessary, replace the connectors.
Noflow	Wrong inlet selected	Select the correct inlet or check the inlet instruc- tions in the method.

Problem	Possible cause	Action
	Empty buffer or sample bottle	Refill the bottle
	Faulty valve	Replace the valve. See Section 5.5.1 Replace ÄKTA go modules, on page 177.

# **Rotary valves**

Problem	Possible cause	Action
Valve not switching or switching to a wrong	Incorrect instruction in the method	Check the method.
position	Hardware error	Restart (power off) the instrument. If the problem persists, replace the module. See Section 5.5.1 Replace ÄKTA go modules, on page 177.

# Pump

Problem	Possible cause	Action
Erratic flow or pres- sure	Air trapped in the pump heads	Purge the pump. Refer to ÄKTA go Operating Instructions (29360951).
	Buffer running out	Check that there is sufficient volume of buffer present in the flask. Prime the inlets.
	Bad piston seal	Replace the piston seal and rinse membrane. See Section 5.4.2 Maintenance of the pump pistons and piston seals, on page 171.
	Bad piston	Replace the piston. See Section 5.4.2 Mainte- nance of the pump pistons and piston seals, on page 171.
	Bad check valve	Replace the check valves. See <i>Replace the pump</i> head check valves, on page 167.
Small pressure pulsa- tions that remain after a proper purge of the pump heads	Air bubble stuck in the pump seal	Run 100% methanol at >5 mL/min and >0.5 MPa for a few minutes. Alternatively, run water at 25 mL/min and >2 MPa for 1 to 2 hours.
Liquid leaking between the pump head and the side panel	Piston seal or rinsing membrane incorrectly fitted or worn	Replace or reinstall the piston seal or membrane. See Section 5.4.2 Maintenance of the pump pistons and piston seals, on page 171.

# 6 Troubleshooting

#### 6.1 Hardware issues

Problem	Possible cause	Action
Strange noise form the pump	Piston spring damaged or incor- rectly mounted	Disassemble the pump head and examine the piston spring. See Section 5.4.2 Maintenance of the pump pistons and piston seals, on page 171.

# **Pressure monitor**

Problem	Possible cause	Action
Pressure offset when the reading should be zero	Uncalibrated pressure sensor	Calibrate the pressure sensors. See Section 5.3.1 Calibrate the pressure sensors, on page 153.
	Changed temperature	Wait until the temperature has stabilized and calibrate the pressure sensors. See Section 5.3.1 Calibrate the pressure sensors, on page 153.

# Mixer

Problem	Possible cause	Action
Leakage	Mixer not properly assembled	Reassemble the mixer. See Section 5.2.3 Mainte- nance of the mixer, on page 137.
	Bad O-ring	Check or replace the O-ring in the mixer. See Section 5.2.3 Maintenance of the mixer, on page 137.
Bad mixing perform- ance	Mixer membrane missing or damaged	Check or replace the membrane in the mixer. See Section 5.2.3 Maintenance of the mixer, on page 137.

# **UV** monitor

Problem	Possible cause	Action
No UV signal	The lamp is turned off	Turn the lamp on in the <b>Manual instructions</b> dialog, in <b>Monitors</b> $\rightarrow$ <b>UV lamp</b> .
Sharp dips or peaks in the UV signal. Ghost peaks	Air in the UV flow cell due to missing flow restrictor	Add the flow restrictor to the flow path. If a pH valve is installed, set the flow restrictor in-line in the <i>Manual instructions</i> dialog, under <i>Flow path</i> $\rightarrow$ <i>pH valve</i> .
Noisy UV signal	Dirt in the UV flow cell	Clean the UV flow cell, see <i>Clean the UV flow cell,</i> on page 140.

Problem	Possible cause	Action
	Dirt in the flow path	Perform a System CIP and Column CIP. Refer to ÄKTA go Operating Instructions (29360951).
	The UV lamp is broken or worn out.	Contact Service.
	Broken UV flow cell	Replace the flow cell, see <i>Instruction, on page 181</i> .
Auto zero out of accepted range	Wrong UV flow cell for current buffer	Change to a shorter UV flow cell or change buffer.
	Incorrectly installed UV flow cell	Check that the UV flow cell is fitted correctly, see <i>Instruction, on page 181</i> .
	Broken UV flow cell	Replace the flow cell, see <i>Instruction, on page 181</i> .
Distorted protein peaks in IEX gradients (e.g., step gradients)	The refractive index of the buffer changes rapidly in quick IEX gradients and disturbs the shape of the protein peaks in the 2 mm flow cell	Run with reversed flow direction through the 2 mm flow cell: connect the inlet tubing at the bottom and the outlet tubing at the top of the flow cell.

# **Conductivity monitor**

Problem	Possible cause	Action
Unstable conductivity	Air bubbles in the conductivity flow cell, due to missing flow restrictor	Add the flow restrictor to the flow path. If a pH valve is installed, set the flow restrictor in-line in the <i>Manual instructions</i> dialog, under <i>Flow path</i> $\rightarrow$ <i>pH valve</i> .
	Air or dirt in the conductivity flow cell	Clean the conductivity flow cell. See Section 5.2.5 Maintenance of the conductivity flow cell, on page 142.
Increasing/decreasing conductivity measure- ment with the same buffer over time	Dirt in the conductivity flow cell	Clean the conductivity flow cell. See Section 5.2.5 Maintenance of the conductivity flow cell, on page 142.
	Changes in ambient temperature	Set the temperature compensation factor, in <b>System Settings</b> $\rightarrow$ <b>Conductivity</b> $\rightarrow$ <b>Cond temp compensation</b> .

# 6 Troubleshooting

#### 6.1 Hardware issues

Problem	Possible cause	Action
	Uncalibrated conduc- tivity monitor	Check the calibration with a solution with known conductivity. Calibrate the conductivity monitor, see Section 5.3.3 Calibrate the conductivity monitor, on page 159.
A "knee" in the begin- ning of a linear gradient	<b>B</b> inlet not properly primed	Prime the <b>B</b> inlet.
Non-linear conduc- tivity response of a programmed linear gradient	Conductivity response is by nature non-linear	N/A
Fluctuating conduc- tivity during a gradient or a Conc %B setting between 0 and 100 %BMissing mixer or missing membrane in the mixerBuffers used are too difficult to mix	Check or replace the membrane in the mixer. See Section 5.2.3 Maintenance of the mixer, on page 137.	
	Change buffers or use an external large mixer.	

# pH monitor and pH valve

Problem		Possible cause and action
Leakage	pH or dummy elec- trode not properly installed in the valve	Remove the dummy electrode and wet it prop- erly with distilled water. Insert the dummy elec- trode into the pH valve and rotate it before securing it with the nut.
No or strange pH signal	Dummy electrode installed	Replace the dummy electrode with a pH elec- trode.
	Electrode cable not connected properly	Connect the electrode cable.
	Faulty or old electrode	Replace the electrode. See <i>Replace the pH elec-</i> <i>trode, on page 147.</i>
Incorrect pH measure- ment	pH not properly cali- brated	Calibrate the pH monitor. See Section 5.3.5 Calibrate the pH monitor, on page 163.
	Bad pH electrode	Replace the electrode. See <i>Replace the pH elec-</i> <i>trode, on page 147.</i>
	Dirty pH electrode	Regenerate the pH electrode. Place the pH elec- trode in water for 30 minutes followed by 30 minutes in a buffer with pH 4. If the problem persists, replace the pH electrode. See Section 5.2.7 Maintenance of the pH valve and electrode, on page 145.

Problem		Possible cause and action	
Not possible to inject calibration solution	Blocked waste tubing or <b>W3</b> outlet	Check connector and tubing.	
Alarm in UNICORN: (Alarm) The pH cell can only be run at pressure below 0.8 MPa.	Post-column pressure is too high	Check the tubing and/or lower the flow rate.	

# **Fraction collector F9-T**

Problem	Possible cause	Action
Fraction collector not detected by UNICORN	The UniNet-9 cable between the Fraction collector and the ÄKTA system is not properly connected	Check that the Fraction collector is properly connected.
	Broken/Bad cable	Replace with a new cable.
	Wrong Node ID	Check that the F9-T Node ID is set to 0 (zero).
Drop sync sensor fails to detect drops and switched off the drop sync	The drop sync sensor needs to be cleaned.	Clean the drop sync sensor. See Section 5.2.8 Maintenance and cleaning of Fraction collector F9-T, on page 149 for cleaning instructions.
	Air in the flow path	Check the flow path for air. Fill system and purge the pump.
		Refer to ÄKTA go Operating Instructions (29360951).
	Too high flow rate	Decrease the flow rate. Refer to <i>Fraction</i> <i>Collector F9-T Operation Instructions (29478336)</i> for appropriate flow rates for different nozzles and plate types.
	Blocked drop sync sensor	Check the drop sync sensor, or if the tubing nozzle is used, that the tubing has correct protru- sion for drop sync sensor functionality.
The drop sync sensor signal LED did not manage to adjust the LED power to get a correct drop sync signal	The drop sync sensor needs to be cleaned.	Clean the drop sync sensor. See Section 5.2.8 Maintenance and cleaning of Fraction collector F9-T, on page 149.

#### 6.1 Hardware issues

Problem	Possible cause	Action	
	Blocked drop sync sensor	Remove what is blocking the drop sync sensor, such as salt deposits.	
		<b>Note:</b> If the tubing nozzle is used, it must extend 4 mm or it may block the sensor.	
Plates or tubes do not fit in the plate or tube holder	Wrong plates or tubes are used	Check recommended plates or tubes in Section 2.3.7 Fraction collector F9-T, on page 43.	
Drops does not hit the tubes inserted in 24 deep well plates	Tubes are positioned incorrectly in the 24 deep well plate	Correct positioning of tubes in 24 deep well plates is described in <i>Section 2.3.7 Fraction</i> <i>collector F9-T, on page 43</i> .	
	Drop position adjust- ment is not correct	Check that drop position is correct. In the System Control software module select System Settings → System → Adjust Drop Position (F9-T).	
The green LED light on the dispenser head is off	The LED is switched off in the UNICORN soft- ware	Switch on the LED in <b>System settings</b> .	
The tubing/nozzle in the Fraction collector is blocked	Salt residuals in the tubing/nozzle	Cleaning of the Fraction collector tubing is described in <i>Fraction Collector F9-T Operation Instructions (29478336).</i>	
	The tubing is bent	Replace the tubing.	
	The nozzle needs to be replaced	Replacing the nozzle is described in <i>Fraction</i> <i>Collector F9-T Operation Instructions</i> (29478336).	
The fraction volume found in the tubes or	Leakage in the flow path	Localize and take care of the leakage, for example by tightening connectors.	
wells are smaller than expected	Air in the flow path	Check the flow path for air. Fill system and purge the pump, refer to ÄKTA go Operating Instruc- tions (29360951) for more information.	
	Bad pump function	See troubleshooting of pumps in <i>Pump, on page 193</i> .	
Liquid on the plate holder or on plates beside wells	The finger tight connector on the nozzle is not tight enough	Tighten the connector. Replace the connector if the leakage is persistent.	
	Wrong plate/tube type set for the selected plate position	Check plate/tube type set in the method or manual run.	

Problem	Possible cause	Action
	Wrong drop position set in <b>System control</b> →Adjust Drop Posi- tion (F9-T)	Check that drop position is correct. In the <b>System Control</b> software module select <b>System Settings</b> $\rightarrow$ <b>System</b> $\rightarrow$ <b>Adjust Drop Position (F9-T)</b> .
	The tubes are flooded	Make sure that the fraction volume is adapted to the well/tube volume or that they have been replaced by empty ones. If using 96 microplates with a well volume of less than $300 \mu$ L, make sure to change to more narrow tubing, with an inner diameter of 0.25 mm or less and adjust delay volume accordingly.
Spillage by the Frac- tion collector during fractionation	The drop sync sensor needs to be cleaned.	Cleaning of Fraction collector drop sync sensor is described in Section 5.2.8 Maintenance and cleaning of Fraction collector F9-T, on page 149.
	The nozzle needs to be cleaned.	Remove the nozzle from the fractionation arm. Unscrew the two nozzle parts to detach the nozzle from the tubing. Clean it in distilled water, mild cleaning agent or 20% ethanol. Replace if necessary.
	Too high flow rate during usage of drop sync function.	See Section 3.3 Fractionation overview, on page 83 for more information on the drop sync func- tion.
	Wrong plate/tube type set for the selected plate position	Check plate/tube type set in the method or manual run.
	Wrong drop position set in <b>System</b> Control	Check that drop position is correct. In the System Control software module select System Settings $\rightarrow$ System $\rightarrow$ Adjust Drop Position (F9-T) Poset it to default value or adjust it to fit the
		chosen plate/tube.
	The combination of high flow rates and liquids with low surface tension might lead to spillage in the Fraction collector	Lower the flow rate when using liquids with low surface tension.

# 6 Troubleshooting

#### 6.1 Hardware issues

Problem	Possible cause	Action
Spillage in Fraction collector when the fractionation arm is moving from the plate in position 1 to that in position 2.	Too high flow rate during fractionation arm move from plate 1 to plate 2	Check the setting in System Control $\rightarrow$ System Settings $\rightarrow$ Fraction collector $\rightarrow$ Flow when moving from plate 1 to plate 2.
Mismatch between chromatogram and actual fraction number	The delay volume has not been properly set	Adjust the delay volume. In the <b>System Control</b> software module, select <b>System</b> $\rightarrow$ <b>Tubing and Delay volumes</b> and adjust the value for <b>Delay volume: Monitor to frac</b> .
		See Delay volumes for different system configu- rations, on page 270 for more information.
The Fraction collector fractionates in the wrong well or tube	Unsupported deep well plate/tubes are used	Check that the used plates or tubes have the right dimensions. See <i>Plates, on page 48</i> and <i>Tubes, on page 49</i> for information on recommended plates and tubes.
	Wrong plate/tube type set for the selected plate position	Check that the set plates/tubes in the method match those placed in the Fraction collector.
	Plates/tubes are not reset when replaced with new	When starting a <i>Method run</i> , check that plates/ tubes are reset in the <i>Start Protocol</i> .
Error upon start of a method run, indicating that the wrong plate/ tubes are in place, even though the correct plate/tubes are present in the Fraction collector	The plate/tube type in the text method is not the same as that set in <b>Method settings</b>	End the Method and make sure that the same plate/tube types are set in the text method as in <i>Method settings</i> .
High pressure alarm when collecting frac- tions with the fraction	Too high flow rate during fractionation	Decrease the flow rate or replace the tubing between the outlet valve and the Fraction collector with tubing of larger inner diameter.
collector		Note:
		Adjust the delay volume. In the <b>System Control</b> software module, select <b>System →Tubing and</b> <b>Delay volumes</b> and adjust the value for <b>Delay</b> <b>volume: Monitor to frac</b> .

Problem	Possible cause	Action
The fractionation arm does not return to <b>Home</b> after completed run	Frac behavior when ending a run is set to Remain without reset in System Settings →Fraction collection.	To change Fraction collector behavior when ending a run, adjust the settings in <b>System</b> <b>Settings</b> $\rightarrow$ <b>Fraction collection</b> $\rightarrow$ <b>Frac</b> <b>behavior when ending a run</b> .
Plates/tubes are not reset after ending a run	Frac behavior when ending a run is set to Remain without reset or Go home without reset in System Settings $\rightarrow$ Fraction collec- tion.	Choose <b>Go home and reset</b> in <b>System</b> Settings → Fraction collection → Frac behavior when ending a run.

# Fraction collector F9-R

Problem	Possible cause	Action	
Fraction collector bowl does not rotate prop- erly	Fraction collector movement blocked	Make sure the Fraction collector bowl can move and is free from obstructions.	
Fraction collector skips tubes	Tube sensor height is not properly set	Adjust the tube sensor height. Refer to the <i>Frac-</i> <i>tion collector F9-R Operating instructions</i> (29656880) document.	
	Broken tube sensor	Contact Service.	
Fraction collector failed to detect a drop	Tubing height not properly set	Adjust the tubing height. See <i>Connect tubing, on</i> page 56.	
	The drop sync sensor needs to be cleaned.	Clean the drop sync sensor. See Section 5.2.9 Maintenance and cleaning of Fraction collector F9-R, on page 151.	
	Too high flow rate	Decrease the flow rate to ≤2 mL/min.	
Fraction numbering error	Incorrect setting	Set the desired fraction number mode in <b>System Settings</b> .	

6.1 Hardware issues

# Air sensor

Problem	Possible cause	Action
Air sensor does not trigger alarm	Incorrect setting	Check the settings in the method. If running manually, set the air sensor alarm in <b>Manual</b> instructions $\rightarrow$ Alarms $\rightarrow$ Alarm air sensor.

# I/O-box

Problem	Possible cause	Action	
Analog signal noise	Cables not connected properly	See the <i>Install I/O-box E9</i> installation instruc- tions for details.	
	Long or unshielded cable between the external equipment and the I/O-box	Use a cable as short as possible. Use a shielded cable. Connect the cable shield to the D-sub connector shield.	
I/O signals do not work as expected	Cables incorrectly connected	See the <i>Install I/O-box E9</i> installation instruc- tions for details.	
	Incorrect setting	See the <i>Install I/O-box E9</i> installation instruc- tions for details.	

# 6.2 Software issues

Software issues, including common connection issues and suggested corrective actions, are covered in the UNICORN documentation. For software error codes refer to *Section 7.11 Error codes, on page 275.* For creating a system error report refer to *ÄKTA go Operating Instructions (29360951).* 

# 6.3 Application issues

If a purification method fails, check and perform the actions listed below:

- Clean and prepare all columns according to the column recommendations.
- Adjust the samples to binding buffer conditions.
- Clarify the samples by centrifugation and/or filtration prior to sample loading.
- Use the correct buffers for the chosen columns and proteins.
- Check the buffers for precipitation.
- Use the buffers at the running temperature of the system. If performing a run in cold environment, use cold buffers.
- Check that the buffers have the correct pH. The pH of some buffers changes with the temperature.
- Use columns suitable for the chosen target proteins.

For further help on troubleshooting a purification application, refer to a suitable chromatography handbook. Handbooks with practical tips on chromatography are available at *cytiva.com/handbooks*.

# 7 Reference information

# About this chapter

This chapter lists the allowed environmental and operational ranges for ÄKTA go. Refer to *ÄKTA go Product Documentation* for detailed technical specifications.

# In this chapter

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#### 7 Reference information

7.1 System specifications

#### System specifications 7.1

# Introduction

This section specifies the operating data of ÄKTA go and its components.

# **System specifications**

Parameter	Data	
System configuration	Benchtop system, external computer	
Control system	UNICORN 7.4 or later <sup>1</sup>	
Connection between PC and instrument	Ethernet	
Dimensions (width × height × depth)	335 × 482 × 464 mm	
	(depth without tray 451 mm, depth without modules 380 mm)	
Weight (excluding computer, columns, buffer bottles)	27 kg	
Supplyvoltage	100 to 240 V ~ autorange	
Maximum voltage fluctuation	± 10% from the nominal voltage	
Frequency	50/60 Hz	
Power consumption	Rated max 300 VA <sup>2</sup>	
	Max with all options 150 W $^3$	
	Typical 100 W	
	Power-save < 20 W	
Enclosure protective class	IP21	
Acoustic noise level	< 60 dB(A)	

<sup>1</sup> UNICORN 7.6 or later is required to run ÄKTA go equipped with F9-T. <sup>2</sup> ÄKTA go can deliver 300 VA.

<sup>3</sup> ÄKTA go equipped with all options consumes 150 W.

# **Tubing and connectors**

Flow path	Tubing	Connectors
Inlet	FEP tubing, i.d. 1.6 mm	Tubing connector 1/8" + Ferrule (yellow),
Pump to Injection valve	PEEK tubing, i.d. 0.75 mm	Fingertight connector, 1/16"

Flow path	Tubing	Connectors
After Injection valve	PEEK tubing, i.d. 0.50 mm	Fingertight connector, 1/16"
Outlet and waste	ETFE tubing, i.d. 1.0 mm	Fingertight connector, 1/16"

# **Environmental ranges**

Parameter	Data
Storage and transport temperature range	-25°C to 60°C, during 48 h
Chemical environment	See Section 7.3 Wetted materials and biocompatibility, on page 219.

# **Operating ranges**

Parameter	Data
Operating temperature range	4°C to 35°C
Relative humidity	20% to 95%, non-condensing

# Pump

Parameter	Data
Pump type	Piston pump (metering type)
Flow rate range	0.01 to 25 mL/min
Pressure range	0 to 5 MPa (0 to 50 bar)
Viscosity range	0.7 to 10 cP, flow rate accuracy not specified above 3 cP
Flow rate accuracy	± 2%
	Conditions: 0.25 to 25 mL/min, 0.7 to 3 cP

# Valves

Parameter	Data
Туре	Rotary valves <sup>1</sup>
Number of valves	Up to 7

#### 7.1 System specifications

Parameter	Data
Functions	Standard: Inlet valve (sample and three buffer inlets), injec- tion valve, and outlet valve (three outlets).
	Optional: A inlet valve, B inlet valve, sample inlet valve, column selection valve for three columns, column selection valve for five columns including pressure sensors, and pH valve.

<sup>1</sup> The **K9** inlet valve is a membrane valve, all other valves in ÄKTA go are rotary valves

# **Inlet options**

Parameter	Data
Ainlet	1 standard or 6 optional inlets
Binlet	1 standard or 6 optional inlets
Cinlet	1 inlet
Sample inlet	1 standard or 6 optional inlets (5 sample inlets and 1 buffer inlet)

# **Outlet options**

Parameter	Data
Number of outlets	3 (waste, outlet, and Fraction collector port)
Delay volume (UV - Fraction collector)	223 $\mu L$ , with standard configuration and Fraction collector F9-R.
	233µL with standard configuration and F9-T.

#### Mixer

Parameter	Data
Mixing principle	Static
Mixer volume	1 mL

# **Gradient formation**

Parameter	Data
Gradient composition range	0.0%B to 100.0%B
Gradient composition accuracy	± 2%
	Conditions: 2% to 98% B, 0.5 to 20 mL/min, 0.7 to 2 cP
Gradient step composition fluctuation	<±0.3%
	Conditions: 2% to 98% B, 0.5 to 20 mL/min, 0.7 to 2 cP
Gradient linearity	within ± 1%
	Conditions: within 10% to 85% B, gradient volume $\ge 20$ mL, 0.5 to 20 mL/min, 0.7 to 2 cP

## **Pressure sensors**

Parameter	Data
Number of sensors	Up to 3
Placement of sensors	Standard: Pressure sensor in pressure monitor <b>R9</b>
	Optional: Pre-column pressure sensor and the post-column pressure sensor integrated in column valve <b>V9-C</b>

# **UV** monitor

Parameter	Data
Number of monitors	1
Wavelength range	280 nm
Absorbance reading range	-6 to 6 AU
Linearity	within ± 5%
	Condition: 0 to 2 AU
Noise	< 0.1 mAU
Operating pressure	0 to 2 MPa (20 bar, 290 psi)

# 7 Reference information

7.1 System specifications

Parameter	Data
Flow cells	Standard: Optical path length 2 mm Illuminated volume 2 µL Total volume 30 µL
	Optional: Optical path length 5mm Illuminated volume 6 µL Total volume 20 µL

# **Conductivity monitor**

Parameter	Data	
Conductivity reading range	0.01 to 999.99 mS/cm	
Conductivity accuracy	± 0.01 mS/cm or ± 2%, whichever is greater	
	Conditions: within 0.3 to 300 mS/cm	
Operating pressure	0 to 2 MPa (20 bar, 290 psi)	
Flow cell volume	22 µL	
Temperature monitor reading range	0°C to 70°C	
Temperature monitor accuracy	±1.5°C	
	Conditions: 4°C to 35°C	

# pH monitor

Parameter	Data
pH reading range	0 to 14
pH accuracy	± 0.1 after calibration Conditions: within pH 2 to 12, within ± 3°C from calibration temperature
pH operating pressure range	0 to 0.5 MPa (5 bar, 72.5 psi)
Flow cell volume	76 µL

# Fraction collector, F9-T

Parameter	Data
Number of Fraction collectors	1
Number of fractions	Up to 192
Plates	2 x 96 well microplate, max vol. 0.3 mL 2 x 96 deep well plate, max vol. 2 mL 2 x 48 deep well plate, max vol. 4.5 mL
	2 x 24 deep well plate, max vol. 9 mL
Tubes	0.5 mL tubes in 2 x 48 position rack 1.5 mL tubes in 2 x 24 deep well plate 2 mL tubes in 2 x 24 deep well plate 4 x 50 mL tubes in Tube rack F9-T
Fraction volumes	0.02 to 50 mL
Spillage-free mode	DropSync
Flammable liquids	Yes
Delay volume (UV - Fraction collector)	$233\mu\text{L}$ with standard tubing and nozzle
Dimensions (W x D x H)	320 x 270 x 190 mm
Weight	4 kg

# Fraction collector, F9-R

Parameter	Data
Number of Fraction collectors	1
Number of fractions	Up to 175
Tubes	175 (3 mL tubes)
	95 (8 or 15 mL tubes)
	40 (50 mL tubes)
Fraction volumes	0.1 to 50 mL
Spillage-free mode	Drop sync
Flammable liquids	Yes
Delay volume (UV - Fraction collector)	223 µL with standard configuration

# 7 Reference information

7.1 System specifications

Parameter	Data
Dimensions (W × H × D)	320 × 250 × 400 mm
Weight	5 kg

# Air sensor

Parameter	Data	
Number of sensors	1	
Placement	L9-1.5 before inlet valve or between inlet valve and pump.	
	<b>L9-1.2</b> before inlet valve only.	
Sensing principle	Ultrasonic	

# I/O-box

Parameter	Data
Number of I/O-boxes	1
Number of ports	2 analog in, 2 analog out 4 digital in, 4 digital out
Analog range	In +/- 2 V Out +/- 1 V
Digital range	Max 5 V

# 7.2 Tubing and connectors

# **Tubing types**

The table below shows the tubing types used in ÄKTA go.

Description	Color	Scope of use	Volume/cm
PEEK, o.d. 1/16", i.d. 0.15 mm	Purple	High resolution columns with an inner diameter of 3.2 mm, used from injection valve to fraction collector.	0.18 µL
PEEK, o.d. 1/16", i.d. 0.25 mm	Blue	High resolution columns with an inner diameter of 4 or 5 mm, used from injection valve to Fraction collector.	0.5 μL
PEEK, o.d. 1/16", i.d. 0.50 mm	Orange	Tubing used from injection valve to outlet valve, and from outlet valve to Frac- tion collector.	2.0 µL
PEEK, o.d. 1/16", i.d. 0.75 mm	Green	Tubing used from pump to injection valve.	4.4 μL
ETFE, o.d. 1/16", i.d. 1.0 mm	Transparent	Outlet and waste tubing.	7.8 µL
FEP, o.d. 1/8", i.d. 1.6 mm	Transparent	Inlet tubing.	20.0 µL
Silicone, o.d. 4.1 mm, i.d. 2.1 mm	Transparent	Pump rinse solution tubing.	35.0 µL

To calculate the internal volume (V) of specific tubing, use the formula:

 $V = L \times \pi \times d^2/4$ 

L = length in mm

d = i.d. in mm

# **Tubing connectors**

The table below shows the tubing connectors used in ÄKTA go.

7.2 Tubing and connectors

Description	Use with tubing
Fingertight connector, 1/16"	PEEK, o.d. 1/16" EFTE, o.d. 1/16"
Tubing connector 1/8" + Ferrule (yellow) 1/8"	FEP, o.d. 1/8"

# **Other connectors**

The table below shows other connectors used in ÄKTA go.

Description	Scope of use
Stop plug 1/16"	Stop plug for valve ports
Luer connector (1/16" Male/Luer Female)	Syringe connector for the injection valve and pH valve
Union 1/16"F / 1/16"F	Union connector for two fingertight connectors 1/16"
Union 1/16"M / 1/16"M	Union connector for direct connection of a column to the UV monitor
Union 1/16" Male, 5/16" Female fitting tubing connector, 5/16" + Ferrule (yellow), 1/8".	Connect air sensor <b>L9-1.2</b> before the <b>K9</b> injection valve.

# **Tubing labels**

The illustration below shows the tubing labels for a system configuration with optional modules.



# **Inlet tubing**

The table below shows the labels, standard diameters, and standard lengths of the inlet tubing.

Label	Description	Tubing	Length (mm)	Volume (mL)
In	From inlet valve <b>K9</b> to pump <b>P9-S</b>	FEP, o.d. 1/8", i.d. 1.6 mm	300	0.6
Α	To inlet valve <b>K9</b>	FEP, o.d. 1/8", i.d. 1.6 mm	1250	2.5
В	To inlet valve <b>K9</b>	FEP, o.d. 1/8", i.d. 1.6 mm	1250	2.5
С	To inlet valve <b>K9</b>	FEP, o.d. 1/8", i.d. 1.6 mm	1250	2.5
Sample	To inlet valve <b>K9</b>	FEP, o.d. 1/8", i.d. 1.6 mm	1250	2.5

# **Standard tubing**

The table below shows the labels, diameters, and standard lengths of the tubing used from the pump to the Fraction collector.

## 7 Reference information

7.2 Tubing and connectors

Label	Description	Tubing	Length (mm)	Volume (µL)
1A	Left pump head to pump restrictor	PEEK, o.d. 1/16", i.d. 0.75 mm	180	80
1B	Right pump head to pump restrictor	PEEK, o.d. 1/16", i.d. 0.75 mm	180	80
2	Pump restrictor to pres- sure monitor	PEEK, o.d. 1/16", i.d. 0.75 mm	100	44
3	Pressure monitor to mixer	PEEK, o.d. 1/16", i.d. 0.75 mm	210	93
4	Mixer to injection valve	PEEK, o.d. 1/16", i.d. 0.75 mm	180	80
5	Injection valve to column or column valve	PEEK, o.d. 1/16", i.d. 0.50 mm	170	33
6	Column or column valve to UV monitor	PEEK, o.d. 1/16", i.d. 0.50 mm	150	30
7	UV monitor to conduc- tivity monitor	PEEK, o.d. 1/16", i.d. 0.50 mm	230	45
8	Conductivity monitor to flow restrictor	PEEK, o.d. 1/16", i.d. 0.50 mm	95	19
9	Flow restrictor to outlet valve	PEEK, o.d. 1/16", i.d. 0.50 mm	135	27
Frac	Outlet valve to Fraction collector (both F9-T and F9-R)	PEEK, o.d. 1/16", i.d. 0.50 mm	400	79
Frac	Outlet valve to Fraction collector F9-T in tunnel	PEEK, o.d. 1/16", i.d. 0.50 mm	800	158

# Tubing to pH valve

The table below shows the labels, diameter, and standard length of the tubing for the pH valve. This tubing is delivered with the pH valve.

Label	Description	Tubing	Length (mm)	Volume (mL)
8рН	Conductivity monitor to pH valve	PEEK, o.d. 1/16", i.d. 0.5 mm	180	35
Label	Description	Tubing	Length (mm)	Volume (mL)
-------	------------------------------------	----------------------------------	----------------	-------------
9рН	pH valve to outlet valve	PEEK, o.d. 1/16", i.d. 0.5 mm	160	31
1R	pH flow cell to flow restrictor	PEEK, o.d. 1/16", i.d. 0.5 mm	80	16
2R	Flow restrictor to pH flow cell	PEEK, o.d. 1/16", i.d. 0.5 mm	80	16

### **Reference capillary**

The table below shows the label, diameter, and standard length of the reference capillary. The capillary is used during the System performance tests.

Label	Description	Tubing	Length (mm)	Volume (µL)
Ref 1	Reference capillary	PEEK, o.d. 1/16", i.d. 0.25 mm	400	20

### **Outlet tubing**

The table below shows the labels, diameters, and standard lengths of the outlet tubing. The tubing is not mounted on delivery.

Label	Description	Tubing	Length (mm)	Volume (mL)
Out	Outlet valve port <b>Out1</b>	ETFE, o.d. 1/16", i.d. 1.0 mm	1500	1.2

### Waste tubing

The table below shows the labels, diameters, and standard lengths of the waste tubing. The waste tubing is mounted on delivery.

Label	Description	Tubing	Length (mm)	Volume (mL)
W1	Pump waste. Connected to injection valve port <b>W1</b> .	ETFE, o.d. 1/16", i.d. 1.0 mm	1800	1.4

7.2 Tubing and connectors

Label	Description	Tubing	Length (mm)	Volume (mL)
W2	Sample loop waste. Connected to injection valve port <b>W2</b> .	ETFE, o.d. 1/16", i.d. 1.0 mm	1800	1.4
W3	pH valve waste. Connected to pH valve port <b>W3</b> .	ETFE, o.d. 1/16", i.d. 1.0 mm	1800	1.4
W	System waste. Connected to outlet valve port <b>W</b> .	ETFE, o.d. 1/16", i.d. 1.0 mm	1400	1.1

# 7.3 Wetted materials and biocompatibility

### Introduction

This section provides information about the wetted materials in, and biocompatibility of the ÄKTA go instrument.

For detailed information about chemical resistance of the instrument to the most commonly used chemicals in liquid chromatography, refer to ÄKTA go Operating Instructions (29360951).

### In this section

Section		See page
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7 Reference information7.3 Wetted materials and biocompatibility7.3.1 Wetted materials

# 7.3.1 Wetted materials

### Introduction

The tables below list the materials that come into contact with process fluids in the ÄKTA go instrument. For details about the wetted materials see ÄKTA go Product Documentation.

### **Primary flow path**

Material	Abbreviation
Ethylene Propylene Diene Monomer	EPDM
Ethylene ChloroTriFluoroEthylene	ECTFE
Ethylene TetraFluoroEthylene	ETFE
Fluorinated Ethylene Propylene	FEP
Fully Fluorinated Propylene Monomer	FFKM
PolyChloroTriFluoroEthylene	PCTFE
PolyEtherEtherKetone	PEEK
PolyPropylene	PP
PolyTetraFluoroEthylene	PTFE
PolyVinylidene DiFluoride	PVDF
UltraHighMolecularWeightPolyEthylene	UHMWPE
Aluminum oxide	
Elgiloy	
Hastelloy™ C-276	
Quartz glass	
Ruby	
Sapphire	
Titanium grade 2	
Titanium grade 5	

### **Pump rinsing system**

Material	Abbreviation
Ethylene Propylene Diene Monomer	EPDM
PolyEtherEtherKetone	PEEK
PolyPropylene	PP
PolyPhenylene Sulfide	PPS
PolyVinylidene DiFluoride	PVDF
Silicone	

7.3 Wetted materials and biocompatibility

7.3.2 General information about biocompatibility and chemical resistance

# 7.3.2 General information about biocompatibility and chemical resistance

### **Biocompatibility**

The ÄKTA go instrument is designed for maximum biocompatibility, with biochemically inert flow paths constructed mainly from titanium, PEEK and highly resistant fluoropolymers and fluoroelastomers. Titanium is used as far as possible to minimize contribution of potentially deactivating metal ions such as iron, nickel and chromium. There is no standard stainless steel in the flow path. Plastics and rubber materials are selected to avoid leakage of monomers, plasticizers or other additives.

### **Cleaning chemicals**

Clean using 2 M sodium hydroxide, 70% acetic acid or the alcohols methanol, ethanol and isopropyl alcohol. Avoid using 1 M hydrochloric acid to not damage the pressure sensors. If cleaning separation media using 1 M hydrochloric acid, use loop injection of the acid and make sure that the column is not mounted on the column valve **V9-C**. The column valve **V9-C** contains a pressure sensor which can be damaged by 1 M hydrochloric acid.

If sodium hypochlorite is used as sanitizing agent instead of 2 M sodium hydroxide, use a concentration of maximum 10%.

#### **Organic solvents**

Chromatography of proteins works with acetonitrile up to 83%.

Strong organic solvents like ethyl acetate, 100% acetone, or chlorinated organic solvents must be avoided. These can cause swelling of plastic material and reduce the pressure tolerance of PEEK tubing. For this reason, flash chromatography and straight ("normal") phase chromatography using these solvents is not recommended on the system.

#### Assumptions made

The chemical resistance ratings above are based on the following assumptions:

- Synergy effects of chemical mixtures have not been taken into account.
- Room temperature and limited overpressure is assumed.
- **Note:** Chemical influences are time and pressure dependent. Unless otherwise stated, all concentrations are 100%.

# 7.4 Predefined methods and phases

#### Introduction

A predefined method contains a set of phases, each phase reflecting a specific stage of a chromatography or maintenance run. You can select additional phases from the phase libraries and add these to an existing method, or remove phases that are not required.

The predefined purification methods have default values with suitable running conditions for the chosen column type such as flow and pressure limits. Other settings (for example sample application technique, sample volume, elution profile and fractionation) are set on the **Phase Properties** pane in the appropriate phases.

This section describes the predefined methods and phases.

A method is built up by a number of phases. Each phase represents a major process step in the method, for example, equilibration or elution. Predefined methods, that include all the phases necessary to run the system, are available for different chromatography techniques and also for system cleaning.

### In this section

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7.4 Predefined methods and phases

7.4.1 Predefined purification methods

# 7.4.1 Predefined purification methods

The *Method Editor* has predefined methods for different separation techniques. The methods include a number of relevant phases.

All methods start with the **Method Settings** phase that defines common parameters used in the subsequent phases.

The table below describes the available predefined purification methods and the phases included.

Predefined purification method	Principle	Included phases
Affinity Chromatog- raphy (AC)	The <i>Equilibration</i> step is followed by <i>Sample Application</i> , where the protein of interest is adsorbed to the column ligand. During <i>Column wash</i> , unbound sample is removed from the column. The <i>Elution</i> step is performed by using a buffer that either contains a competitor to displace the protein of interest, or changes the pH or ionic strength. Finally, <i>Re-Equilibration</i> fills the column with start buffer.	Method Settings         Equilibration         Sample Application         Column Wash - Wash Out Unbound Sample         Column Wash - Wash Out Unbound Sample         Elution         Re-Equilibration
Anion Exchange Chro- matography (AIEX)	The <i>Equilibration</i> step is followed by <i>Sample Application</i> , where negatively charged proteins are adsorbed to the column ligand. During <i>Column wash</i> , unbound sample is removed from the column. The <i>Elution</i> step is performed using a gradient of increasing salt concentration (of e.g., NaCl). A second <i>Column wash</i> is performed with a high salt concentration to regenerate the column. Finally, <i>Re-Equilibration</i> fills the column with start buffer.	Method Settings Equilibration  Equilibration  Sample Application  Column Wash - Wash Out Unbound Sample  Elution  Column Wash - Clean After Elution  Re-Equilibration

7.4 Predefined methods and phases7.4.1 Predefined purification methods

Predefined purification method	Principle	Included phases
Cation Exchange Chro- matography (CIEX)	The <b>Equilibration</b> step is followed by <b>Sample Application</b> , where positively	Method Settings
	charged proteins are adsorbed to the column ligand. During <b>Column wash</b> .	Equilibration
	column ligand. During <b>Column wash</b> , unbound sample is removed from the column. The <b>Elution</b> step is performed using a gradient of increasing salt concentration of, for example, NaCl. A second <b>Column wash</b> is performed with a high salt concentration to regenerate the column. Finally, <b>Re-Equilibration</b> fills the column with start buffer.	
Desalting (DS)	After <b>Equilibration</b> and <b>Sample Appli</b> cation, the proteins are eluted isocratically, during the <b>Elution</b> step. This technique is commonly used for buffer exchange.	Method Settings Equilibration Sample Application Elution
Hydrophobic Interac- tion Chromatography (HIC)	The <b>Equilibration</b> step is followed by <b>Sample Application</b> , where hydro- phobic proteins are adsorbed to the column ligand, using a buffer containing a high salt concentration (e.g., 2 M ammo- nium sulfate). During <b>Column wash</b> , unbound sample is removed from the column. The <b>Elution</b> step is performed using a gradient of decreasing salt concentration. Finally, a second <b>Column</b> <b>wash</b> is performed.	Method Settings         Equilibration         Sample Application         Column Wash - Wash Out Unbound Sample         Elution         Column Wash - Clean After Elution

7.4 Predefined methods and phases

7.4.1 Predefined purification methods

Predefined purification method	Principle	Included phases					
Segmented Gradient Elution (example)	The <i>Equilibration</i> step is followed by <i>Sample Application</i> where the proteins	Method Settings					
	in the sample are adsorbed to the resin. After <b>Column Wash</b> to remove unbound sample, <b>Elution</b> is performed using a	in the sample are adsorbed to the resin. After <b>Column Wash</b> to remove unbound sample, <b>Elution</b> is performed using a	Equilibration				
			Sample Application				
	concentration of for example NaCl						
	Three elution segments are included and	Column Wash - Wash Out Unbound Sample					
	followed by a new <b>Column wash</b> and a	•					
	Re-Equilibration step to fill the column	Elution - Segment 1					
	with start buffer.						
		Elution - Segment 2					
		Elution - Segment 3					
		▼					
		Column Wash - Clean After Elution					
		•					
		Re-Equilibration					
Size Exclusion Chroma- tography (SEC)	The <b>Equilibration</b> step is followed by <b>Sample Application</b> where the proteins	Method Settings					
	at different speeds, depending on the size	at different speeds, depending on the size	at different speeds, depending on the size	at different speeds, depending on the size	In the sample travel through the column at different speeds, depending on the size	In the sample travel through the column at different speeds, depending on the size	Equilibration
	of the molecule. Directly after, the						
	Elution step isocratically elutes the	Sample Application					
	proteins according to their size (largest	▼					
	first).	Elution					
		<u></u>					

### 7.4.2 Predefined maintenance methods

Two predefined methods for preparation and cleaning are available. These maintenance methods are used to prepare, clean, and fill the system or a column with storage solution.

The table below describes the available predefined maintenance methods.

Predefined mainte- nance method	Principle	Included phases
Column CIP	The method cleans a column by filling it with a cleaning solution. Select the appropriate inlet positions, and enter the solution identity, volume, flow rate and incubation time. By adding steps to the method, several cleaning solutions can be used. Suggestions for cleaning steps are available for a number of column types.	Method Sottings Column CIP
System CIP	The system is filled with cleaning solution. Select for example inlets, outlets and column positions to be cleaned. Five <b>System CIP</b> phases are included in the method to facilitate the use of four different cleaning solutions. Additional <b>System CIP</b> phases can be added from the <b>Phase Library</b> if desired.	Method Phases Method Settings System CIP - Water System CIP - 1 M NaOH System CIP - 0.1 M NaCI System CIP - 0.1 M NaCI System CIP - 0.1 M VaCI

7.4 Predefined methods and phases

7.4.3 Predefined column performance test method

# 7.4.3 Predefined column performance test method

A predefined method to test column performance is available. The table below describes this method.

Predefined column performance test method	Principle	Included phases
Column Performance Test	After <i>Equilibration</i> of the column, sample is injected via a loop and eluted isocratically. A non-adsorbing sample like acetone or salt should be used. After the run, calculate column performance in the <i>Evaluation</i> module. The efficiency of the column is determined in terms of height equivalent to a theoretical plate (HETP), and the peak asymmetry factor (A <sub>s</sub> ). The result is logged in the column logbook.	Method Settings Equilibration Column Performance Test

# 7.4.4 Predefined phases

The table below describes the predefined phases.

Phase name	Description	
Method Settings	The first, and mandatory phase in any method. Defines common parame- ters used in the subsequent phases.	
	The <i>Method Settings</i> phase defines:	
	Column Type	
	<b>Note:</b> The <b>Column Type</b> list can be filtered in two steps:	
	<ol> <li>Select the chromatography technique to be used in the list Show by Technique.</li> </ol>	
	<ol><li>Select Only show suggested to show the columns with a diameter suitable for the chosen ÄKTA system.</li></ol>	
	Column Volume	
	Pressure Limit(s)	
	• Flow Rate	
	Option to control the flow to avoid overpressure	
	Column Position	
	Flow restrictor use	
	Unit Selection for Method Base and Flow Rate	
	Enable pH Monitoring	
	Settings for <b>Column Logbook</b>	
	• Plate types for <b>F9-T</b> .	
	Note:	
	Default values for pressure limits and flow rate are given for the selected column type.	
	Some of these options may not be required by certain methods.	
Equilibration	Equilibrates the column before purification, or re-equilibrates the column after purification.	
Sample Application	Applies sample to the column. Defines the sample application technique, the sample volume, and the handling of flowthrough.	
Column Wash	Washes out unbound sample after sample application or removes strongly bound proteins after elution.	
Elution	Elutes the sample from the column. Defines parameters for the elution and fractionation settings.	

- 7.4 Predefined methods and phases
- 7.4.4 Predefined phases

Phase name	Description
Column CIP	Cleans the column after purification runs by rinsing the column with a cleaning solution to remove nonspecifically bound proteins. By adding steps, several cleaning solutions can be used sequentially.
System CIP	Cleans the system after purification runs by rinsing the system with a cleaning solution. One cleaning solution is used per phase.
Column perform- ance test	Tests the efficiency of a packed column in terms of height equivalent to a theoretical plate (HETP), and the peak asymmetry factor ( $A_S$ ).
Miscellaneous	Can be added to any method at suitable places. The instructions can help the user to better organize the graphical output of the results or introduce a controlled delay in the method run.

# 7.5 System settings

### Introduction

The system settings are used to set the parameters for the available instructions. The **System Settings** dialog can be accessed by selecting **System**  $\rightarrow$  **Settings**, in the **System Control** module. The following subsections list the system settings available for ÄKTA go.

### In this section

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# 7.5.1 System settings - UV

The table below describes the UV related system settings available for ÄKTA go.

Instruction name	Description
Alarm UV	<b>Alarm UV</b> enables or disables the alarm for the UV signal. When enabled, it sets the alarm limits for the UV signal from UV monitor <b>U9-L</b> . When the UV signal falls outside the set limits, an alarm will be triggered and the run will be paused.
Noise reduction UV	<b>Noise reduction UV</b> filters the noise in the UV signal from <b>U9-L</b> . A column-specific averaging time is set automatically when a column is defined in a method run and <b>Averaging time</b> is set as a variable.

# 7.5.2 System settings - Conductivity

The table below describes the conductivity related system settings available for  $\ddot{\mathsf{A}}\mathsf{KTA}$  go.

Instruction name	Description
Alarm conductivity	<i>Alarm conductivity</i> enables or disables the conductivity alarm. When enabled, it sets the alarm limits for the conductivity signal. When the conductivity falls outside the set limits, an alarm will be triggered and the run will be paused.
Relative scale cond	Relative scale cond facilitates monitoring of a gradient, for which the user sets the conductivity values for 0% and 100%. The <b>Rela-</b> <i>tive scale cond</i> can be set in ascending manner (0% for low and 100% for high conductivity) or in descending manner (0% for high and 100% for low conductivity). <b>Note:</b> The <b>Relative scale cond</b> in descending manner is especially useful for conductivity visualization in HIC, where the conductivity
	curve is reversed compared to the concentration curve (i.e., high conductivity at 0% B and low conductivity at 100% B).
Cond temp condensation	<b>Cond temp compensation</b> is used to adjust the conductivity values to a reference temperature in order to compare conductivity values between runs that have been performed at different temperatures, or to prevent fluctuation of conductivity signals due to temperature changes, such as in a refrigerator. Setting the compensation factor to 0% turns this function off.

7 Reference information 7.5 System settings 7.5.3 System settings - pH

# 7.5.3 System settings - pH

The table below describes the pH related system settings available for ÄKTA go.

Instruction name	Description
AlarmpH	<b>Alarm pH</b> enables or disables the pH alarm. When enabled, it sets the alarm limits for the pH signal. When the pH falls outside the set limits, an alarm will be triggered and the run will be paused.

# 7.5.4 System settings - Pressure alarms

The table below describes the pressure alarm related system settings available for  $\ddot{\mathsf{A}}\mathsf{KTA}$  go.

Instruction name	Description
Alarm pressure	<b>Alarm pressure</b> sets the alarm limits for the pressure. When enabled and the pressure falls outside the set pressure limits, an alarm will be triggered and the run will be paused. When a column is selected in the run, the alarm limits are automatically set to the values in the column list. For methods without column valve <b>V9-C</b> , <b>Column pressure limit</b> is also set as a variable. <b>Low alarm</b> is only triggered if the pressure first exceeds the <b>Low alarm</b> limit for ten seconds continuously and then falls below the <b>Low alarm</b> limit.
	Note:
	Setting the <b>Low alarm</b> or the system flow rate to 0 deactivates the low pressure alarm.
Alarm pre-column pressure	<b>Alarm pre-column pressure</b> sets the alarm limits for the pre- column pressure. When enabled and the pre-column pressure falls outside the set pressure limits, an alarm is issued and the run will be paused. When a column is selected in the run, the alarm limits are automatically set to the values in the column list. For methods, <b>Pre-column pressure limit</b> is also set as a variable. <b>Low alarm</b> is only triggered if the pressure first exceeds the <b>Low alarm</b> limit for ten seconds continuously then falls below the <b>Low alarm</b> limit.
	Note:
	<ul> <li>Setting the Low alarm to 0 deactivates the low pressure alarm.</li> <li>Instruction Alarm pre-column pressure is available only when Column valve V9-C is installed and selected in the compo- nent list.</li> </ul>

7.5 System settings

7.5.4 System settings - Pressure alarms

Instruction name	Description
Alarm delta column pres- sure	<ul> <li>Alarm delta column pressure sets the alarm limits for the delta-column pressure. When enabled and the delta-column pressure falls outside the set pressure limits, an alarm will be triggered and the run will be paused. When a column is selected in the run, the alarm limits are automatically set to the values in the column list. When creating a method, <i>Delta column pressure limit</i> is also set as a variable. <i>Low alarm</i> is only triggered if the pressure first exceeds the <i>Low alarm</i> limit.</li> <li>Note:</li> <li>Setting the Low alarm to 0 deactivates the low pressure alarm.</li> <li>Instruction Alarm delta column pressure is available only when Column valve V9-C is installed and selected in the component list.</li> </ul>
Alarm Superloop pressure	<i>Alarm Superloop pressure</i> is set to avoid overpressure in a Superloop, when a Superloop is in use. If the pressure signal falls outside the set limits, an alarm is triggered and the run is paused. <i>High alarm</i> sets the top limit for the Superloop pressure alarm. <i>Note:</i> <i>Alarm Superloop pressure is connected to the Pressure value. If</i> <i>Alarm pressure is also enabled, an alarm will be triggered by the</i> <i>lowest set value between Alarm pressure and Alarm Superloop</i> <i>pressure. By default Alarm Superloop pressure/High alarm is</i> <i>set to 5.0 MPa.</i>

# 7.5.5 System settings - Air sensor

The table below describes the air sensor related system settings available for ÄKTA go.

Instruction name	Description	
Alarm air sensor	<b>Alarm air sensor</b> enables or disables the alarm for the air sensor. If the alarm is enabled and air is detected, an alarm will be trig- gered and the run will be paused.	
Sensitivity air sensor	<b>Sensitivity air sensor</b> is used together with the <b>Alarm air sensor</b> instruction and sets the sensitivity of the external air sensor. <b>Normal</b> (100 $\mu$ L) is used to detect when a buffer or sample vessel is empty. <b>High</b> (30 $\mu$ L) is used to detect small air bubbles.	
	By default, the sensitivity is set to <b>Normal</b> .	

# 7.5.6 System settings - Fraction collection F9-T

The tables below describe the Fraction collector F9-T system settings available for  $\ddot{\mathsf{A}}\mathsf{KTA}$  go.

Instruction name	Description
Peak frac parameters UV	<b>Peak frac parameters UV</b> sets the detection parameters for peak collection, i.e. it determines when a peak starts and ends. This information is used by the instruction <b>Peak fractionation</b> in order to start/end the peak collection.
Delay volume →Detector - Frac	<b>Delay volume</b> → <b>Detector - Frac</b> , defines the delay volume between the UV monitor and the Fraction collector, excluding the pH valve but including the tubing to the pH valve.
Delay volume →Restrictor volume	<b>Delay volume</b> → <b>Restrictor volume</b> , defines the volume of the flow restrictor that is added to the delay volume when the pH valve is installed, and the flow restrictor is in-line. This volume includes the volume of the tubing to the flow restrictor.
Delay volume →pH cell volume	<b>Delay volume</b> $\rightarrow$ <b>pH cell volume</b> , defines the volume of the pH valve that is added to the delay volume when the pH electrode is in-line.
Drop sync	Drop sync synchronize movement after drop release. The available settings are <b>On</b> , <b>Off</b> , and <b>Auto</b> . The Auto setting turns the drop synchronization on for flow rates below 3 mL/min, and turns it off for flows above 3 mL/min. This is done for all plate types except 96 deep well, where the limit is 5 mL/min. Do not use the Auto setting together with a micro nozzle because the micro nozzle only form drops up to 1.5 mL/min.

Instruction name	Description
Collection of pre-fractionation volume	The options for <b>Collection of pre-</b> fractionation volume are:
	• Auto
	<b>Auto</b> sets the pre-fractionation volume to be collected in a separate well/tube position if the fraction volume is below 5 mL. If the fraction volume is 5 mL or more, the pre-frac- tionation volume will be collected in the same tube/well as the first frac- tion.
	• <b>Combined with first</b> fraction is used when the pre-fractionation volume shall be collected in the same position as the first fraction.
	Note:
	If the sum of pre-fractionation plus fraction volume exceed the maximum volume for the well/tube, the pre-fractionation volume will automatically be collected in a sepa- rate well/tube.
	• Separate position is used when the pre-fractionation volume shall be collected in a position that is different from the first fraction.

7.5 System settings

7.5.6 System settings - Fraction collection F9-T

Instruction name	Description
Last position filled action	<i>Last position filled</i> action determines what action should be taken after the last position has been filled during fractionation.
	The options for <i>Last position filled</i> <i>action</i> are:
	• <b>Out 1</b> directs the flow to outlet 1 after the last position has been filled.
	• <b>Pause</b> sets the method to pause after the last position has been filled.
	• <b>Waste</b> directs the flow to waste after the last position has been filled.
	Note:
	It is possible to enable collection in more than two plates when <b>Last position</b> filled action is set to <b>Pause</b> .
Flow when moving from plate 1 to plate 2	<i>Flow when moving from plate 1 to plate 2</i> sets system behavior when moving between plates.
	The options for <b>Flow when moving</b> from plate 1 to plate 2 are:
	• <b>Keep flow</b> , keeps the flow rate when the fractionation arm is moving from plate 1 to plate 2. For high flow rates, drops may be lost during movement.
	• <b>Direct flow to waste,</b> directs the flow to waste when the fractionation arm is moving from plate 1 to plate 2. When the fractionation arm reaches its position the outlet valve will turn to frac again.
	• Auto chooses Keep flow or Direct flow to waste depending on flow rate. Keep flow is selected for flow rates below 1.5 mL/min, Direct flow to waste for flow rates above or equal to 1.5 mL/min.

Instruction name	Description
Frac behavior when ending a run	<b>Frac behavior when ending a run</b> sets what shall happen after the run has ended. The options for <b>Frac behavior when</b> ending a run are:
	• <b>Go home without reset</b> moves the fractionation arm to its home position without resetting the plate(s).
	• <b>Go home and reset</b> moves the fractionation arm to the home position and resets the plate(s).
	• <b>Remain without reset</b> keeps the fractionation arm at its last position without resetting the plate.
LED mode	<b>LED mode</b> turns the green light in the fractionation arm On or Off during fractionation.

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# 7.5.7 System settings - Fraction collection F9-R

The tables below describe the Fraction collector F9-R system settings available for  $\ddot{\mathsf{A}}\mathsf{KTA}$  go.

Instruction name	Description
Fractionation settings	Drop sync synchronizes tube change to drop release. The available settings are <b>On</b> or <b>Off</b> . It is recommended to use <b>Drop sync</b> for flow rates below 2 mL/min. Higher flow rates can however be used, depending on the properties (for example viscosity) of the liquid.
Peak frac parameters UV	<b>Peak frac parameters UV</b> sets the detection parameters for peak collection, i.e. it determines when a peak starts and ends. This information is used by the instruction <b>Peak fractionation</b> in order to start/end the peak collection.
Fraction numbering mode	Determines whether the fraction number is reset at the end of a method or not.
	Note:
	The default setting is <b>Reset</b> .
Delay volume →Detector - Frac	<b>Delay volume</b> $\rightarrow$ <b>Detector - Frac</b> is used to define the delay volume between the monitor and the Fraction collector. The instruction is used to make sure that the collected fractions correspond to the fractions indicated in the chromatogram. The instruction is available only when the <b>Fraction collector</b> is selected in the component list.
Delay volume →Restrictor volume	<b>Delay volume</b> $\rightarrow$ <b>Restrictor volume</b> , defines the volume of the flow restrictor that is added to the delay volume when the pH valve is installed, and the flow restrictor is in-line. This volume includes the volume of the tubing to the flow restrictor.
Delay volume →pH cell volume	<b>Delay volume</b> $\rightarrow$ <b>pH cell volume</b> is used to set the pH cell volume. This volume is added to the volume defined in <b>Delay volume</b> $\rightarrow$ <b>Detector - Frac</b> when the pH electrode is in-line.

# 7.5.8 System settings - Wash settings

The table below describes the wash related system settings available for ÄKTA go.

Instruction name	Description
Pump wash settings	<b>Pump wash settings</b> sets the flow rate and the wash volume used during <b>Pump wash</b> .
System wash settings	<b>System wash settings</b> sets the flow rate and post wash volume used for <b>System wash</b> .

# 7.5.9 System settings - Watch parameters

The table below describes the watch parameter settings available for ÄKTA go.

Instruction name	Description
Watch UV parameters	Watch UV parameters sets the Accepted fluctuation and Delta peak limit of the UV signal for some of the tests in the Watch and Hold until instructions.
Watch cond parameters	Watch cond parameters sets the Accepted fluctuation and Delta peak limit of the conductivity signal for some of the tests in the Watch and Hold until instructions.
Watch pressure parameters	<b>Watch pressure parameters</b> sets the value for the accepted fluctuation of the pressure signals used for the test <b>Stable signal</b> in the instructions <b>Watch</b> and <b>Hold until</b> .
Watch flow parameters	<b>Watch flow parameters</b> sets the value for the accepted fluctua- tion of the flow rate signal used for the test <b>Stable signal</b> in the instructions <b>Watch</b> and <b>Hold until</b> .
Watch pH parameters	<b>Watch pH parameters</b> sets the value for the accepted fluctua- tion of the pH signal used for the test <b>Stable signal</b> in the instruc- tions <b>Watch</b> and <b>Hold until</b> .
Watch analog in parameters	<b>Watch analog in parameters</b> sets the accepted signal fluctua- tion and <b>Delta peak</b> limit of the analog signal for some of the tests in the <b>Watch</b> and <b>Hold until</b> instructions.

7 Reference information 7.5 System settings 7.5.10 System settings - I/O-box

# 7.5.10 System settings - I/O-box

The table below describes the I/O-box related system settings available for ÄKTA go.

Instruction name	Description
Noise reduction analog in X	<b>Noise reduction analog in X</b> filters the noise in the analog signal in port number X.
Digital out X	<b>Digital out X</b> sets the value of the signal sent out by digital port number X to either 0 or 1. The default value is 1.
Alarm analog in X	<b>Alarm analog in X</b> enables or disables the alarm for the analog signal in port number X. When enabled, it sets the alarm limits for the analog signal. If the alarm is enabled and the analog signal falls outside the set limits, an alarm will be triggered and the run will be paused.
Alarm digital in X	<b>Alarm digital in X</b> enables or disables the alarm for the signal in digital port number X. The alarm can be triggered by either of the signal values, 0 or 1. If the alarm is enabled and the condition set in <b>Value</b> occurs, an alarm will be triggered and the run will be paused.
Configure analog out X	<b>Configure analog out X</b> enables the user to send one of the pre- defined signals (UV signal, conductivity, temperature, pH or concentration of eluent B) to the analog out port number X, and also to set the range of that signal.

# 7.5.11 System settings - Advanced

The table below describes the advanced system settings available for ÄKTA go.

Instruction name	Description
Power-save	<b>Power-save</b> sets the instrument into power saving mode. When the function is enabled, the instrument enters power-saving mode after having been in state <b>Ready</b> for a certain time period. The instrument turns into state <b>Ready</b> when a method run, a method queue or a manual run ends. The time interval before the instru- ment enters power-saving mode is defined by the user.
Instrument control panel	<b>Instrument control panel</b> locks/unlocks the control panel located on the front side of the instrument. When unlocked, the buttons on the instrument control panel are active and can be used to control a few basic functions of the instrument. When the instrument control panel is locked, no functions are available.
Pressure control parame- ters	<b>Pressure control</b> regulates the flow rate if the pressure reaches close to the defined limit. <b>Pressure control</b> can be used to avoid method stops due to pressure alarms. <b>Pressure control</b> is enabled in the instruction <b>Flow</b> . <b>Pressure control parameters</b> provides the P and I factors used in the regulator and can be adjusted for different columns.
<i>Max flow during valve turn</i>	<i>Max flow during valve turn</i> sets the maximum flow rate used during the turning of the injection and outlet valve in order to avoid high pressure alarms. If the flow rate in the run is higher than this value, the flow rate is lowered during valve turn.

# 7.5.12 System settings - Data collection

The table below describes the data collection related system settings available for  $\ddot{\mathsf{A}}\mathsf{KTA}$  go.

Instruction name	Description
Data collection:	The <b>Data collection</b> settings determine the maximum number of
• UV	data points collected for a given curve. Data reduction occurs if the maximum number of data points is exceeded. To avoid data reduc-
Conductivity	tion, set the maximum number of data points to be collected to
• ConcB	180000 or insert a <b>New Chromatogram</b> instruction in the
• Flow	method.
• pH	Note:
Pressure	The default setting is 54000 data points, which corresponds to 1.5 h
Temperature	for a signal of 10 Hz.
Linear Flow	Tip:
• % Cond	The <b>New Chromatogram</b> instruction can be accessed from the
UV cell path length	Miscellaneous phase.

# 7.6 Manual instructions

### In this section

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7 Reference information 7.6 Manual instructions 7.6.1 Executing Manual instructions

### 7.6.1 Executing Manual instructions

To manually interact with the system using *Manual instructions*, follow the steps below.

Step	Action
1	In the <b>System Control</b> module:
	• select Manual → Execute Manual Instructions
	or
	• use the shortcut <b>Ctrl + m</b> .
	Result:
	The <i>Manual instructions</i> dialog opens.
2	In the <i>Manual instructions</i> dialog:
	<b>a.</b> Click the <b>+</b> symbol to show the instructions for the instruction group that you want to excecute.
	<b>b.</b> Select the instruction that you want to excecute.
	c. Enter the parameters for the instruction.
3	To execute several instructions at the same breakpoint, select and choose parameters for the instruction and click <b>Insert</b> . Repeat for several instructions.
4	To adjust parameter fields during method run, check the <b>Auto update of</b> <b>parameters during run</b> box.
5	To perform the instructions, click <i>Execute</i> .

All available manual instructions are described in the following subsections.

7 Reference information7.6 Manual instructions7.6.2 Manual instructions - Pump

### 7.6.2 Manual instructions - Pump

The table below describes the pump-related manual instructions available for ÄKTA go.

Instruction name	Description
Flow	<i>Flow</i> defines the system flow rate.
	The flow rate can be set either as volumetric, linear, or column flow. A column type must be selected before using linear or column flow.
Gradient	<b>Gradient</b> sets a gradient (linear or stepwise) using the pump and inlet valve.
	Note:
	Set gradient length value to 0 to perform a step gradient.
System wash	<b>System wash</b> is used to fill the system with the selected buffer composition. If a column valve is installed, the flow can be directed to the waste position of either the injection valve or the outlet valve. The flow is directed to the end of the flow path if outlet valve is not present.
	Note:
	• Pressing <b>End</b> during <b>System wash</b> will terminate both the wash and the run immediately.
	<ul> <li>Pressing Continue during System wash will terminate the wash and the run will continue from the point at which the System wash instruction was executed.</li> </ul>
	<ul> <li>If System wash is performed during a gradient operation, the current component B concentration is maintained during the wash.</li> </ul>
	<ul> <li>An instruction issued during System wash cannot be executed until the wash is completely finished and all valves have turned back to the previous positions.</li> </ul>
	• <b>System wash</b> cannot be executed when the system is in state <b>Hold</b> .

Instruction name	Description
Pump wash	<b>Pump wash</b> is used to change buffers in the specified inlet tubing, and in the pump and mixer. It is also used to wash away previously used buffers and sample.
	Note:
	<ul> <li>Pressing End during Pump wash will terminate both the wash and the run immediately.</li> </ul>
	<ul> <li>Pressing Continue during Pump wash will terminate the wash and the run will continue from the point at which the Pump wash instruction was executed.</li> </ul>
	<ul> <li>An instruction issued while a <b>Pump wash</b> is in progress will not be executed until the wash is completely finished and all valves have turned back to the previous positions.</li> </ul>
	• <b>Pump wash</b> cannot be executed when the system is in state <b>Hold</b> .

7 Reference information7.6 Manual instructions7.6.3 Manual instructions - Flow path

# 7.6.3 Manual instructions - Flow path

The table below describes the flow path related manual instructions available for ÄKTA go.

Instruction name	Description
Inlet	<i>Inlet</i> opens the selected inlet port. Positions <i>A</i> , <i>B</i> , <i>C</i> , or <i>Sample</i> can be selected.
Optional A inlet	A inlet turns inlet valve A to the selected position.
Optional B inlet	<b>B inlet</b> turns inlet valve <b>B</b> to the selected position.
Optional Sample inlet	Sample inlet turns the sample inlet valve to the selected position.
Injection valve	<i>Injection valve</i> turns the injection valve to the selected position. Positions <i>Load</i> , <i>Inject</i> , or <i>Waste</i> can be selected. An injection mark appears in the chromatogram when the inlet valve switches to <i>Inject</i> .
pH valve	<i>pH valve</i> sets the pH cell and the flow restrictor to position <i>In-line</i> or <i>Off-line</i> .
	The pH valve also has a calibration position. This position is only available when performing calibration of the pH monitor (in <b>System control</b> select <b>System</b> $\rightarrow$ <b>Calibrate</b> ). The calibration position can also be used to fill the pH cell with storage solution since the pH valve is in open position.
	Note:
	• It is not possible to turn the pH valve during any type of fractio- nation as it affects the delay volume.
	<ul> <li>The <b>pH valve</b> instruction can be given during the delay volume of the different stop fractionation instructions, but it is executed only after the set delay volume has been collected.</li> </ul>
Outlet valve	<b>Outlet valve</b> turns the outlet valve to the selected position. The instruction gives a mark in the chromatogram when the valve is switched to the selected position.
Injection mark	<i>Injection mark</i> makes an injection mark in the chromatogram at the point where this instruction is executed.
7 Reference information 7.6 Manual instructions 7.6.4 Manual instructions - Alarm

# 7.6.4 Manual instructions - Alarm

The table below describes the alarm related manual instructions available for ÄKTA go.

Instruction name	Description
Alarm pressure	<b>Alarm pressure</b> sets the alarm limits for the pressure. When enabled and the pressure falls outside the set pressure limits, an alarm will be triggered and the run will be paused. When a column is selected in the run, the alarm limits are automatically set to the values in the column list. For methods without column valve <b>V9-C</b> . <b>Column pressure limit</b> is also set as a variable. <b>Low alarm</b> is only triggered if the pressure first exceeds the <b>Low alarm</b> limit for ten seconds continuously and then falls below the <b>Low alarm</b> limit.
	Note:
	Setting the <b>Low alarm</b> or the system flow rate to <b>0</b> deactivates the low pressure alarm.
Alarm pre-column pressure	<b>Alarm pre-column pressure</b> sets the alarm limits for the pre- column pressure. When enabled and the pre-column pressure falls outside the set pressure limits, an alarm is issued and the run will be paused. When a column is selected in the run, the alarm limits are automatically set to the values in the column list. For methods, <b>Pre-column pressure limit</b> is also set as a variable. <b>Low alarm</b> is only triggered if the pressure first exceeds the <b>Low alarm</b> limit for ten seconds continuously then falls below the <b>Low alarm</b> limit.
	Note:
	<ul> <li>Setting the Low alarm to 0 deactivates the low pressure alarm.</li> <li>Instruction Alarm pre-column pressure is available only when Column valve V9-C is installed and selected in the compo- nent list.</li> </ul>

#### 7 Reference information

7.6 Manual instructions

7.6.4 Manual instructions - Alarm

Instruction name	Description
Alarm delta column pres- sure	<b>Alarm delta column pressure</b> sets the alarm limits for the delta- column pressure. When enabled and the delta-column pressure falls outside the set pressure limits, an alarm will be triggered and the run will be paused. When a column is selected in the run, the alarm limits are automatically set to the values in the column list. When creating a method, <b>Delta column pressure limit</b> is also set as a variable. <b>Low alarm</b> is only triggered if the pressure first exceeds the <b>Low alarm</b> limit for ten seconds continuously then falls below the <b>Low alarm</b> limit.
	Note:
	• Setting the <b>Low alarm</b> to 0 deactivates the low pressure alarm.
	<ul> <li>Instruction Alarm delta column pressure is available only when column valve V9-C is installed and selected in the compo- nent list.</li> </ul>
Alarm UV	<b>Alarm UV</b> enables or disables the alarm for the UV signal. When enabled, it sets the alarm limits for the UV signal from UV monitor <b>U9-L</b> . When the UV signal falls outside the set limits, an alarm will be triggered and the run will be paused.
Alarm conductivity	<b>Alarm conductivity</b> enables or disables the conductivity alarm. When enabled, it sets the alarm limits for the conductivity signal. When the conductivity falls outside the set limits, an alarm will be triggered and the run will be paused.
AlarmpH	<b>Alarm pH</b> enables or disables the pH alarm. When enabled, it sets the alarm limits for the pH signal. When the pH falls outside the set limits, an alarm will be triggered and the run will be paused.
Alarm air sensor	<b>Alarm air sensor</b> enables or disables the alarm for the external air sensor. If the alarm is enabled and air is detected, an alarm will be triggered and the run will be paused.
Alarm analog in X	<b>Alarm analog in X</b> enables or disables the alarm for the analog signal in port number X. When enabled, it sets the alarm limits for the analog signal. If the alarm is enabled and the analog signal falls outside the set limits, an alarm will be triggered and the run will be paused.
Alarm digital in X	<b>Alarm digital in X</b> enables or disables the alarm for the signal in digital port number X. The alarm can be triggered by either of the signal values, 0 or 1. If the alarm is enabled and the condition set in <b>Value</b> occurs, an alarm will be triggered and the run will be paused.

Instruction name	Description
Alarm Superloop pressure	<i>Alarm Superloop pressure</i> is set to avoid overpressure in a Superloop, when a Superloop is in use. If the pressure signal falls outside the set limits, an alarm is triggered and the run is paused. <i>High alarm</i> sets the top limit for the Superloop pressure alarm.
	Note: Alarm Superloop pressure is connected to the Pressure value. If Alarm pressure is also enabled, an alarm will be triggered by the lowest set value between Alarm pressure and Alarm Superloop pressure. By default Alarm Superloop pressure/High alarm is set to 5.0 MPa.

7 Reference information7.6 Manual instructions7.6.5 Manual instructions - Monitors

# 7.6.5 Manual instructions - Monitors

The table below describes the monitor related manual instructions available for ÄKTA go.

Instruction name	Description
Auto zero UV	Auto zero UV sets the UV signals from U9-L to 0 mAU.
UVlamp	Sets the UV lamp <b>On</b> or <b>Off</b> . Default is <b>On</b> . The UV lamp is turned <b>On</b> when the system changes state to <b>Run</b> , <b>Hold</b> , or <b>Wash</b> .
Noise reduction UV	<b>Noise reduction UV</b> filters the noise in the UV signal from UV monitor <b>U9-L</b> . When a column is selected in a method run, column-specific averaging time is set automatically and <b>Aver-aging time</b> is set as a variable.
Relative scale cond	<b>Relative scale cond</b> facilitates monitoring of a gradient, for which the user sets the conductivity values for 0% and 100%. The <b>Rela-</b> <i>tive scale cond</i> can be set in ascending manner (0% for low and 100% for high conductivity) or in descending manner (0% for high and 100% for low conductivity).
	Note:
	The <b>Relative scale cond</b> in descending manner is especially useful for conductivity visualization in HIC, where the conductivity curve is reversed compared to the concentration curve (i.e., high conductivity at 0% B and low conductivity at 100% B).
Reset auto zero UV	<b>Reset auto zero UV</b> resets the UV signal to the actual measured value, by removing the offset generated when <b>Auto zero UV</b> was performed.

# 7.6.6 Manual instructions - I/O-box

The table below describes the I/O-box related manual instructions available for  $\ddot{\mathsf{A}}\mathsf{KTA}$  go.

Instruction name	Description
Auto zero analog in X	Auto zero analog in $X$ sets the value of the analog signal in the analog port number X to 0 mV.
Reset auto zero analog in X	<b>Reset auto zero analog in X</b> sets the signal in analog port number X to its current value, i.e. the actual voltage in the analog port number X.
Noise reduction analog in X	<b>Noise reduction analog in X</b> filters the noise in the analog signal in port number X.
Digital out X	<b>Digital out X</b> sets the value of the signal sent out by digital port number X to either 0 or 1. The default value is 1.
Pulse digital out X	<b>Pulse digital out X</b> generates a pulsed signal in digital port number X. The signal changes from the initial state (0 or 1) to the opposite state and returns to the initial state after the defined length of time.
Configure analog out X	<b>Configure analog out X</b> enables the user to send one of the pre- defined signals (UV signal, conductivity, temperature, pH or concentration of eluent B) to the analog out port number X, and also to set the range of that signal.

7 Reference information7.6 Manual instructions7.6.7 Manual instructions - Fraction collection F9-T

# 7.6.7 Manual instructions - Fraction collection F9-T

The tables below describe the Fraction collector F9-T manual instructions available for  $\ddot{\mathsf{A}}\mathsf{KTA}$  go.

Instruction name	Description
Fractionation	<i>Fractionation</i> sets the fractionation parameters when collecting fractions with the Fraction collector.
	Plate type sets the type of plates or tubes used.
	<i>Fraction size</i> sets the size of the fraction in each well/tube. The fractionation size can be set in time or in volume.
	Start position sets at which position fractionation shall start.
	The options for <b>Start position</b> are:
	• <b>Set A. 1</b> ; resets the plate position and fractionation starts in A1 in the first plate of the chosen type.
	• <i>First available position</i> ; fractionation starts in the first available position.
	• <i>First available row;</i> fractionation starts in the first available row.
	<ul> <li>First available plate; fractionation starts in the first available plate.</li> </ul>
	• <b>Skip two positions</b> sets the start position two positions ahead of the first available position.
	• <b>Set position</b> enables you to set any position as start position. You choose your start position in a plate by typing the plate number, the letter of the corresponding row and the column number, e.g. 2.A.1. When setting a start position in a 50 mL tube, type the letter and number of the corresponding tube, e.g. T.1. The start position can also be set by clicking the position in the <b>Process</b> picture. If the chosen start position is used the plate will be reset.
Peakfractionation	<b>Peak fractionation</b> enables collection of only those peaks that fulfill the conditions set in the <b>Peak fractionation parameters UV</b> instruction described below.
	Fraction marks are set in the chromatogram for each collected peak.
	The settings are the same as for <i>Fractionation</i>

Instruction name	Description
Combined fractionation	<b>Combined fractionation</b> combines fractionation and peak frac- tionation. This enables a more dynamic fractionation where a fixed fractionation is performed between detected peaks. When a peak start is detected, peak fractionation starts and continues until peak end is detected. At peak end, the fixed fractionation is resumed until a new peak start is detected. Fraction marks are set in the chromatogram for each new fraction collected.
Ston fractionation	Ston fractionation ends the fractionation after the set delay
	volume has been collected. The outlet valve is then turned to posi- tion <b>Waste</b> .
Next position	The fractionation goes to the next position after the delay volume has been collected. When no fractionation is ongoing, <b>Next posi-</b> <i>tion</i> moves the fractionation arm instantly.
	When issuing <b>Next position</b> while the flow is directed to Waste or Out 1, the fractionation arm will move over the positions. These positions will be marked in the chromatogram, but no fractions are collected in these positions.
Next plate	The fractionation goes to the next plate after the set delay volume has been collected. Next plate can only be issued when plate 1 and plate 2 are of the same type.
Gohome	<b>Go home</b> makes the fractionation arm move to its home position. Using this command is only possible when fractionation is not ongoing, e.g. Pause during ongoing fractionation.
Go to right	<b>Go to right</b> makes the fractionation arm move as far right as possible. Using this command is only possible when fractionation is not ongoing, e.g. Pause during ongoing fractionation.
Reset plate	<b>Reset plate</b> resets all positions in plates or racks and sets them as available for fractionation. The command cannot be performed for the current plate/rack when fractionation is ongoing.
Flow when moving from plate 1 to plate 2	<i>Flow when moving from plate 1 to plate 2</i> sets what shall happen when the fractionation arm is moving between plates.
Frac behavior when ending a run	<i>Frac behavior when ending a run</i> sets what shall happen after the run has ended.
Peak frac parameters UV	<b>Peak frac parameters UV</b> sets the detection parameters for peak collection, i.e. it determines when a peak starts and ends. This information is used by the instruction <b>Peak fractionation</b> in order to start/end the peak collection.

# 7.6.8 Manual instructions - F9-R Fraction collection

The tables below describe the Fraction collector F9-Rmanual instructions available for ÄKTA go.

Instruction name	Description
Fractionation	<i>Fractionation</i> is used to set the fractions size when collecting fractions with a Fraction collector. The fractions size can be set in time or in volume.
Stop fractionation	Stop fractionation       ends the fractionation after the set delay volume (specified in System Settings → Fraction collection → Delay volume) has been collected. The outlet valve is then turned to position Waste.         Note:       If Stop fractionation is issued when both Fractionation and Peak fractionation are active, fractionation is stopped after the
	set delay volume has been collected. The outlet valve remains in position <b>Frac</b> and peak fractionation continues.
Feed tube	<b>Feed tube</b> moves the tube rack forward one tube after the set delay volume has been collected and a fraction mark is set. When fractionation or peak fractionation is not ongoing, <b>Feed tube</b> moves the rack instantly and no fraction mark is set.
Reset frac number	Sets fraction numbers to restart from 1. The restart occurs when the instruction is issued. The instruction overrides the continuous numbering mode if <i>Fractionation numbering mode</i> is set to <i>Continue</i> in <i>System Settings</i> .
Peakfractionation	<b>Peak fractionation</b> enables collection of only those peaks that fulfill the conditions set in the <b>Peak fractionation parameters UV</b> instruction described below.
Stop peak fractionation	<b>Stop peak fractionation</b> ends the peak fractionation after the set delay volume (specified in <b>System Settings</b> $\rightarrow$ <b>Fraction collection</b> $\rightarrow$ <b>Delay volume</b> ) has been collected. The outlet value is then turned to position Waste.
Peak frac parameters UV	<b>Peak frac parameters UV</b> sets the detection parameters for peak collection, i.e. it determines when a peak starts and ends. This information is used by the instruction <b>Peak fractionation</b> in order to start/end the peak collection.

# 7.6.9 Manual instructions - Wash settings

The table below describes the wash related manual instructions available for ÄKTA go.

Stage	Description
Pump wash settings	<b>Pump wash settings</b> sets the flow rate and wash volume used during pump washes.
	Note:
	The flow rate should not exceed 10 mL/min if narrow inlet tubing (i.d. 0.75 mm) is used.

# 7.6.10 Manual instructions - Watch parameters

The table below describes the watch parameter instructions available for ÄKTA go.

Instruction name	Description
Watch UV parameters	Watch UV parameters sets the accepted signal fluctuation and Delta peak limit of the UV signal for some of the tests in the Watch and Hold until instructions.
Watch cond parameters	<b>Watch cond parameters</b> sets the accepted fluctuation and <b>Delta peak</b> limit of the conductivity signal for some of the tests in the <b>Watch</b> and <b>Hold until</b> instructions.
Watch pressure parameters	<b>Watch pressure parameters</b> sets the value for the accepted fluctuation of the pressure signals used for the test <b>Stable signal</b> in the instructions <b>Watch</b> and <b>Hold until</b> .
Watch flow parameters	<b>Watch flow parameters</b> sets the value for the accepted fluctua- tion of the flow rate signal used for the test <b>Stable signal</b> in the instructions <b>Watch</b> and <b>Hold until</b> .
Watch pH parameters	<b>Watch pH parameters</b> sets the value for the accepted fluctua- tion of the pH signal used for the test <b>Stable signal</b> in the instruc- tions <b>Watch</b> and <b>Hold until</b> .
Watch analog in parameters	<b>Watch analog in parameters</b> sets the accepted signal fluctua- tion and <b>Delta peak</b> limit of the analog signal for some of the tests in the <b>Watch</b> and <b>Hold until</b> instructions.

# 7.6.11 Manual instructions - Advanced

The table below describes the advanced manual instructions available for ÄKTA go.

Instruction name	Description
Pressure control parame- ters	By using <b>Pressure control parameters</b> the method can be run with the set flow rate without the risk of method stop due to pres- sure alarm. <b>Pressure control parameters</b> is enabled in the instruction <b>Flow</b> . <b>Pressure control parameters</b> provides the P and I factors used in the regulator and can be adjusted for different columns.
	<i>Min allowed flow rate</i> can be set either as volumetric or as linear flow. A column type must be selected before using linear flow.
Start volume count	<b>Start volume count</b> starts the volume counter function. The counted volume is saved into a memory.
	This instruction is best used in combination with <b>Watch</b> instruc- tions.
Stop volume count	<b>Stop volume count</b> stops the volume counter function. The counted volume is stored in the memory and can be recalled with the instruction <i>Hold counted volume</i> . The counted volume can also be recalled in following runs and is stored until a new <b>Stop volume count</b> instruction is issued. This instruction is best used in combination with <i>Watch</i> instruction
Set trigger X	Triggers are used together with a <b>Watch</b> instruction to trigger a user defined action. There are 12 triggers available and each trigger can have a value of 0 or 1. The instruction <b>Set trigger X</b> sets the value of the selected trigger to 0 or 1 (0 is default). In a second step, <b>Watch</b> can be used to monitor the trigger. When
	parameter of the watch to <b>Trigger X</b> will make the watch monitor the value of the specified trigger. The watch will be activated when the value (0 or 1) of the trigger equals the value specified in <b>Watch</b> , i.e. when the watch condition is fulfilled. The action specified by the user when setting up <b>Watch</b> will at this point be performed.
	Note:
	Watch is only available as a method instruction.

# 7.6.12 Manual instructions - Other

The table below describes the other manual instructions available for ÄKTA go.

Instruction name	Description
Set mark	<b>Set mark</b> inserts a mark into the current chromatogram with the text entered for the parameter <b>Mark text</b> .
Timer	<i>Timer</i> sets the system to pause or end after a set volume or time has passed. <i>Select base</i> sets the base to either accumulated time or accumulated volume. <i>Timeout</i> sets the volume or time. <i>Action</i> sets the action to perform (pause or end)

# 7.7 Available run data

The table below lists all available **Run data** for ÄKTA go.

Run Data	Range/Unit	Description
Connection	N/A	System connection to the software.
System state	N/A	Status of connection and run.
Acc. Volume	mL	Total accumulated volume in the current method or manual run.
Block volume	mL	Accumulated volume in the current block (method run only).
Acc. Time	min	Total accumulated time in the current method or manual run.
Block time	min	Accumulated time in the current block (method run only).
Scouting no.	N/A	The current scouting number in the scouting scheme.
Flow	0.01 to 25 mL/min	The set flow rate of the pump.
Linear Flow	cm/h	The set flow velocity of the pump. Only available if a column is selected.
Pressure	0 to 5 MPa	The pressure signal (at the pump).
PreC pressure	0 to 5 MPa	The pre-column pressure signal.
DeltaC pressure	0 to 5 MPa	The delta-column pressure signal.
PostC pressure	0 to 5 MPa	The post-column pressure signal.
Conc B	0.0% to 100.0% B	The set concentration B or the current value during a gradient.
Air Sensor	No air, Air	The current state of the air alarm for the air sensor.
UV	-6000.000 to 6000.000 mAu	The UV absorbance signal of the UV monitor.
Conductivity	0.00 to 999.99 mS/cm	The conductivity signal.
% Cond	0.0% to 100.0%	The conductivity signal as a percentage of a set range.
Temperature	0.0°C to 99.0°C	The temperature signal (in the conduc- tivity flow cell).

## 7 Reference information

7.7 Available run data

Run Data	Range/Unit	Description
рН	0.00 to 14.00	The pH signal.
Inlet	Closed, A, B, C, Sample	The set position of inlet valve <b>K9</b> .
Optional A inlet	A1-A6	The set position of the A inlet valve.
Optional B inlet	B1 – B6	The set position of the B inlet valve.
Optional sample inlet	S1 – S5, Buffer	The set position of the sample inlet valve.
Injection valve	N/A	The set position of the injection valve.
Column position	N/A	The set position of the column valve.
pH valve	N/A	The set position of the pH valve.
Outlet	N/A	The set position of the outlet valve.
Frac position	N/A	The current tube position of the Fraction collector.
Analog In 1, to	-2000.0 to 2000.0 mV	The I/O-box analog input signals.
Analog In 2		
Digital In 1, to	0, 1	The I/O-box digital input signals.
Digital In 4		
Digital Out_1, to Digital Out_4	0, 1	The set value of the I/O-box digital output signals.

# 7.8 Available curves

Curve	Range	Sampling frequency	Description
UV	-6000.000 to 6000.000 mAU	10 Hz	The UV absorbance signal of the UV monitor.
Conductivity	0.00 to 999.99 mS/cm	5 Hz	The conductivity signal.
ConcB	0.0% to 100.0%	1 Hz	The set concentration B or the current value during a gradient.
Flow	0.01 to 25.00 mL/min	1 Hz	The set flow rate of the pump.
рН	0.00 to 14.00	1 Hz	The pH signal.
Pressure	0.00 to 5.00 MPa	2 Hz	The pressure signal (at the pump).
Temperature	0.0°C to 99.0°C	0.5 Hz	The temperature signal (in the conductivity flow cell).
Pre Column Pressure	0.00 to 5.00 MPa	1 Hz	The pre-column pressure signal.
Delta Column Pressure	0.00 to 5.00 MPa	1 Hz	The delta-column pressure signal.
Post Column Pressure	0.00 to 5.00 MPa	1 Hz	The post-column pressure signal.
Fraction	N/A	N/A	The fraction marks and number.
Injection	N/A	N/A	The position mark for an injec- tion instruction.
Linear Flow	cm/h	1 Hz	The set flow velocity of the pump. Only available if a column is selected.
% Cond	0.0% to 100.0%	1 Hz	The conductivity signal as a percentage of a set range.
Run Log	N/A	N/A	Shows all registered actions.
Analog In 1, to Analog In 2	-2000.0 to 2000.0 mV	10 Hz	The I/O-box analog input signals.
UV cell path length	2 or 5 mm	1 Hz	The nominal cell path length of the UV monitor.

The table below lists all available curves for ÄKTA go.

## 7 Reference information

#### 7.8 Available curves

Curve	Range	Sampling frequency	Description
Digital In 1, to Digital In 4	0, 1	10 Hz	The I/O-box digital input signals.
Digital Out_1, to Digital Out_4	0, 1	10 Hz	The I/O-box digital output signals.
Flow (CV/h)	CV/h	1 Hz	The set flow velocity of the pump. Only available if a column is selected.

# 7.9 Delay volumes

#### Introduction

The delay volumes of a system can be determined by performing a theoretical determination of delay volumes. Delay volumes for standard configurations are listed in *Delay volumes for different system configurations, on page 270.* 

#### **Explanation of delay volume**

During fractionation there is a delay in retention between when the fractionation mark is seen in the UV to when the same liquid is dispensed in the fraction collector. This delay, called delay volume, corresponds to the volume of the flow path from the UV monitor to the Fraction collector, which has to be dispensed before what is seen in the UV reaches the Fraction collector.

The delay volume should be correctly set in UNICORN to make sure that the collected fractions correspond to the fractions indicated in the chromatogram.

By default, the delay volume is set for a system with standard configuration and 40 cm tubing length between the outlet valve and the Fraction collector. If a pH valve is installed, change the delay volume setting according to *Delay volumes for different* system configurations, on page 270. Depending on the position of the valve, volumes for the pH cell and restrictor will be added automatically.

#### **Pre-fractionation volume**

The first delay volume dispensed during fractionation is called the pre-fractionation volume. This is the liquid present in the flow path from UV to Fraction collector when the fractionation starts.

#### Set the delay volume in UNICORN

The delay volume is set in System Settings for the following options:

- **Detector Frac**, defines the delay volume between the UV monitor and the Fraction collector, excluding the pH valve but including the tubing to the pH valve.
- **Restrictor volume**, defines the volume of the flow restrictor that is added to the delay volume when the pH valve is installed and the flow restrictor is in-line. This volume includes the volume if the tubing to the flow restrictor.
- **pH cell volume**, defines the volume of the pH valve that is added to the delay volume when the pH electrode is in-line.

Follow the steps below to set the delay volume.

**Note:** Change the delay volumes only if the system configuration has changed. Changing tubing dimensions or modules between the UV monitor and the Fraction collector affects the delay volume.

Step	Action		
1	Open the <b>System Setti</b> System Control modu		tings in the
2	Select <b>Fraction collect</b> field: System Settings - ÄKTA go	t <b>ion</b> → <b>Delay volume</b> and type in the vo	olume in each
	Conductivity DH Pressure alarm Fraction collection Fraction settings Peak frac parameters UV Fraction numbering mode Delsy volume Wash settings Watch parameters Advanced Data collection	Detector - Frac [0 - 7999] 223	
	0	Set Parameters To Strategy Default Values OK	Cancel

3 Click **OK** to save the new delay volumes.

# Delay volumes for different system configurations

The tables below list the delay volumes between the UV detector and the Fraction collector for different system configurations.

**Note:** The values are based on the 2 mm flow cell for the UV monitor. When using the 5 mm flow cell, subtract 5 μL to the volumes shown.

# Delay volumes for a system without a pH valve installed

Nozzle	Placement of fraction collector, tubing length	Standard tubing (i.d. 0.5 mm)	Tubing (i.d. 0.25 mm)	Tubing (i.d. 0.75 mm)
F9-T standard nozzle	Next to system, 40 cm	233 µL	106 µL	434 µL
F9-T standard nozzle	In tunnel under ÄKTA go, 80 cm	311 µL	126 µL	611 µL

Nozzle	Placement of fraction collector, tubing length	Standard tubing (i.d. 0.5 mm)	Tubing (i.d. 0.25 mm)	Tubing (i.d. 0.75 mm)
F9-R tubing holder	Next to system, 40 cm	223 µL	96 µL	424 µL
F9-T flexible nozzle	Next to system, 40 cm	223 µL	96 µL	424 µL
F9-T flexible nozzle	In tunnel under ÄKTA go, 80 cm	301 µL	116 µL	601 µL
F9-T micro nozzle	Next to system, 40 cm	224 µl Not recom- mended <sup>1</sup>	97 µL	Not recom- mended <sup>1</sup>
F9-T micro nozzle	In tunnel under ÄKTA go, 80 cm	302 µl Not recom- mended <sup>1</sup>	117 µL	Not recom- mended <sup>1</sup>

<sup>1</sup> Not recommended due to large delay volumes, not suitable for micro application.

# Delay volumes to add for a system with a pH valve installed

Input field in UNICORN	Standard tubing (i.d. 0.5 mm)	Tubing (i.d. 0.25 mm)	Tubing (i.d. 0.75 mm)
Detector - Frac <sup>1</sup>	26 µL	11 µL	54 µL
Restrictor volume	48 µL	25 µL	88 µL
pH cell volume	76 µL	76 µL	76 µL

<sup>1</sup> Add the volume specified here for Detector to Frac to the correct volume for Detector to Frac found in table above. The volume specified here is the extra volume in the tubing needed to connect the pH valve in the flow path between the conductivity monitor and the outlet valve.

# Theoretical determination of delay volumes

A theoretical determination is performed as described in the steps below:

#### Action Step 1 Identify all components in the system flow path that contribute to the delay volume. 2 Determine the internal volumes of all hardware modules and tubing. See Section 7.10 Internal volumes, on page 273 for information about theoretical module volumes and Section 7.2 Tubing and connectors, on page 213 for information about tubing lengths and dimensions. 3 To obtain the total delay volume, sum up half of the flow cell volume of the UV monitor with all volumes of tubing and modules that are located after the monitor in the flow path. Note: For pH valve **V9-pH** always use the volume for the valve in bypass position (15 µL). The system automatically adds the volume for the flow restrictor and the pH

flow cell, depending on the position of the valve.

# 7.10 Internal volumes

The table below shows the flow path volumes of the modules and components in ÄKTA go. For tubing volumes refer to *Tubing types, on page 213*.

Component	Volume (µL)
Inlet valve <b>K9</b>	A inlet: 350
	<b>B</b> inlet: 300
	<b>C</b> inlet: 250
	Sample inlet: 200
Inlet valves V9-ImA, V9-ImB, or V9-ImS	88
Air sensor L9-1.5	35
Air sensor L9-1.2	20
Pump <b>P9-S</b> (total volume for two heads, including inlet manifold and check valves)	1392
Pump restrictor	30
Pressure monitor <b>R9</b>	45
Mixer	1000
Injection valve <b>V9-J</b>	5
Column valve <b>V9-C</b>	110
Column valve V9-Cm	17
UV monitor <b>U9-L</b> : Flow cell 2 mm	30
UV monitor <b>U9-L</b> : Flow cell 5 mm	20
Conductivity monitor <b>C9</b> flow cell	22
Flow restrictor <b>FR-902</b>	10
pH valve <b>V9-pH</b> , in <b>By-pass</b> position	15
pH flow cell	76
Outlet valve V9-Os	9
F9-T standard nozzle	10
F9-T micro nozzle	1
F9-T tubing nozzle	0
F9-R tubing holder	0

**Note:** The valve volumes shown above are average values. The actual internal volume might differ depending on the chosen flow path.

# 7.11 Error codes

#### Introduction

This section describes the software error codes for each module, and suggested corrective actions.

#### **All modules**

Error code	Description	Action
0 - 19	Internal instrument error	Restart the instrument. If recurrent, contact Service.

#### Instrument control unit

Error code	Description	Action
21-69	Internal instrument error	Restart the instrument. If recurrent, contact Service.

#### Valve

Error code	Description	Action
20, 24	Internal instrument error	Restart the instrument. If recurrent, contact Service.
22	Valve not finding position	Restart the instrument. If recurrent, contact Service.
23	Faulty air sensor	Restart the instrument. If recurrent, contact Service.
25	High temperature	See Section 6.1 Hardware issues, on page 190.

# Inlet valve K9

Error code	Description	Action
51	High temperature	See Section 6.1 Hardware issues, on page 190.
52, 53	Internal instrument error	Restart the instrument. If recurrent contact Service.

### 7 Reference information

7.11 Error codes

## Pump

Error code	Description	Action
51 - 53	Internal pump error	Check that there is no blockage of the pump outlet. Restart the instrument. If recurrent contact Service.
54	High temperature	See Section 6.1 Hardware issues, on page 190.

## **Pressure monitor**

Error code	Description	Action
20, 21, 24 - 27	Internal instrument error	Restart the instrument. If recurrent contact Service.
23	High temperature	See Section 6.1 Hardware issues, on page 190.

# **UV** monitor

Error code	Description	Action
51	High temperature	See Section 6.1 Hardware issues, on page 190.
52, 55	Low lamp intensity	Contact Service
54	Auto zero out of range	Auto zero requested when AU value is larger than 2.
58	Low light intensity, S channel	No light through flow cell. Check solution absorption and that the cell is fitted correctly.
59,60	Internal instrument error	Restart the instrument. If recurrent contact Service.
61	Measurement error	Restart the instrument. If recurrent contact Service.

# **Conductivity monitor**

Error code	Description	Action
20-27	Internal instrument error	Restart the instrument. If recurrent contact Service.
28	High temperature	See Section 6.1 Hardware issues, on page 190.

Error code	Description	Action
29	Temperature data error	Restart the instrument. If recurrent contact Service.
32 - 34	No factory calibra- tion	Contact Service

# pH monitor

Error code	Description	Action
20, 21	Internal instrument error	Restart the instrument. If recurrent contact Service.
25	No factory calibra- tion	Contact Service
26	High temperature	See Section 6.1 Hardware issues, on page 190.

# Fraction collector F9-T

Error code	Description	Action
20	The internal temper- ature of the Fraction collector is too high.	See Section 6.1 Hardware issues, on page 190.
21	The Fraction collector failed to detect a drop.	See Fraction collector F9-T, on page 197.
23	The fractionation volume is too small compared to the delay volume. New position changes will be lost.	Increase fractionation volume
26	Delayed position change.	Avoid mixing fast and slow longer movements of the fraction collector. Check also that the drop sync works properly.
27	An invalid mode change was requested.	Restart the instrument with the power switch at the next convenient occasion. If this warning recurs, generate a System error report refer to ÄKTA go Operating Instructions (29360951)) and contact service.

# 7 Reference information

#### 7.11 Error codes

Error code	Description	Action
28	Too fast position changes.	Reduce flow or increase the fractionation volume.
29	Axis not verified. Start fractionation was attempted without a successful Find Zero Position.	Restart the instrument with the power switch at the next convenient occasion. If this warning recurs, generate a System error report (refer to ÄKTA go Operating Instruc- tions (29360951)) and contact service.
30	Position filled.	Reduce fractionation time or flow.
31	Error in one or both optical sensors on the y axis.	Restart the instrument with the power switch at the next convenient occasion. If this warning recurs, generate a System error report (refer to ÄKTA go Operating Instruc- tions (29360951)) and contact service.
32	Restart position error. An error occurred when trying to move to the next start position.	Restart the instrument with the power switch at the next convenient occasion. If this warning recurs, generate a System error report (refer to ÄKTA go Operating Instruc- tions (29360951)) and contact service.
33	Encoder has identi- fied a loss of steps.	Restart the instrument with the power switch at the next convenient occasion. If this warning recurs, generate a System error report (refer to ÄKTA go Operating Instruc- tions (29360951)) and contact service.
35	Find zero position failed.	Restart the instrument with the power switch at the next convenient occasion. If this warning recurs, generate a System error report (see Section 7.5.6 System settings - Fraction collection F9-T, on page 238) and contact service.
36	Drops were detected during two move- ments in a row.	Decrease the flow rate or turn drop sync off, see Section 7.5.6 System settings - Fraction collection F9-T, on page 238.

# Fraction collector F9-R

Error code	Description	Action
20	High temperature	See Section 6.1 Hardware issues, on page 190.
21	Drop sync sensor warning	Clean the drop sync sensor and remove air bubbles from the flow path.

Error code	Description	Action
22	Tube sensor error	Check that the tube sensor is adjusted prop- erly.
24, 26	Internal instrument error	Restart the instrument. If recurrent contact Service.
25, 28	Fast tube change	Increase the fraction size or lower the flow rate.
27	Drop sync sensor error	Clean the drop sync sensor.

# Instrument control panel

Error code	Description	Action
25	High temperature	See Section 6.1 Hardware issues, on page 190.

## Air sensor

Error code	Description	Action
20	High temperature	See Section 6.1 Hardware issues, on page 190.

# I/O-box

Error code	Description	Action
20	High temperature	See Section 6.1 Hardware issues, on page 190.
21	Analog in signal below -2 V	Check the external equipment connected to the I/O-box.
22	Analog in signal above 2 V	Check the external equipment connected to the I/O-box.
23-28	Internal instrument error	Restart the instrument. If recurrent contact Service.

# 7.12 Node ID

#### Node ID for core modules

The table below lists the Node ID for the core modules.

Core module	Label	Node ID
Pump	P9-S	0
Pressure monitor	R9	0
Inlet valve	К9	0
Injection valve	V9-J	25
UV monitor	U9-L	0
Conductivity monitor	C9	0
Outlet valve	V9-Os	19

## Node ID for optional modules

The table below lists the Node ID for the optional modules.

Module	Label	Node ID
A inlet valve	V9-ImA	27
B inlet valve	V9-ImB	28
Sample inlet valve	V9-ImS	29
Column valve (3-columns)	V9-Cm	26
Column valve (5-columns)	V9-C	5
Pre-column pressure sensor	N/A	2
Post-column pressure sensor	N/A	3
pH valve	V9-рН	11
Air sensor	L9-1.5	0
Air sensor	L9-1.2	0
Fraction collector	F9-R, F9-T	0
I/O-box	E9	0

#### Check/Change Node ID

Step	Action
1	Remove the module according to the respective instruction.
2	The Node ID of a module is set by the position of an arrow on a rotating switch at the back of the module.

- **a.** The first switch, labeled **A**, sets the tens.
- **b.** The second switch, labeled **B**, sets the units.

Valve modules have two rotating switches, as shown in the image below:

For example, to set the Node ID to 6 for a valve module, switch **A** is set to **0** and switch **B** is set to **6**.



- 3 Check the Node ID and compare it with the listed Node IDs in the tables above.
- 4 To change the Node ID, use a screwdriver to set the arrows of the switches to the desired number.
- 5 If applicable, re-install the module in the instrument.

# 8 Ordering information

## About this chapter

This chapter lists accessories and user replaceable spare parts available for ÄKTA go.

# Tubing

Item	Code no.
PEEK tubing, i.d. 0.15 mm, 2 m	18115659
PEEK tubing, i.d. 0.25 mm, 2 m	18112095
PEEK tubing, i.d. 0.50 mm, 2 m	18111368
PEEK tubing, i.d. 0.75 mm, 2 m	18111974
FEP tubing, i.d. 1.6 mm, 3 m	18112116
ETFE tubing, i.d. 1.0 mm, 2 m	18111583
Reference capillary <b>Ref 1</b>	28950749
Pump rinsing system tubing	29011348
Union 1/16" male/male, i.d. 0.5 mm (5-pack)	28954326
Tubing cutter	18111246
Inlet filter holder kit	11000407
Inlet filter set	11000414

### Holders

Item	Code no.
Adapter for air sensor	28956342
Bottle holder	28956327
Column clamp	28956319
Column holder	28956282
Multidirectional column clamp	29383530
Column holder rod	28956270
Flexible column holder	28956295

Item	Code no.
Multi-purpose holder	29011349
Rail extension	29011352
Tube holder (5-pack)	28954329
Tubing holder comb	28956286
Tubing holder spool	28956274
Inlet filter holder kit	11000407
Screw lid GL45 kit	11000410

## Pump P9-S

Item	Code no.
Pump <b>P9-S</b>	29658265
P9 Seal kit 65 mL	28960250
P9 Piston kit 100 mL	18111213
Check valve kit	28979364

# **Pressure monitor**

Item	Code no.
Pressure monitor <b>R9-1n</b>	29383536

#### Mixer

Item	Code no.
Mixer	29383537
Mixer O-rings and membrane	29393228

### 8 Ordering information

## Valves

Item	Code no.
Inlet valve <b>K9</b>	29383535
Injection valve <b>V9-J</b>	29298324
Column valve kit <b>V9-C</b>	29011367
Column valve V9-Cm	29383526
A Inlet valve V9-ImA	29383527
B Inlet valve <b>V9-ImB</b>	29383528
Sample inlet valve V9-ImS	29383529
Outlet valve kit <b>V9-Os</b>	29011356
pH valve kit <b>V9-pH</b>	29011359

# Injection valve accessories

Item	Code no.
Sample loop 10 µL	18112039
Sample loop 50 µL	29325047
Sample loop 100 µL	18111398
Sample loop 500 µL (mounted at delivery)	18111399
Sample loop 1 mL	18111401
Sample loop 2 mL	18111402
Sample loop 10 mL	18116124
Superloop 10 mL	19758501
Superloop 50 mL	18111382
Superloop 150 mL	18102385
Fill port	18112766
Injection kit	18111089
Connector 1/16" male and Luer female	28985812

## UV monitor

Item	Code no.
UV monitor <b>U9-L</b>	29011360
UV flow cell 2 mm	29011325
UV flow cell 5 mm	18112824
UV Test Kit, 1 and 2 mm	29276997
UV Test Kit, 5 and 10 mm	29276998

# **Conductivity monitor**

Item	Code no.
Conductivity monitor <b>C9n</b>	29011363

## pH valve

Item	Code no.
pH electrode	28954215
O-ring 5.3 × 2.4 mm	28956497

# **Flow restrictor**

Item	Code no.
Flow restrictor <b>FR-902</b>	18112135

## **Fraction collector F9-T**

Item	Code no.
Fraction collector F9-T	29454032
F9-Ttunnel	29476924
Tube rack F9-T <sup>1</sup>	29418016
F9-T standard nozzle <sup>1</sup>	29477967
F9-T micro nozzle	29501534
F9-T tubing nozzle <sup>1</sup>	29510082

Item	Code no.
Tubing guide for nozzle <sup>2</sup>	29507802
Microplate holder F9-T	29476921
Tube rack - 0.5 mL tubes	29491085

Included with Fraction collector F9-T
 Included with F9-T tunnel

# **Fraction collector F9-R**

Item	Code no.
Fraction collector F9-R	29011362
Tube Rack Complete, 175 x 12 mm	19868403
Tube Rack Complete, 95 x 10 18 mm	18305003
Tube Rack Complete, 40 x 30 mm	18112467
Bowl	18305103
Tube support	18305402
Tube holder	18646401
Tube rack upgrade kit, 175 x 12 mm	19724202
Tube rack upgrade kit, 95 x 18 mm	19868902
Tube rack upgrade kit, 40 x 30 mm	18112468
Drive sleeve	19606702

# I/O box

Item	Code no.
I/O box <b>E9</b>	29011361

## Air sensor

Item	Code no.
Air sensor L9-1.5	28956500
Air sensor L9-1.2	28956502

#### **Online filter**

Item	Code no.
Filter 10PP	56302238

#### **Module components**

Item	Code no.
Module Panel	29011364
Extension box	29110806
Note: Includes external module cables.	

#### Cables

Item	Code no.
Jumper 1 IEC 1394 (F-type)	28956489
External module cable, short (F-type)	29012474
External module cable, long (F-type)	29011366

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