GE Healthcare Life Sciences

# ÄKTA™ avant Installation Guide

Original instructions





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## 1 Introduction

#### About the Installation Guide

The purpose of the Installation Guide is to give the necessary instructions to enable users and service personnel to:

- unpack an ÄKTA avant system delivered from the factory
- install the instrument
- install the computer
- install the software
- verify functionality after installation

Read the entire Installation Guide before starting to install the ÄKTA avant system.

#### In this chapter

This chapter contains the following sections:

Section	See page
1.1 Important user information	6
1.2 Regulatory information	8

### 1.1 Important user information

## Read this before using the ÄKTA avant system



## All users must read the entire ÄKTA avant Installation Guide and Getting Started with ÄKTA avant, before installing, operating, or maintaining the instrument.

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

#### Intended use

ÄKTA avant is a liquid chromatography system intended for process development. The system can be used to screen for optimal choice of columns, media and running parameters to purify selected proteins.

The ÄKTA avant system is intended for research use only, and shall not be used in any clinical procedures, or for diagnostic purposes.

#### **Safety notices**

This user documentation contains WARNINGS, CAUTIONS and NOTICES concerning the safe use of the product. See definitions below.

#### Warnings



#### 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.

#### Cautions



#### CAUTION

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

#### Notices



#### NOTICE

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

#### Notes and tips

Note:	A Note is used to indicate information that is important for trouble-free and optimal use of the product.
Tip:	A tip contains useful information that can improve or optimize your procedures.

#### **Typographical conventions**

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

Hardware controls, indicators and connections are identified in the text by **bold** text (e.g., **Power** switch).

Text entries that UNICORN™ generates or that the user must type are represented by a monotype typeface(e.g., \Program Files\GE Healthcare\UNICORN\bin\UNI-CORN Instrument Server.exe.config).

## 1.2 Regulatory information

#### Introduction

This section describes the directives and standards that are fulfilled by ÄKTA avant.

#### **Manufacturing information**

The table below summarizes the required manufacturing information. For further information, see the EC Declaration of Conformity document.

Requirement	Content
Name and address of	GE Healthcare Bio-Sciences AB, Björkgatan 30, SE-751
manufacturer	84 Uppsala, Sweden

#### **CE conformity**

This product complies with the European directives listed in the table below, by fulfilling the corresponding harmonized standards. For further information, see the EC Declaration of Conformity document.

Directive	Title
2006/42/EC	Machinery Directive (MD)
2006/95/EC	Low Voltage Directive (LVD)
2004/108/EC	ElectroMagnetic Compatibility (EMC) Directive
1999/5/EC	Radio Equipment and Telecommunications Terminal Equipment (R&TTE) Directive

#### **CE marking**

## CE

The **CE** marking and the corresponding EC Declaration of Conformity, is valid for the instrument when it is:

• used as a stand-alone unit, or

- connected to other GE Healthcare instruments, or
- connected to other products recommended or described in the user documentation, and
- used in the same state as it was delivered from GE Healthcare, except for alterations described in the user documentation.

#### **International standards**

This product fulfills the requirements of the following standards:

Standard	Description	Notes
EN ISO 12100	Safety of machinery. General princi- ples for design. Risk assessment and risk reduction.	EN ISO standard is har- monized with EU direc- tive 2006/42/EC
EN 61010-1, IEC 61010-1, UL 61010-1, CAN/CSA C22.2 No. 61010-1	Safety requirements for electrical equipment for measurement, con- trol, and laboratory use.	EN standard is harmo- nized with EU directive 2006/95/EC
EN 61326-1, IEC 61326-1 (Emission accord- ing to CISPR 11, Group 1, class A)	Electrical equipment for measure- ment, control and laboratory use - EMC requirements	EN standard is harmo- nized with EU directive 2004/108/EC
EN 301 489-1, EN 301 489-3	Electromagnetic compatibility and Radio spectrum Matters (ERM); ElectroMagnetic Compatibility (EMC) standard for radio equipment and services.	EN standard is harmo- nized with EU directives 1999/5/EC and 2004/108/EC.
EN 300 330-2	Electromagnetic compatibility and Radio spectrum Matters (ERM); Short Range Devices (SRD); Radio equip- ment in the frequency range 9 kHz to 25 MHz and inductive loop sys- tems in the frequency range 9 kHz to 30 MHz.	EN standard is harmo- nized with EU directive 1999/5/EC.

#### **FCC statement**

The ÄKTA avant instrument, excluding transmitted radio frequency energy from the Mixer **M9** and UV detector **U9-D**, complies with FCC 47 CFR Part 15b (Federal Communications Commission (FCC) title 47 of the Code of Federal Regulations (CFR), Part 15b, Radio Frequency Devices).

**Note:** This equipment has been tested and found to comply with the limits for a Class A digital device, pursuant to part 15 of the FCC Rules. These limits are designed to provide reasonable protection against harmful interference when the equipment is operated in a commercial environment. This equipment generates, uses and can radiate radio frequency energy and, if not installed and used in accordance with the instruction manual, may cause harmful interference to radio communications. Operation of this equipment in a residential area is likely to cause harmful interference in which case the user will be required to correct the interference at his own expense.

The modules Mixer M9 and UV detector U9-D, comply with FCC 47 CFR Part 15c.

- **Note:** This device complies with Part 15c rules. Operation is subject to the following two conditions:
  - This device may not cause harmful interference, and
  - This device must accept any interference received, including interference that may cause undesired operation.

The user is cautioned that any changes or modifications not expressly approved by the manufacturer could void the user's authority to operate the equipment.

## Software declaration of conformity

UNICORN 6 is technically compatible with all relevant sections of FDA 21 CFR Part 11.

A part 11-system assessment checklist is available on request through the local GEHC representative.

## Regulatory compliance of connected equipment

Any equipment connected to ÄKTA avant should meet the safety requirements of EN 61010-1/IEC 61010-1, or other relevant harmonized standards. Within EU, connected equipment must be CE marked.

#### **Environmental Conformity**

Regulation	Title
2011/65/EU	Restriction of Hazardous Substances (RoHS) Directive
2002/96/EC	Waste Electrical and Electronic Equipment (WEEE) Direc- tive
Regulation (EC) No 1907/2006	Registration, Evaluation, Authorization and restriction of CHemicals (REACH)
ACPEIP	Administration on the Control of Pollution Caused by Electronic Information Products, China Restriction of Hazardous Substances (RoHS)

## 2 Introduction to ÄKTA avant

#### About this chapter

The ÄKTA avant system comprises the ÄKTA avant instrument, the UNICORN software and accessories. This chapter gives an overview of the ÄKTA avant system and describes the location of indicators and controls on the instrument.

#### Illustration of the system

The illustration below shows the ÄKTA avant instrument with UNICORN software installed on a computer.



#### In this chapter

This chapter contains the following sections:

Section	See page
2.1 The ÄKTA avant instrument	13
2.2 Indicators and controls	14
2.3 Connectors	17
2.4 Network architecture	18

### 2.1 The ÄKTA avant instrument

#### Introduction

This section provides an overview of the ÄKTA avant instrument.

## Illustration of the main parts of the instrument

The illustration below shows the location of the main parts of the instrument.



Part	Function	Part	Function
1	Fraction collector	7	Holder rails
2	Buffer tray	8	Swivel foot Lock/Unlock knob
3	Instrument display	9	Swivel foot
4	Wet side	10	Swing out toolbox
5	Foldable door	11	Power switch
6	Pump cover		

### 2.2 Indicators and controls

#### Introduction

This section describes the indicators and controls that are available to the user of the ÄKTA avant system.

#### Illustration

The illustration below shows the location of indicators and controls.



Part	Function
1	Power switch
2	Swivel foot <b>Lock/Unlock</b> knob
3	Instrument display (See description below)

## Illustration of the Instrument display

The illustration below shows the Instrument display with the system state *Ready* showing.



## Instrument display indicators and buttons

The Instrument display is a touch screen that shows the current system status. The Instrument display includes the following indicators and buttons

Indicator/Button	Description	
e <sup>n</sup>	Indicates if the Instrument display buttons are unlocked or locked. The buttons can be locked from UNICORN <b>Sys-</b> <i>tem Control</i> .	
ll Pause	Pauses the run and stops all pumps.	
<ul> <li>▶ Continue</li> <li>Resumes instrument operation from the following</li> <li>Wash</li> <li>Pause</li> <li>Hold</li> </ul>		

Indicator/Button	Description
ŧ	Indicates that fractionation is ongoing. Do <i>not</i> open the Frac drawer during fractionation.

### 2.3 Connectors

#### Introduction

This section describes the connectors for power and communication on the ÄKTA avant instrument.

#### Illustration

The illustration below shows the location of the connectors.



Part	Function
1	Power input connector
2	Network connector (Ethernet)
3	UniNet-9 connectors
	Note:
	Termination plugs must be connected to the connectors that are not in use.

Other connectors are for use by authorized service engineers only.



#### NOTICE

**Misuse of UniNet-9 connectors**. The **UniNet-9** connectors at the rear panel should not be mistaken for Firewire connectors. Do not connect any external equipment to the **UniNet-9** connectors. Do not disconnect or move the **UniNet-9** bus cable.

### 2.4 Network architecture

#### Introduction

The ÄKTA avant instrument is connected to the UNICORN computer through the Network connector (Ethernet) at the back of the instrument. There are two possible network configurations:

- Workstation configuration: local network and a local database
- **Network** configuration: distributed network using TCP/IP, and a central database. Office computers can be used to set up runs, view and evaluate run data.

In this guide, only the workstation configuration is described. For further information about network configuration, please refer to UNICORN Administration and Technical Manual.

## Illustration, Workstation configuration

The illustration below shows a typical workstation configuration.



## Illustration, Network configuration

The illustration below shows a possible network configuration.



**Note:** Griffin is a software tool that can only be used by GE Healthcare service staff. This service tool is used for diagnostic, testing and quality control.

## 3 Site preparation

#### About this chapter

This chapter describes the site planning and the preparations necessary to perform before installation of an ÄKTA avant system. The purpose is to provide planners and technical staff with the data needed to prepare the laboratory for the installation.

#### In this chapter

This chapter contains the following sections:

Section	See page
3.1 Introduction	21
3.2 Delivery and storage	22
3.3 Room requirements	24
3.4 Site environment	28
3.5 Power requirements	29
3.6 Computer requirements	31
3.7 Required materials	32

### 3.1 Introduction

#### Why site preparation?

The laboratory site must be planned and prepared before installing the ÄKTA avant system. The performance specifications of the system can be met only if the laboratory environment fulfills the requirements stated in this chapter. The time spent in preparing the laboratory will contribute to the long term performance of the systems.

#### **Required knowledge**

Personnel that will install the system must have:

- A general understanding of how a PC and Microsoft<sup>™</sup> Windows<sup>™</sup> operating system work. In most cases universal computer functions will not be explained.
- An understanding of the concepts of liquid chromatography. Terminology and functions will be explained only when they differ from normal practice.
- A general understanding of risks associated with handling chemicals and substances for use in liquid chromatography.

### 3.2 Delivery and storage

#### Introduction

This section describes the requirements for receiving the delivery box and storing the instrument before installation.



#### WARNING

**Heavy object**. The ÄKTA avant instrument weighs about 116 kg. Use proper lifting equipment, or use four or more persons when moving the instrument. All lifting and moving must be performed in accordance with local regulations.

#### When you receive the delivery

- Record on the receiving documents if there is any apparent damage on the delivery box. Inform your GE Healthcare representative of such damage.
- Move the delivery box to a protected location indoors.

#### **Delivery box**

ÄKTA avant instruments are shipped in a delivery box with the following dimensions and weight:

Contents	Dimensions (mm)	Weight
ÄKTA avant instrument with ac- cessories	w1000 × h900 × d800	155 kg

#### **Storage requirements**

The delivery boxes should be stored at a protected place indoors. The following storage requirements must be fulfilled for the unopened boxes:

Parameter	Allowed range
Ambient temperature, storage	-25°C to +60°C
Relative humidity	20% to 95%, non condensing

#### **Equipment for transportation**

 Equipment
 Specifications

 Pallet mover
 Suitable for a lightweight pallet 80 × 100 cm

 Image: Control of the instrument to the lab
 Suitable for a lightweight pallet 80 × 100 cm

The following equipment is recommended for handling the delivery boxes:

### 3.3 Room requirements

#### Introduction

This section describes the requirements for the transportation route and the room where the ÄKTA avant instrument is placed.



#### **Transportation route**

Doors, corridors and elevators must have a minimum width of 65 cm to allow for transporting the instrument. Allow additional space for moving around corners.

#### **Space requirements**



The illustration below shows the space recommended for the ÄKTA avant system.

#### Allow space on the laboratory bench for:

- handling of samples and buffers (2 × 30 cm)
- computer and monitor (80 cm)
- access for service (see below)

#### Service access

To access the rear panel, the instrument can be rotated on a swivel foot. There must be at least 20 cm additional space on the bench to allow for free rotation.





#### WARNING

**Rotating the instrument.** Make sure that there is always at least 20 cm of free space around the ÄKTA avant instrument to allow for sufficient ventilation and rotation on the swivel foot. When rotating the instrument, take care not to stretch or squeeze tubing or cables. A disconnected cable may cause power interruption or network interruption. Stretched tubing may cause bottles to fall, resulting in liquid spillage and shattered glass. Squeezed tubing may cause increase in pressure, or block liquid flow. To avoid the risk of knocking over bottles, always place bottles on the buffer tray, and close the doors before rotating the instrument.

#### Laboratory bench

The bench must be clean, flat and stable to support the weight of the ÄKTA avant system, see table *Equipment weight* below.

#### **Equipment dimensions**



The outer dimensions of the ÄKTA avant instrument are shown in the illustration below.

#### **Equipment weight**

Item	Weight
ÄKTA avant instrument	116 kg
Computer	9 kg
Monitor	3 kg
Total	128 kg

### 3.4 Site environment

#### Introduction

This section describes the environmental requirements for installation of ÄKTA avant.

#### **Room climate**

The following requirements must be fulfilled:

- The room must have exhaust ventilation.
- The instrument should not be exposed to direct sunlight.
- Dust in the atmosphere should be kept to a minimum.

Allowed temperature and humidity ranges are specified in the table below.

Parameter	Allowed range
Ambient temperature, operating	4°C to 35°C
Ambient temperature, storage	-25°C to +60°C
Relative humidity, operating	20% to 95%, non condensing

#### Heat output

The heat output data is listed in the table below.

Component	Heat output
ÄKTA avant instrument	800 W
Computer, incl. monitor and printer	300 W
Total	1100 W

### 3.5 Power requirements

#### Introduction

This section describes the power supply requirements for ÄKTA avant.



#### WARNING

**Protective ground.** The ÄKTA avant instrument must always be connected to a grounded power outlet.



#### WARNING

Only use grounded power cords delivered or approved by GE Healthcare.



#### WARNING

Do not block access to the power switch and power cord. The power switch must always be easy to access. The power cord with plug must always be easy to disconnect.



#### WARNING

**Supply voltage.** Make sure that the supply voltage at the wall outlet corresponds to the marking on the instrument, before connecting the power cord.

#### Requirements

The table below specifies the power requirements.

Parameter	Requirement
Supply voltage	100-240 V ~
Frequency	50-60 Hz
Max power consump- tion	800 VA

Parameter	Requirement
Number of sockets	1 socket per instrument, up to 3 sockets for computer equipment
Type of sockets	EU or US plugs. Grounded mains sockets, fused or protect- ed by equivalent circuit breaker.
Location of sockets	Maximum 2 m from the instrument (due to length of mains cable). Extension cables can be used if required.

#### Quality of power

The mains power supply must be stable and conform to specifications at all times to ensure reliable operation of ÄKTA avant. There should be no transient or slow changes in average voltage outside the limits specified above.

### 3.6 Computer requirements

#### Introduction

ÄKTA avant systems are controlled by UNICORN software running on a PC. The PC can be part of the delivery or be supplied locally.

The PC used must fulfill the recommendations stated in this section.

#### **Computer specifications**

Refer to UNICORN Administration and Technical Manual for computer specifications.

### 3.7 Required materials

#### Introduction

This section describes the accessories required for the installation and operation of ÄKTA avant.

#### **Buffers and solutions**

The buffers and solutions listed below are required during the installation procedure and should be provided at the installation site.

Buffer/solution	Required volume	Scope of use
Distilled water	1 liter	Air sensor test, Fraction collector test, Q valve test, System test
1% acetone in distilled water	0.5 liter	Q valve test
1% acetone and 1 M NaCl in distilled water	0.5 liter	System test
20% ethanol	200 ml	Priming of the pump piston rinsing system

#### Laboratory equipment

The equipment listed below is required during the installation procedure and should be provided at the installation site.

Equipment	Specification
Flasks, liquid containers	For buffers and waste
Gloves	For protection
Protective glasses	For protection

#### **Fraction collector tubes**

Tube size [ml]	Diameter [mm]		Height (mm)		Max.	Examples of
	Min.	Max.	Min.	Max.	volume [ml]	manufacturers
3	10.5	11.5	50	56	3	NUNC
8	12	13.3	96	102	8	BD Biosciences, VWR
15	16	17	114	120	15	BD Biosciences
50	28	30	110	116	50	BD Biosciences

The tubes used in the Fraction collector of ÄKTA avant must fulfill the requirements listed in the table below. Examples of manufacturers are also listed in the table.

#### **Deep well plates**

#### Requirements

The deep well plates used in the Fraction collector of ÄKTA avant must fulfill the requirements listed in the table below.

Property	Specification
No. of wells	24, 48, or 96
Shape of wells	Square, not cylindrical
Well volume	10, 5, or 2 ml

#### Approved deep well plates

The plates listed in the table below are tested and approved by GE Healthcare to be used with ÄKTA avant.

Plate type	Manufacturer	Part no.
96 deep well plate	Eppendorf™	951033405/0030501.306
	BD Biosciences	353966
	Greiner Bio-One	780270
	Porvair Sciences	219009
	Seahorse Bioscience™	S30009
	Whatman™	7701-5200
48 deep well plate	Seahorse Bioscience	S30004
	Whatman	7701-5500
24 deep well plate	Seahorse Bioscience	S30024
	Whatman	7701-5102

## 4 Hardware installation

#### About this chapter

This chapter describes the installation procedure of an ÄKTA avant system.

#### In this chapter

This chapter contains the following sections:

Section	See page
4.1 Unpack the instrument	36
4.2 Install the computer equipment	46
4.3 Connect system units	47
4.4 Install the instrument	50

### 4.1 Unpack the instrument

#### Introduction

This section describes how to unpack the ÄKTA avant instrument, and how to lift the instrument onto the bench.



#### WARNING

**Heavy object**. The ÄKTA avant instrument weighs about 116 kg. Use proper lifting equipment, or use four or more persons when moving the instrument. All lifting and moving must be performed in accordance with local regulations.
## Lift the instrument onto the bench using an overhead crane

It is recommended to use an overhead crane when lifting the ÄKTA avant instrument. Follow the instruction below to lift the instrument onto the bench using an overhead crane and to remove the transport fixations. If an overhead crane is not available, see *Lift the instrument onto the bench by hand, on page 38.* 

#### Step Action

1 Lift off the cardboard hood.



- 2 Make sure that the safety hooks of the straps are properly connected to the lifting yokes located in each of the four corners of the plywood board under the instrument.
- 3 Connect both straps to the hook of the overhead crane.



5 Disconnect the safety hooks of the straps from the lifting yokes of the plywood board.

## Lift the instrument onto the bench by hand

If an overhead crane is not available, lift off the cardboard hood and disconnect the safety hooks of the straps from the lifting yokes of the plywood board under the instrument. Lift the instrument by hand using the handles illustrated in steps 6-7 in instruction *Remove transport fixations, on page 39.* 

#### **Remove transport fixations**

Follow the instruction below to remove the transport fixations from the instrument.

Step	Action
1	Lift off the plywood board from the top of the instrument.
2	Remove the pieces of foam from around the instrument.
3	Check the contents in the Buffer tray according to the illustration <i>Accessories packages, on page 42,</i> and lift off the packages from the tray.

4 Unscrew the screws from the Buffer tray securing plate, and remove the plate. Fasten the screws back into place.



#### Step Action

5

Remove the pieces of adhesive tape placed on the locations marked in the illustrations below.







6

Open the pump cover, and lift off the tray on the wet side and the tray on the front of the instrument to access the instrument handles.



#### Step Action

7 Grip the handles on the front and wet side of the instrument. On the back and left-hand side, grip the metal plates located at the bottom of the instrument.





8

#### Step Action

Lift the instrument, and at the same time pull away the plywood board from under the instrument.



#### **Accessories packages**

The illustration below shows the accessories packages placed in the Buffer tray at delivery.



Part	Description
1	Cassette tray
2	Manual box
3	Fraction collector cassettes
4	pH electrode
5	Accessories box

## Remove transport fixations from inside the Fraction collector

Follow the instruction below to remove the transport fixations from inside the Fraction collector.

#### Step Action

1 Pull out the deep well plates, the Waste funnel and the Button cover from the holes in the piece of foam located in the Frac chamber.



2

Fold forward the vertical section of the foam, and fold over the right edge of the foam.

5



- 4 Pull out the F-shaped piece of foam that holds the Frac arm in position during transportation.
  - Attach the Waste funnel in position inside the Frac chamber.





#### 4.2 Install the computer equipment

#### Introduction

The computer is either:

- Supplied as a part of the ÄKTA avant delivery
- Supplied locally

#### Unpacking and installing

Unpack and install the computer according to the manufacturer's instructions.

#### 4.3 Connect system units

#### Introduction

The following interconnections must be made:

- Power supply to the ÄKTA avant instrument
- Power supply to the computer equipment
- Network connection between the computer and the ÄKTA avant instrument.



#### WARNING

Only use grounded power cords delivered or approved by GE Healthcare.



#### WARNING

**Supply voltage.** Make sure that the supply voltage at the wall outlet corresponds to the marking on the instrument, before connecting the power cord.

## Connect power to the ÄKTA avant instrument

Follow the instruction below to connect power to the ÄKTA avant instrument.

Step	Action
1	Select the correct power cord to be used. Each instrument is delivered with 2 alternative power cords:
	• Power cord with US-plug, 2 m
	• Power cord with EU-plug, 2 m
	Discard the unused power cord.
2	Connect the power cord to the <b>Power</b> input connector on the back of the instrument and to a grounded wall outlet $100-240 V \sim$ , $50/60 Hz$ .

StepAction3Attach the power cord to the rear of the instrument using the cable clip.



## Connect power to computer equipment

Follow the manufacturer's instructions to connect power to the:

- computer
- monitor
- local printer, if used

#### **Connect to network**

Follow the instructions below to make network connections.

Step	Action
1	Connect a network cable between the network connector (Ethernet) on the back of the instrument and the computer network card marked for ÄKTA.
	The illustration below shows the symbol of the Ethernet connector.
	머미

# Step Action 2 If the computer is to be connected to an external network, connect a network cable between the main network card of the computer and a network wall outlet. Note: If the computer has not been supplied by GE Healthcare and if network configuration is to be used, see Administration and Technical Manual for further information on network settings.

#### 4.4 Install the instrument

#### Introduction

This section describes how to install the Barcode scanner and the pH electrode, and how to prime the pump piston rinsing systems.

#### Install the 2D Barcode scanner

Connect the cable of the 2D Barcode scanner to the scanner and to a USB port on the computer.

#### Install the pH electrode

If pH monitoring is to be used, you need to replace the dummy electrode mounted at delivery with a pH electrode.



#### CAUTION

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

Follow the instruction below to install the pH electrode.

Step	Action
1	Unpack the pH electrode. Make sure that the electrode is not broken or dry.
2	Unscrew the dummy electrode from the flow cell.
3	Pull off the plug from the connector on the front of the pH valve, and store the plug together with the dummy electrode.
4	Remove the cover from the tip of the pH electrode.
5	Carefully insert the electrode in the flow cell. Tighten the locking ring by hand to secure the electrode.
6	Connect the pH electrode cable to the connector on front of the pH valve.

## Illustration of the pump piston rinsing systems

The illustration below shows the tubing configuration of the pump piston rinsing systems.

Part	Description
1	Inlet tubing to the Sample pump piston rinsing system
2	Outlet tubing from the Sample pump piston rinsing system
3	Inlet tubing to the System pump piston rinsing system
4	Outlet tubing from the System pump piston rinsing system

0-

## Prime the pump piston rinsing system

Follow the instruction below to fill the pump piston rinsing systems with rinsing solution. See the tubing configuration of the rinsing systems in *Illustration of the pump piston rinsing systems, on page 51.* 

#### Step Action

1

Unscrew the rinsing system tubes from the holders.



- 2 Fill each of the rinsing system tubes with 50 ml of 20% ethanol.
- 3 Screw the rinsing solution tubes back in the holders.
- 4 Immerse the inlet tubing to the System pump piston rinsing system in one of the rinsing solution tubes.

#### Note:

Make sure that the inlet tubing reaches to the very bottom of the rinsing solution tube.

5 Immerse the inlet tubing to the Sample pump piston rinsing system in the other rinsing solution tube.

#### Note:

Make sure that the inlet tubing reaches to the very bottom of the rinsing solution tube.

#### Step Action

6

Connect a 25-30 ml syringe to the outlet tubing of the System pump piston rinsing system. Draw liquid slowly into the syringe.



- 7 Disconnect the syringe and discard its contents.
- 8 Immerse the outlet tubing in the rinsing solution tube where the inlet tubing of the System pump piston rinsing system is immersed.
- 9 Connect a 25-30 ml syringe to the outlet tubing from the Sample pump piston rinsing system. Draw liquid slowly into the syringe.
- 10 Disconnect the syringe and discard its contents.
- 11 Immerse the outlet tubing in the rinsing solution tube where the inlet tubing of the Sample pump piston rinsing system is immersed.
- 12 Fill the rinsing solution tubes so that each of the tubes contains 50 ml of 20% ethanol.

#### Location of waste tubing

All waste tubing is found on the rear of the instrument, see the illustration below.



Part	Description
1	Waste tubing from the Injection valve, the pH valve and the Outlet valve (W, W1, W2 and W3).
2	Waste tubing from the Fraction collector and the Buffer tray.

#### Prepare the waste tubing

Follow the instruction below to prepare the waste tubing.

#### Step Action

1

2

Place the four pieces of waste tubing from the Injection valve, the pH valve and the Outlet valve (**W**, **W1**, **W2** and **W3**) in a vessel placed below the bench.



#### NOTICE

The maximum level of the waste vessel for the waste tubing from the valves must be lower than 30 cm above the lab bench.

Place the three pieces of waste tubing from the Fraction collector and the Buffer tray in a waste vessel placed below the bench.



#### NOTICE

The maximum level of the waste vessel for the waste tubing from the Fraction collector and the Buffer tray must be lower than the bench height.

- 3
- Cut the waste tubing from the Fraction collector and the Buffer tray to appropriate length. It is important that the tubing is not bent and will not be submerged in liquid during the run.



#### Note:

If the tubing is too short, replace it with new tubing. Do not lengthen the tubing as this might cause obstruction of the tubing and flooding in the Frac chamber.



#### CAUTION

Make sure that the waste vessels will hold all the produced volume of the run. For ÄKTA avant 25, a suitable waste vessel should typically have a volume of 2 to 10 liters. For ÄKTA avant 150, a waste vessel should have a volume of 40 liters.

## 5 Start the instrument and the computer

#### Introduction

This section describes how to start the instrument and the computer.

#### Instruction

Follow the instructions below to start the instrument and the computer.

#### Step Action





*Result*: The instrument starts and the Instrument display states **Not connect-ed**.

2 Turn on the computer and monitor according to the manufacturer's instructions.

## 6 Software installation

#### Introduction

This section gives an overview of the different UNICORN installation types.

Detailed information about software installation and configuration is available in the UNICORN Administration and Technical Manual.

#### Software installations

UNICORN can be installed as listed below:

- a complete UNICORN installation on a stand-alone workstation (Full installation)
- a UNICORN database and license server (Custom installation) and
- UNICORN software client and instrument server software on a network client station (Custom installation).

It is also possible to:

- define a system as part of the installation
- configure E-licenses
- configure Windows settings necessary for the UNICORN process pictures in a network deployment
- configure firewall settings, when necessary
- upgrade UNICORN
- remove UNICORN installations and
- set up a system printer.

## 7 Start UNICORN and connect to system

#### Introduction

This section describes how to start and  $\log$  on to UNICORN and how to connect the instrument to UNICORN.

#### Start UNICORN and log on

Follow the instructions below to start UNICORN and log on to the program.

Step	Action
1	Double-click the UNICORN icon on the desktop.
	<i>Result</i> : The <i>Log On</i> dialog opens.
	Note:

If there is no connection to the database it is still possible to log on to UNICORN and control a running system. The **Log On** dialog will give the option to start **System Control** without a database. Click **Start System Control** to proceed to the next **Log On** dialog.

## Step Action 2 In the Log On dialog: • select User Name.

-----

• enter **Password**.

#### Note:

It is also possible to select the **Use Windows Authentication** checkbox and enter a network ID in the **User Name** field.

利 Log On - I	JNICORN 🔀
🔲 Use <u>W</u> ind	ows Authentication
<u>U</u> ser Name:	Eric
<u>P</u> assword:	XXXXXXXX
<u>D</u> omain:	<b></b>
Access Group:	Administrators 💌
۲	<u> <u> D</u>K <u> C</u>ancel <u> Dptions</u> &gt;&gt;</u>

• click the **Options** button, and select which UNICORN modules to start.

Start:	VNICORN Administration	System Control
	Method Editor	Evaluation

• click OK.

Result: The selected UNICORN modules open.



#### **Connect to system**

Follow the instructions below to connect the instrument to UNICORN.

Ste	р	Α	cti	on	

1

2

In the System Control module, click the Connect to Systems icon.



Result: The Connect to Systems dialog opens.

Connect to Systems		
Connected systems (1 selected, max 3)		
System name	Control	View
🔲 🔳 System1		۲
🔲 📕 System2		۲
🔣 🔳 System3	۲	0
🛗 🔳 System4		
🔲 📕 System 5		
🔲 📕 System6		۲
🔲 🔳 System7		۲
Connected Users	ОК	Cancel

In the Connect to Systems dialog:

- Select a system.
- Select Control mode.
- Click OK.

Result: The selected instrument can now be controlled by the software.

## 8 Prime inlets and purge pumps

#### About this chapter

Before starting the performance tests described in *Chapter 9 Performance tests, on page 81* it is important to:

- 1 Prime the inlets (fill the inlets with liquid)
- 2 Purge the pumps (remove air from the pumps)

This chapter describes how to prime the buffer inlets, sample inlets, and Q inlets, and how to purge the System pumps and the Sample pump.

#### In this chapter

This chapter contains the following sections:

Section	See page
8.1 Prime buffer inlets and purge System pump A and System pump B	63
8.2 Prime sample inlets and purge the Sample pump	71
8.3 Prime Q inlets and purge System pump A and System pump B	76

## 8.1 Prime buffer inlets and purge System pump A and System pump B

#### Overview

The procedure consists of the following stages:

- 1 Prime all inlet tubing B to be used during the run
- 2 Prime all inlet tubing A to be used during the run
- 3 Prepare the system before purging the System pumps
- 4 Purge System pump B
- 5 Purge System pump A
- 6 End the run

#### Prime inlet tubing B

Follow the instructions below to fill all B inlet tubing to be used in the run with appropriate buffer/solution.

Step	Action
1	Make sure that all B inlet tubing that is to be used during the method run is immersed in the correct buffers.

8.1 Prime buffer inlets and purge System pump A and System pump B

### Step Action

- 2 In the *Manual instructions* dialog:
  - Select *Flow path:Inlet B*.
  - Select the *Position* of the inlet to be filled from the drop-down list. Start at the inlet position with the highest number and end at the inlet position with the lowest number.

Manual instructions - System3		
Instructions:	Selected column type:	Select
Pumps and pressures     Flow path     Injection valve     Column position     Inlet A     pH valve     Sample inlet     Outlet valve     Injection mark     Monitors     Fraction collection	Parameters for Inlet 8 Position B1 V	~
Save result as:		Browse
Auto update of parameters duri	ing run	

Click Execute

*Result:* Inlet valve B switches to the selected port.

3

Connect a 25-30 ml syringe to the purge valve of one of the pump heads of System pump B. Make sure that the syringe fits tightly into the purge connector.



4 Open the purge valve by turning it counter-clockwise about 3 quarters of a turn. Draw liquid slowly into the syringe until the liquid reaches the pump.

Step	Action
5	Close the purge valve by turning it clockwise. Disconnect the syringe and discard its contents.
6	Repeat steps 2-5 for each piece of inlet tubing B that is to be used during the run.

#### Prime inlet tubing A

Follow the instruction below to fill all A inlet tubing, to be used in the run, with appropriate buffer/solution.

Step	Action			
1	Make sure that all A inlet tubing that is to be used during the method run is immersed in the correct buffers.			
2	In the <b>Manual instructions</b> dialog:			
	• Select <i>Flow path:Inlet A</i> .			
	• Select the <i>Position</i> of the inlet to be filled from the drop-down list.			
	Click Execute			
	Result: Inlet valve A switches to the selected port.			
3	Connect a 25-30 ml syringe to the purge valve of one of the pump heads of System pump A. Make sure that the syringe fits tightly into the purge con- nector.			
4	Open the purge valve by turning it counter-clockwise about 3 quarters of a turn. Draw liquid slowly into the syringe until the liquid reaches the pump.			
5	Close the purge valve by turning it clockwise. Disconnect the syringe and discard its contents.			
6	Repeat steps 2-5 for each piece of inlet tubing A that is to be used during the run.			

## Prepare the system before purging the System pump heads

Follow the instructions below to prepare the system.

Step	Action
1	Make sure that the piece of waste tubing connected to Injection valve port <b>W1</b> is placed in a waste vessel.
2	Open the System Control module and select Manual:Execute Manual In- structions.
	Result: The Manual instructions dialog opens.
3	In the Manual instructions dialog:
	<ul> <li>Select <i>Flow path:Injection valve</i> and select <i>System pump waste</i> from the <i>Position</i> drop-down list. Click Execute.</li> <li><i>Result</i>: The Injection valve switches to waste position. This is necessary to achieve a low backpressure during the purge procedure.</li> <li>Select <i>Pumps and pressures:System flow</i>. For ÄKTA avant 25, set the <i>Flow rate</i> to 1.0 ml/min. For ÄKTA avant 150, set the <i>Flow rate</i> to 10.0 ml/min. Click Execute.</li> <li><i>Result</i>: A system flow starts.</li> </ul>

#### Purge System pump B

Follow the instruction below to purge both pump heads of System pump B.

Step	Actio	in			
1	• S	e <b>Manual instructions</b> elect <b>Pumps and press</b> et <b>Target</b> to 100% B ar	ures:Gradient.		
		Annual instructions - System3 Instructions:  Pumps and pressures System flow Sample flow Gradient Pump wash System wash Quaternary start concentrations Quaternary gradient BufferPro pH Column packing flow	Selected column type:	[0.0 - 100.0] 100.0 ≩ %8 [0.00 - 100000.0] 0.00 ≩ min	Select

~

۷	Auto update of parameters during run

Click Execute

Monitors

Save result as:

*Result:* Only System pump B is active.

- 2 In the *Manual instructions* dialog:
  - Select Flow path:Inlet B.
  - Select the *Position* of one of the inlets to be used from the drop-down list.

Instructions: E Pumps and pressures	~	Selected column type: Parameters for Inlet B Position	Select.
☐ Flow path Injection valve Column position Inlet & Phi valve Sample inlet Outlet valve Injection mark I Fraction collection		B1	~
Save result as:	<u>×</u>		Browse
Auto update of parameter	s during rur	1	

Result: Inlet valve B switches to the selected port.

Browse.

8.1 Prime buffer inlets and purge System pump A and System pump B





- 4 Open the purge valve by turning it counter-clockwise about 3 quarters of a turn. Draw a small volume of liquid slowly into the syringe (with a rate of about 1 ml per second).
- 5 Close the purge valve by turning it clockwise. Disconnect the syringe and discard its contents.
- 6 Connect the syringe to the purge valve on the right pump head of System pump B, and repeat steps 4 and 5. Keep the system flow running.



#### Purge System pump A

Follow the instructions below to purge both pump heads of System pump A.

Step	Action
1	In the <b>Manual instructions</b> dialog:
	Select Pumps and pressures:Gradient.
	• Set <i>Target</i> to 0% B and <i>Length</i> to 0 min.
	Click Execute
	Result: Only System pump A is active.
2	In the <b>Manual instructions</b> dialog:
	• Select <i>Flow path:Inlet A</i> .
	• Select the <i>Position</i> of one of the inlets to be used from the drop-down list.
	Click Execute

Result: Inlet valve A switches to the selected port.

3 Connect a 25-30 ml syringe to the purge valve of the left pump head of System pump A. Make sure that the syringe fits tightly into the purge connector.



4

Open the purge valve by turning it counter-clockwise about 3 quarters of a turn. Draw a small volume of liquid slowly into the syringe (with a rate of about 1 ml per second).

8.1 Prime buffer inlets and purge System pump A and System pump B

Step	Action
5	Close the purge valve by turning it clockwise. Disconnect the syringe and discard its contents.
6	Connect the syringe to the purge valve on the right pump head of System pump A, and repeat steps 5-6. Keep the system flow running.

#### End the run

Click the *End* icon in the *System Control* toolbar to end the run.



#### 8.2 Prime sample inlets and purge the Sample pump

#### **Overview**

The procedure consists of the following steps:

- 1 Prime all sample inlet tubing to be used during the run
- 2 Prepare the system before purging the Sample pump
- 3 Purge the Sample pump
- 4 End the run

#### Prime sample inlet

Follow the instructions below to fill all sample inlet tubing, to be used in the run, with appropriate buffer/solution.

Step	Action
1	Make sure that all sample inlet tubing that is to be used during the method
	run is immersed in the correct buffers.

- 2 In the **Manual instructions** dialog:
  - Select Flow path:Sample inlet.
  - Select the **Position** of the inlet to be filled from the drop-down list.



Click Execute

*Result:* The Sample valve switches to the selected port.

8.2 Prime sample inlets and purge the Sample pump





4	Open the purge valve by turning it counter-clockwise about 3 quarters of a turn. Draw liquid slowly into the syringe until the liquid reaches the pump.
5	Close the purge valve by turning it clockwise. Disconnect the syringe and discard its contents.
6	Repeat steps 2-5 for each sample inlet that is to be used in the method run.

## Prepare the system before purging the Sample pump

Follow the instruction below to prepare the system.

Step	Action
1	Make sure that the piece of waste tubing connected to Injection valve port <b>W1</b> is placed in a waste vessel.
2	Open the System Control module, and select Manual:Execute Manual In- structions.
	Result: The Manual instructions dialog opens.
Step	Action
------	---
3	In the <b>Manual instructions</b> dialog:
	<ul> <li>Select <i>Flow path:Injection valve</i> and select <i>Sample pump waste</i> from the <i>Position</i> drop-down list. Click <a href="#">Execute</a>.</li> <li><i>Result:</i> The Injection valve switches to waste position. This is necessary to achieve a low backpressure during the purge procedure.</li> </ul>
	<ul> <li>Select <i>Pumps and pressures:Sample flow</i>. For ÄKTA avant 25, set the <i>Flow rate</i> to 1.0 ml/min. For ÄKTA avant 150, set the <i>Flow rate</i> to 10.0 ml/min. Click Execute.</li> <li><i>Result</i>: A sample flow starts.</li> </ul>

#### Purge the Sample pump

Follow the instruction below to purge both the pump heads of the Sample pump.

Step	Action
1	In the <b>Manual instructions</b> dialog:
	Select Flow path:Sample inlet.
	• Select <i>Buffer</i> from the <i>Position</i> drop-down list.
	• Click Execute.
	<i>Result</i> : The Sample inlet valve switches to <i>Buffer</i> port.
2	Connect a 25-30 ml syringe to the purge valve of the left pump head of the Sample pump. Make sure that the syringe fits tightly into the purge connector.

5

8.2 Prime sample inlets and purge the Sample pump

#### Step Action

3 Open the purge valve by turning it counter-clockwise about 3 quarters of a turn. Draw a small volume of liquid slowly into the syringe (with a rate of about 1 ml per second).



- 4 Close the purge valve by turning it clockwise. Disconnect the syringe and discard its contents.
  - Connect the syringe to the purge valve on the right pump head of the Sample pump, and repeat steps 2-3. Keep the sample flow running.



#### End the run

Click the *End* icon in the *System Control* toolbar to end the run.



#### 8.3 Prime Q inlets and purge System pump A and System pump B

# 8.3 Prime Q inlets and purge System pump A and System pump B

#### Overview

The procedure consists of the following steps:

- 1 Prepare the system before priming the Q inlets and purging the System pumps
- 2 Prime all Q inlet tubing
- 3 Purge the Quaternary valve and the System pumps
- 4 End the run

#### Prepare the system before priming the Q inlets and purging the System pumps

Note:	Inlet <b>A1</b> and <b>B1</b> must be immersed in buffer or water. When the System pumps are synchronized, the Inlet valves are positioned to <b>A1</b> and <b>B1</b> for a short moment.		
Step	Action		
1	Make sure that the piece of waste tubing connected to Injection valve port <b>W1</b> of the Injection valve is placed in a waste vessel.		
2	Open the System Control module, and select Manual:Execute Manual In- structions.		
	Result: The Manual instructions dialog opens.		
3	In the <b>Manual instructions</b> dialog:		
	<ul> <li>Select <i>Flow path:Injection valve</i> and</li> </ul>		
	choose <i>System pump waste</i> from the <i>Position</i> drop-down list.		
	Click Execute		
	Result: The injection valve switches to the waste position.		

#### Note:

This is necessary in order to achieve a low backpressure during the purge procedure.

Step	Action
4	Select Pumps and pressures:System flow:
	For ÄKTA avant 25:
	Set the <i>Flow rate</i> to 1.0 ml/min.
	For ÄKTA avant 150:
	Set the <i>Flow rate</i> to 10.0 ml/min.
	Click Execute
	<i>Result:</i> A system flow starts.

#### **Prime the Q inlets**

Follow the instruction below to prime the Q inlets.

Step	Action
1	Make sure that the pieces of inlet tubing marked <b>A1</b> , <b>B1</b> and <b>Q1-Q4</b> are immersed in the correct buffers.

- 2 In the *Manual instructions* dialog:
  - Select Pumps and pressures:Quaternary start concentrations.
  - Set *Start concentration Q1* to 100%. Make sure that the other start concentrations are set to 0%.

Manual instructions - System3		
Instructions: Pumps and pressures System flow Sample flow Gradient Pump wash System wash Quaternay stat concentrations Quaternay gradient BufferPo pH Column packing flow Flow path Monitors	Selected column type: Parameters for Quaternary start concentrations Start concentration Q1 [0.0 · 100.0] 100.0 $\stackrel{6}{=}$ % Start concentration Q2 [0.0 · 100.0] 0.0 $\stackrel{6}{=}$ % Start concentration Q3 [0.0 · 100.0] 0.0 $\stackrel{6}{=}$ %	Select
Save result as:	ing run	Browse

Click Execute

3

8.3 Prime Q inlets and purge System pump A and System pump B

Step Action

Connect a 25-30 ml syringe to one of the purge valves of either of the system pumps. Make sure that the syringe fits tightly into the purge connector.



- 4 Open the purge valve by turning it counterclockwise about 3 quarters of a turn. Draw 10 ml of liquid into the syringe. Check that the **Q1** inlet is filled with liquid.
- 5 Close the purge valve by turning it clockwise. Disconnect the syringe and discard its contents.
- 6 Repeat steps 2-5 for Q2, Q3 and Q4 respectively by setting the respective *Quaternary start concentration* to 100%.

#### Tip:

The piece of inlet tubing that is immersed in distilled water should be the last piece of inlet tubing to be primed.

# Purge the Quaternary valve and the System pumps

Follow the instruction below to purge the Quaternary valve, System pump A and System pump B. Note that both pump heads of each System pump have to be purged.

Step	Action
SIED	ACTION

•

- 1 In the *Manual instructions* dialog:
  - Select *Pumps and pressures:Pump wash*, and select *All* from the *BufferPro / Q inlets* drop-down list.

nstructions:		column type:	Select
Pumps and pressures System flow Sample flow Gradient Pump wash System wash Quaternay start concentrations Quaternay gradient Butfer/Pto pH Column packing flow Flow path Monitors	<ul> <li>Paramet</li> <li>Variante</li> </ul>	Inlet A Inlet A Off Inlet B Off BufferPro / Q inlets Al Sample inlet Off Off	•
Save result as:	ing run		Browse

*Result:* A simultaneous pump wash of all the Q inlets is started. This will remove air from the Quaternary valve.

2 Wait until the pump wash is completed.

3

8.3 Prime Q inlets and purge System pump A and System pump B

Step Action

Connect a 25-30 ml syringe to the left purge valve of the selected System pump. Make sure that the syringe fits tightly into the purge connector.



- 4 Open the purge valve by turning it counterclockwise about 3 quarters of a turn. Draw 10 ml of liquid slowly into the syringe with a rate of about 1 ml per second.
- 5 Close the purge valve by turning it clockwise. Disconnect the syringe and discard its contents.
- 6 Repeat steps 3-5 for the other three purge valves of the System pumps to get rid of air in all pump heads. Keep the system flow running.

#### End the run

Click the *End* icon in the *System Control* toolbar to end the run.



## 9 Performance tests

#### About this chapter

Performance tests should be run after installation to check the function of the ÄKTA avant system. Performance tests can also be used at any time to check the condition of the system, for example, after a prolonged stop. This chapter describes how to prepare, run, and evaluate the four performance tests available.

#### In this chapter

This chapter contains the following sections:

Section	See page
9.1 Air sensor test	82
9.2 Fraction collector test	85
9.3 Q valve test	90
9.4 System test	96
9.5 Test protocol for Q valve test	107
9.6 Test protocol for System test	109

## 9.1 Air sensor test

#### **Method description**

The Air sensors A, B, and S are integrated in Inlet valve A, Inlet valve B, and Sample inlet valve. The Air sensor test checks if the air sensors detect air. The method run takes approximately 5 minutes.

#### **Required material**

The following material is required:

- Syringe, 25-30 ml
- Distilled water

#### Prepare the test

Follow the instruction below to prepare the system before method start.

Step	Action
1	Immerse the pieces of inlet tubing marked <b>A1</b> , <b>B1</b> , and the piece of sample inlet tubing marked <b>Buff</b> in distilled water.
2	Prime the inlets <b>A1</b> , <b>B1</b> , and the sample inlet <b>Buff</b> , and purge the pumps. See Section 8.1 Prime buffer inlets and purge System pump A and System pump B, on page 63 and Section 8.2 Prime sample inlets and purge the Sample pump, on page 71.
3	Disconnect the inlet tubing connected to the inlet valve positions <b>A4</b> , <b>B4</b> , and <b>S4</b> . During the test method air is introduced into the inlet valves through these inlet ports to test the function of the air sensors.

#### Start the test

Follow the instruction below to start the Air sensor test.

Step	Action
1	In the System Control module, select System:Performance Test and Report.
	Result: The System Performance Test and Report dialog opens.

Step	Action
2	In the System Performance Test and Report dialog:
	Select <i>Air Sensor Test</i>
	Click the <i>Run Performance Method</i> button
	Result: The Start Protocol of the Air sensor test opens.
3	Click <b>Next</b> in the dialogs of the <b>Start Protocol</b> to open the next dialog. The dialogs are described in table <i>Overview of the Start Protocol dialogs, on page</i> 83.
4	In the last dialog of the <b>Start Protocol, Result name and location</b> , click <b>Finish</b> .
	Result: The Air sensor test starts.

# Overview of the Start Protocol dialogs

The table below describes the dialogs of the Start Protocol.

Dialog	Description
Notes	Displays the <i>Method Notes</i> of the method. The <i>Method Notes</i> contains a method description and instructions on how to run the method. This dialog also allows the user to enter <i>Start Notes</i> .
Evaluation Procedures	Allows the user to select to save the report to file (recommended) and/or to print the report.
Result Name and Loca- tion	Allows the user to change result name and result location.

#### During the run

A *Message* dialog opens during the run. Read the messages in the dialog, and make sure that necessary preparations have been performed. Click the *Confirm and Continue* button in the *Message* dialog to change system state from *System Pause* to *Run* and proceed with the test. Alternatively, click the *Confirm* button in the *Message* dialog and click the *Continue* button on the Instrument display.

#### Automatic evaluation

The system automatically generates a report when the test is finished. The report can be printed in two ways:

If the option Save the report to file was selected in the Evaluation Procedures dialog
in the Start Protocol of the test, the report is saved in location C:\Program
Files\GE Healthcare\UNICORN\Temp. Open the report and print it using
your default Windows printer. (Recommended)

**Note:** The exact search path depends on the location of the UNICORN installation folder.

• If the option *Print report* was selected in the *Evaluation Procedures* dialog in the *Start Protocol* of the test, the report is automatically printed on the system printer. Note that a system printer must have been set up, see *UNICORN Administration and Technical Manual*.

Print the report and check the status of the tests. For each of the tests the report states "The test passed" or "The test failed".

#### Possible causes of a failed test

The table below describes possible causes of a failed test. When possible sources of error have been checked and taken care of, run the test once again.

Cause	Action
Faulty air sensor	For further information, see User Manual, chapter Troubleshooting.
Incorrect preparation of tubing	Make sure that the tubing was correctly prepared, see <i>Prepare the test, on page 82.</i>

## 9.2 Fraction collector test

#### **Method description**

The Fraction collector test checks the functionality of the Fraction collector. The method run takes approximately 10 minutes. For ÄKTA avant 25, fractionation of 2 ml is performed in three sequential wells in each of two 96 deep well plates. For ÄKTA avant 150, fractionation of 20 ml is performed in three sequential tubes placed in each of two Cassettes for 50 ml tubes.

#### **Required material**

The following material is required:

- Distilled water
- Syringe, 25-30 ml
- For ÄKTA avant 25:
  - Cassettes for deep well plate, 2 pcs
  - 96 deep well plates, 2 pcs
- For ÄKTA avant 150:
  - Cassettes for 50 ml tubes, 2 pcs
  - 50 ml tubes, 12 pcs

#### **Prepare the test**

Follow the instruction below to prepare the system before method start.

Step	Action
1	Immerse the piece of inlet tubing marked <b>A1</b> in distilled water.
2	Prime inlet <b>A1</b> and purge System pump A. See Section 8.1 Prime buffer inlets and purge System pump A and System pump B, on page 63.
3	Place the deep well plates or tubes in the cassettes. Make sure that the deep well plates are rotated so that the well marked <b>A1</b> is positioned above the <b>A1</b> marking on the Cassette.

5

# Step Action 4 Place the Cassettes on the Cassette tray, one on Cassette position 1 and one on Cassette position 6. Make sure that the Cassette type codes (see illustration below) faces the front of the tray marked with the GE logo. Image: Comparison of the tray of the tray marked with the GE logo.

Open the Frac drawer by pressing the handle upwards, and pulling out the drawer.





- 7 Close the Frac drawer. Make sure that it snaps into closed position.
- 8 In the *System Control* module, select *View:Fraction collection content*. In the *Fraction collector content* dialog, check that the automatic scanning procedures have been performed correctly.
- 9 In the **System Control** module, select **System:Settings**. *Result:* The **System Settings** dialog opens.
- 10 In the **System Settings** dialog:
  - Select Fraction collector:Fractionation settings.
  - For ÄKTA avant 25: In the *Fractionation mode* drop-down list, select *Accumulator*.
  - In the *Fractionation order* drop-down list, select *Row-by-row*.
  - Click OK.

#### Start the test

Follow the instruction below to start the Fraction collector test.

Step	Action
1	In the <b>System Control</b> module, select <b>System:Performance Test and Report</b> . <i>Result</i> : The <b>System Performance Test and Report</b> dialog opens.
2	In the System Performance Test and Report dialog:
	Select Fraction Collector Test
	Click the <i>Run Performance Method</i> button
	Result: The Start Protocol of the Fraction collector test opens.
3	Click <b>Next</b> in the dialogs of the <b>Start Protocol</b> to open the next dialog. The dialogs are described in table <i>Overview of the Start Protocol dialogs, on page</i> 88.
4	In the last dialog of the <b>Start Protocol</b> , <b>Result name and location</b> , click <b>Finish</b> .
	Result: The Fraction collector test starts.

# Overview of the Start Protocol dialogs

The table below describes the dialogs of the Start Protocol.

Dialog	Description
Notes	Displays the <i>Method Notes</i> of the method. The <i>Method Notes</i> contains a method description and instructions on how to run the method. This dialog also allows the user to enter <i>Start Notes</i> .
Result Name and Loca- tion	Allows the user to change result name and result location.

#### **During the run**

Check that an accumulator wash is performed at the beginning of the run. During the accumulator wash the Dispenser head is positioned over the Waste funnel and liquid is squirted from the nozzle several times.

#### **Evaluate the result**

For ÄKTA avant 25: Check that correct volume, 2 ml, is collected in each of the wells **1A1-1A3** and **6A1-6A3**. Also, check that the fractionation marks in the chromatogram correspond to the filled wells.

For ÄKTA avant 150: Check that correct volume, 20 ml, is collected in each of the tubes **1A1-1A3** and **6A1-6A3**. Also, check that the fractionation marks in the chromatogram correspond to the filled tubes.

#### Possible causes of a failed test

The table below describes possible causes of a failed test. When possible sources of error have been checked and taken care of, run the test once again.

Cause	Action
Wrong volumes collect- ed in the wells or tubes, and disturbances on the system pressure curves: - Air trapped in System	Air in pumps: Make sure to prime inlet tubing <b>A1</b> and purge System pump A before method start, see Section 8.1 Prime buffer inlets and purge System pump A and System pump B, on page 63. Faulty pump: See User Manual, chapter Troubleshooting.
pump A - Faulty System pump A	
Liquid is collected in the wrong wells or tubes: - Incorrect fractionation settings	Check the fractionation settings in the <b>System Settings</b> dialog.
Liquid is collected in wrong Cassette: - Incorrect preparation of the Fraction collector	Make sure that only two Cassettes are placed on the Cassette tray, and that the Cassettes are positioned on the cassette positions 1 and 6.
Fractionation interrupt- ed: - Impurities on the Dis- penser head of the Fraction collector	Clean the Fraction collector. See User Manual, chapter Maintenance.
Incorrect preparation of buffer and tubing	Make sure that the system was correctly prepared, see <i>Prepare the test, on page</i> 85.

## 9.3 Q valve test

#### **Method description**

The Q valve test checks the functionality of the Quaternary valve. Correct gradient formation is tested by producing a series of gradient steps. The method run takes approximately 30 minutes for ÄKTA avant 25 and 70 minutes for ÄKTA avant 150.

#### **Required material**

The following material is required:

- 1% acetone in distilled water
- Distilled water
- Capillary loop Ref 1
- Syringe, 25-30 ml
- For ÄKTA avant 25: Mixer, 1.4 ml
- For ÄKTA avant 150: Mixer, 5 ml

#### Prepare the test

Follow the instructions below to prepare the system before method start.

Step	Action
1	Immerse the pieces of inlet tubing marked <b>Q1</b> and <b>Q3</b> in 1% acetone in dis- tilled water.
2	Immerse the pieces of inlet tubing marked <b>Q2</b> and <b>Q4</b> in distilled water.
3	Connect the capillary loop marked <b>Ref 1</b> between Column valve ports <b>1A</b> and <b>1B</b> to generate a backpressure.
4	For ÄKTA avant 25: Make sure that the Mixer with a chamber volume of 1.4 ml is installed.
	For ÄKTA avant 150: Make sure that the Mixer with a chamber volume of 5 ml is installed.
	For further information, see User Manual chapter Maintenance.
5	Prime all Q inlets and purge the System pumps. See Section 8.3 Prime Q inlets and purge System pump A and System pump B, on page 76.

#### Start the test

Follow the instruction below to start the Q valve test.

Step	Action
1	In the <b>System Control</b> module, select <b>System:Performance Test and Report</b> .
	<i>Result</i> : The <b>System Performance Test and Report</b> dialog opens.
2	In the System Performance Test and Report dialog:
	• Select <b>Q Valve Test</b> .
	Click the <i>Run Performance Method</i> button.
	Result: The Start Protocol of the Q valve test opens.
3	Click <b>Next</b> in the dialogs of the <b>Start Protocol</b> to open the next dialog. The dialogs are described in table <i>Overview of the Start Protocol, on page</i> 91.
4	In the last dialog of the <b>Start Protocol, Result name and location</b> , click <b>Finish</b> .
	<i>Result</i> : The Q valve test starts.

#### **Overview of the Start Protocol**

The table below describes the dialogs of the Start Protocol.

Dialog	Description
Notes	Displays the <i>Method Notes</i> of the method. The <i>Method Notes</i> contains a method description and instructions on how to run the method. This dialog also allows the user to enter <i>Start Notes</i> .
Evaluation Procedures	Allows the user to select to save the report to file (recom- mended) and/or to print the report.
Result Name and Loca- tion	Allows the user to change result name and result location.

#### During the run

A **Message** dialog opens during the run. Read the messages in the dialog, and make sure that necessary preparations have been performed. Click the **Confirm and Continue** button in the **Message** dialog to change system state from **System Pause** to **Run** and proceed with the test. Alternatively, click the **Confirm** button in the **Message** dialog and click the **Continue** button on the Instrument display.

#### **Automatic evaluation**

The system automatically generates a report when the test is finished. The report can be printed in two ways:

- If the option Save the report to file was selected in the Evaluation Procedures dialog
  in the Start Protocol of the test, the report is saved in location C:\Program
  Files\GE Healthcare\UNICORN\Temp. Open the report and print it using
  your default Windows printer. (Recommended)
  - **Note:** The exact search path depends on the location of the UNICORN installation folder.
- If the option *Print report* was selected in the *Evaluation Procedures* dialog in the *Start Protocol* of the test, the report is automatically printed on the system printer. Note that a system printer must have been set up, see *UNICORN Administration and Technical Manual*.

Print the report and check the status of the tests. For each of the tests the report states "The test passed" or "The test failed".

#### **Manual evaluation - Preparation**

If you prefer to evaluate your results manually, follow the instructions below.

Step	Action
1	In the <i>Evaluation</i> module in UNICORN, click the <i>Open Result Navigator</i> icon.
	Result: The <b>Result Navigator</b> opens.

2 In the *Result Navigator*, double-click the test result name to open the test result.

#### Step Action

3 In the **Chromatogram** pane of the **Evaluation** module:

- Select the curve UV1\_265.
- Right-click and select Vertical marker.

4 Click the vertical marker and drag it to the constant section of each plateau of the curve UV1\_265. Read the corresponding absorbance values in the upper left corner of the *Chromatogram* pane. Enter the values in column 2 in the tables in *Section 9.5 Test protocol for Q valve test, on page 107* as follows.

Enter the absorbance value at	in table
0% UV (100% Q2)	Step response test result - Q1, Q2 Step response test result - Q3, Q4 Step response test result - Q1, Q2, Q3, Q4
100% UV (100% Q1)	Step response test result - Q1, Q2 Step response test result - Q3, Q4 Step response test result - Q1, Q2, Q3, Q4
5% Q1, 95% Q2	Step response test result - Q1, Q2
95% Q1, 5% Q2	Step response test result - Q1, Q2
5% Q3, 95% Q4	Step response test result - Q3, Q4
95% Q3, 5% Q4	Step response test result - Q3, Q4
50% UV (25% Q1, 25% Q2, 25% Q3, 25% Q4)	Step response test result - Q1, Q2, Q3, Q4

#### Illustration of chromatogram

The illustration below shows a chromatogram from a Q valve test. The gradient steps are marked in the illustration.



**Note:** The illustration shows a chromatogram from a Q valve test on ÄKTA avant 25. A chromatogram from a Q valve test on ÄKTA avant 150 has a similar appearance, but a different scale on the y-axis.

## Manual evaluation - Evaluate the step response

Follow the instruction below to calculate the relative absorption plateau heights for curve UV1\_265. Perform the calculations for each of the three tables in *Section 9.5 Test protocol for Q valve test, on page 107.* 

Step	Action
1	Subtract the baseline value (0% UV) from each of the values in column 2. Enter the results in column 3.

Step	Action
2	Divide each value in column 3 by the baseline corrected value corresponding to 100% UV, multiply by 100, and enter the results in column 4.
3	Check that all values in column 4 fall within the intervals given in column 5.

#### Possible causes of a failed test

The table below describes possible causes of a failed test. When possible sources of error have been checked and taken care of, run the test once again.

Cause	Action
Disturbances caused by air trapped in any of the System pumps	Make sure to prime all Q inlets and purge the System pumps before method start, see <i>Section 8.3 Prime Q inlets</i> <i>and purge System pump A and System pump B, on</i> <i>page 76.</i>
Disturbances caused by damaged pump piston seals.	Replace piston seals. See User Manual, chapter Mainte- nance.
Unstable or incorrect UV signal, or drifting base line - Faulty UV monitor	See User Manual, chapter Troubleshooting.
Wrong mixer chamber size or faulty mixer	Replace the mixer. See User Manual, chapter Maintenance.
Faulty Quaternary valve	See User Manual, chapter Troubleshooting.
Faulty System pumps	See User Manual, chapter Troubleshooting.
Incorrect preparation of buffer and tubing	Make sure that the system was correctly prepared, see <i>Prepare the test, on page</i> 90.

## 9.4 System test

#### About this section

The System test checks the solvent delivery, the functionality of the UV and conductivity monitoring systems, and the valve functionality. The method run takes approximately 30 minutes. This section describes how to prepare, run, and evaluate a System test.

#### In this section

This section contains the following subsections:

Section	See page
9.4.1 Prepare the test	97
9.4.2 Run the test	98
9.4.3 Evaluate the test	100
9.4.4 Possible causes of a failed test	105

#### 9.4.1 Prepare the test

#### **Required material**

The following material is required:

- Distilled water
- 1% acetone and 1 M NaCl in distilled water
- Capillary loop Ref 1
- Syringe, 25-30 ml
- For ÄKTA avant 25: Mixer, 1.4 ml
- For ÄKTA avant 150: Mixer, 5 ml

#### Prepare the test

Follow the instructions below to prepare the system before method start.

Step	Action
1	Immerse the piece of inlet tubing marked <b>A1</b> in distilled water.
2	Immerse the piece of inlet tubing marked <b>B1</b> in 1% acetone and 1 M NaCl in distilled water.
3	Immerse the piece of inlet tubing marked ${\bf Buff}$ in 1% acetone and 1 ${\sf M}$ NaCl in distilled water.
4	Connect the capillary loop marked <b>Ref 1</b> between Column valve ports <b>1A</b> and <b>1B</b> to generate a backpressure.
5	For ÄKTA avant 25: Make sure that the Mixer with a chamber volume of 1.4 ml is installed.
	For ÄKTA avant 150: Make sure that the Mixer with a chamber volume of 5 ml is installed.
	For further information, see User Manual chapter Maintenance.
6	Prime the buffer inlets and purge System pump A and System pump B. See Section 8.1 Prime buffer inlets and purge System pump A and System pump B, on page 63 and Section 8.2 Prime sample inlets and purge the Sample pump, on page 71.

## 9.4.2 Run the test

#### Start the test

Follow the instruction below to start the System test.

Step	Action
1	In the <b>System Control</b> module, select <b>System:Performance Test and Report</b> .
	Result: The <b>System Performance Test and Report</b> dialog opens.
2	In the System Performance Test and Report dialog.
	Select System Test
	Click the <i>Run Performance Method</i> button.
	Result: The Start Protocol of the System test opens.
3	Click <b>Next</b> in the dialogs of the <b>Start Protocol</b> to open the next dialog. The dialogs are described in table <i>Overview of the Start Protocol, on page</i> 98.
4	In the last dialog of the <b>Start Protocol, Result name and location</b> , click <b>Start</b> . <i>Result</i> : The System test starts.

#### **Overview of the Start Protocol**

The table below describes the dialogs of the *Start Protocol*.

Dialog	Description
Notes	Displays the <i>Method Notes</i> of the method. The <i>Method Notes</i> contains a method description and instructions on how to run the method. This dialog also allows the user to enter <i>Start Notes</i> .
Evaluation Procedures	Allows the user to select to save the report to file (recom- mended) and/or to print the report.
Result Name and Loca- tion	Allows the user to change result name and result location.

#### During the run

A *Message* dialog opens during the run. Read the messages in the dialog, and make sure that necessary preparations have been performed. Click the *Confirm and Continue* button in the *Message* dialog to change system state from *System Pause* to *Run* and proceed with the test. Alternatively, click the *Confirm* button in the *Message* dialog and click the *Continue* button on the Instrument display.

#### Illustration of chromatogram



The illustration below shows a chromatogram from a System test.

**Note:** The illustration shows a chromatogram from a System test on ÄKTA avant 25. A chromatogram from a System test on ÄKTA avant 150 has a similar appearance, but a different scale on the y-axis.

## 9.4.3 Evaluate the test

#### **Automatic evaluation**

The system automatically generates a report when the test is finished. The report can be printed in two ways:

- If the option Save the report to file was selected in the Evaluation Procedures dialog
  in the Start Protocol of the test, the report is saved in location C:\Program
  Files\GE Healthcare\UNICORN\Temp. Open the report and print it using
  your default Windows printer. (Recommended)
  - **Note:** The exact search path depends on the location of the UNICORN installation folder.
- If the option *Print report* was selected in the *Evaluation Procedures* dialog in the *Start Protocol* of the test, the report is automatically printed on the system printer. Note that a system printer must have been set up, see *UNICORN Administration and Technical Manual*.

Print the report and check the status of the tests. For each of the tests the report states "The test passed" or "The test failed".

#### **Manual evaluation - Preparation**

If you prefer to evaluate your results manually, follow the instructions below.

Step	Action
1	In the <b>Evaluation</b> module in UNICORN, click the <b>Open Result Navigator</b> icon.
	Result: The <b>Result Navigator</b> opens.
2	In the <b>Result Navigator</b> , double-click the test result name to open the test result.

3 Click the **Customize** icon.



Result: The Customize dialog opens.

4 In the **Customize** dialog, select the **Curves** tab.

Step	Action	
5	In the <b>Curves</b> tab,	
	Select the following curves to be displayed:	
	- UV1_265	
	- UV2_254	
	- UV3_280	
	- Cond	
	- Conc B	
	• Click OK.	
6	In the <b>Chromatogram</b> pane of the <b>Evaluation</b> module,	
	• Select the curve UV1_265.	
	Right-click and select <i>Vertical marker</i> .	
7	Click the vertical marker and drag it to the constant section of each plateau of the curve UV1_265. Read the absorbance values of each plateau in the upper left corner of the <i>Chromatogram</i> pane, and enter the values in the column 2 in table <i>Step response test result, on page 109</i> .	
8	Read the absorbance values for the plateaus corresponding to 0% B and 100% B for the curves listed below. Click the curve name in <i>Chromatogram</i> pane to change the curve reading.	
	• UV1_265	
	• UV2_254	
	• UV3_280	
	Enter the absorbance values in column 2 in table <i>UV response test result, on page 109</i> .	

Step	Action
9	Click the <b>Print</b> icon.



Result: The Print Chromatograms dialog opens.

Print Ch	romat	ograms			
Printer:		oft Office Docu	ment Image Writer		Properties
L	L		MM		
			Chromatograms in e Chromatograms in e		1
<b>V</b> U:	se thick	lines		C	Preview
0				ОК	Cancel

10 In the **Print Chromatograms** dialog:

- Select **Printer**.
- Click OK.

Result: Your chromatogram is printed.

# Manual evaluation - Evaluate the gradient

Follow the instruction below to evaluate the gradient for curve UV1\_265.

Step	Action
1	Place a ruler along the gradient part of curve UV1_265 in the printed report.
2	Check that the curve is linear between 5% B and 90% B and void of discon- tinuities.
3	Enter the start and stop values of the linear section of the curve in <i>Gradient test result, on page 109.</i>

# Manual evaluation - Evaluate the step response

Follow the instruction below to calculate the relative absorption plateau heights for curve UV1\_265.

Step	Action
1	Subtract the baseline value (0% B) from each of the values in column 2 in table <i>Step response test result, on page 109</i> . Enter the results in column 3.
2	Divide each value in column 3 by the baseline corrected value corresponding to 100% B, multiply by 100, and enter the results in column 4.
3	Check that all values in column 4 fall within the intervals given in column 5.

# Manual evaluation - Evaluate the UV response

Follow the instruction below to calculate the UV response.

Step	Action
1	Subtract the baseline values (0% B) corresponding to each UV curve from the values corresponding to 100% B in column 2 in table <i>UV response test result</i> , <i>on page 109</i> . Enter the results in column 3.
2	Calculate the absorbance ratios 265/254 nm and 265/280 nm using the values in column 3. Enter the values in column 4.
3	Check that the obtained ratios fall within the intervals given in column 5.

#### Manual evaluation - Evaluate the Sample Peak and Conductivity Test Result

Follow the instruction below to inspect the two sample peaks of the Cond curve in the chromatogram.

Step	Action
1	Check that the Cond curve has a shape similar to the shape of the UV curves.

Step	Action				
2	Check that the baseline of the Cond curve looks correct and is not drifting.				
	• If the baseline is not drifting: Integrate the curve using the function <i>Zero baseline</i> .				
	<ul> <li>If the baseline is drifting: Correct the baseline before integrating the curve.</li> </ul>				
	For further information on peak integration, see Evaluation Manual.				
3	Enter the area of each peak in column 2 in table <i>Sample peak and conduc-</i> <i>tivity test result</i> , <i>on page 110</i> . Add the areas of peak 1 and peak 2, and divide the area of each peak with the total peak area. Enter the results in column 3.				
4	Check that the obtained percentages fall within the intervals given in column 4.				

## 9.4.4 Possible causes of a failed test

#### Introduction

The tables in this section describe possible causes of a failed test. When possible sources of error have been checked and taken care of, run the test once again.

#### **Faulty Gradient Test Result**

Cause	Action
Disturbances caused by air trapped in any of the pumps	Make sure to prime the buffer inlets and to purge the System pumps and the Sample pump before method start. See Section 8.1 Prime buffer inlets and purge System pump A and System pump B, on page 63 and Section 8.2 Prime sample inlets and purge the Sample pump, on page 71.
Disturbances caused by damaged pump piston seals.	Replace piston seals. See User Manual chapter Mainte- nance.
Unstable or incorrect UV signal, or drifting base line - Faulty UV monitor	See User Manual chapter Troubleshooting
Wrong Mixer chamber size or faulty Mixer	Replace the Mixer. See User Manual chapter Maintenance.

#### Faulty Step Response Result

Cause	Action
If all values are faulty - air in the pump or a faulty pump	Air in pumps: Make sure to prime the buffer inlets and to purge the System pumps and the Sample pump before method start. See Section 8.1 Prime buffer inlets and purge System pump A and System pump B, on page 63 and Section 8.2 Prime sample inlets and purge the Sample pump, on page 71. Faulty pump: See User Manual chapter Troubleshooting.
Faulty values at 5% - damaged pump piston seal in System pump B	Replace piston seals. See User Manual chapter Mainte- nance.

Cause	Action
Faulty values at 95% - damaged pump piston seal in System pump A	Replace piston seals. See User Manual chapter Mainte- nance.

#### Faulty UV Response Test Result

Cause	Action	
Faulty UV monitor	Restart the instrument to calibrate the UV monitor.	
	See User Manual chapter Troubleshooting.	

#### Faulty Runlog Event Test Result

Cause	Action
Any of the valves do not switch	See User Manual chapter Troubleshooting.

#### Faulty Sample Peak and Conductivity Test Result

Cause	Action		
If the shape of the Cond curve differs from the shape of the UV curves - faulty Conductivity monitor	See User Manual, chapter Troubleshooting.		
Unproportional peaks - faulty Sample pump	See User Manual, chapter Troubleshooting.		
Faulty baseline	Perform a manual peak integration of the Cond curve. See <i>Evaluation Manual</i> .		

## 9.5 Test protocol for Q valve test

#### Step response test result - Q1, Q2

1 Programmed Conc.	2 Value read (mAU)	3 Baseline corrected value	4 Normalized value	5 Allowed interval
100% UV (100% Q1)				
95% UV (95% Q1, 5% Q2)				93.6 - 96.4
5% UV (5% Q1, 95% Q2)				3.6 - 6.4
0% UV (100% Q2)				

#### Step response test result - Q3, Q4

1 Programmed Conc.	2 Value read (mAU)	3 Baseline corrected value	4 Normalized value	5 Allowed interval
100% UV (100% Q1)				
95% UV (95% Q3, 5% Q4)				93.6 - 96.4
5% UV (5% Q3, 95% Q4)				3.6 - 6.4
0% UV (100% Q2)				

#### Step response test result - Q1, Q2, Q3, Q4

1 Programmed Conc.	2 Value read (mAU)	3 Baseline corrected value	4 Normalized value	5 Allowed interval
100% UV (100% Q1)				
50% UV (25% Q1, 25% Q2, 25% Q3, 25% Q4)				48.6 - 51.4
0% UV (100% Q2)				
# 9.6 Test protocol for System test

#### **Gradient test result**

#### Step response test result

1 Programmed Conc. %B	2 Value read (mAU)	3 Baseline corrected value	4 Normalized value	5 Allowed interval
100			-	-
95				94.00 - 96.00
50				49.00 - 51.00
5				4.00 - 6.00
0			-	-

#### UV response test result

1 Wavelength (nm)	2 Value rec		3 Baseline corrected value	4 Normalized value	5 Allowed interval
	100% B	0% B			
254				-	-
265/254	-	-	-		1.10 - 1.23
265				-	-
265/280	-	-	-		1.30 - 1.58
280				-	-

# Sample peak and conductivity test result

1	2	3	4
Peak no.	Area	Area/ Peak area (%)	Allowed interval (%)
1			65.00-67.00
2			33.00-35.00

# 10 Reference information

#### About this chapter

This chapter lists the technical specifications of the ÄKTA avant. The chapter also includes a list of wetted materials and a chemical resistance guide.

#### In this chapter

This chapter contains the following sections:

Section	See page
10.1 System specifications	112
10.2 Component specifications	114
10.3 Wetted materials	121
10.4 Chemical resistance guide	124

# 10.1 System specifications

### Introduction

This section lists the system specification data of ÄKTA avant. For components data see *Section 10.2 Component specifications, on page 114.* 

## System specifications

Parameter	Data
System configuration	Benchtop system, external computer
Control system	For ÄKTA avant 25: UNICORN 6.0 or higher version For ÄKTA avant 150: UNICORN 6.1 or higher version
Connection between PC and instrument	Ethernet
Dimensions (W x D x H)	860 x 710 x 660 mm
Weight (excluding computer)	116 kg
Power supply	100-240 V ~, 50-60 Hz
Power consumption	800 VA
Enclosure protective class	IP 21, wet side IP 22

Parameter	Data
Tubing and connectors	ÄKTA avant 25:
	<ul> <li>Inlet: FEP tubing, i.d. 1.6 mm, Tubing connector 5/16" + Ferrule (yellow), 1/8"</li> </ul>
	<ul> <li>Pump to Injection valve: PEEK tubing, i.d. 0.75 mm, Fingertight connector, 1/16"</li> </ul>
	<ul> <li>After Injection valve: PEEK tubing, i.d. 0.50 mm, Fingertight connector, 1/16"</li> <li>ÄKTA avant 150:</li> </ul>
	• Inlet: FEP tubing, i.d. 2.9 mm, Tubing connector 5/16" + Ferrule (blue), 3/16"
	• After pumps: PEEK tubing, 1.0 mm i.d., Fingertight connector, 1/16"

## **Environmental ranges**

Parameter	Data
Storage and transport temperature range	-25°C to +60°C
Chemical environment	See Section 10.4 Chemical resistance guide, on page 124.

# **Operating range**

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

# 10.2 Component specifications

## Introduction

This section specifies the operating data of the components in ÄKTA avant. For general data for the system see *Section 10.1 System specifications, on page 112.* 

#### System pumps

Parameter	Data
Pump type	Piston pump, metering type
Flow rate range	ÄKTA avant 25: 0.001 to 25 ml/min ÄKTA avant 150: 0.01 to 150 ml/min
Pressure range	ÄKTA avant 25: 0 to 20 MPa (2900 psi) ÄKTA avant 150: 0 to 5 MPa (725 psi)
Viscosity range	ÄKTA avant 25: 0.35 to 10 cP ÄKTA avant 150: 0.35 to 5 cP
Flow rate specifications	<ul> <li>ÄKTA avant 25:</li> <li>Accuracy: ± 1.2%</li> <li>Precision: RSD &lt; 0.5%</li> <li>(Conditions: 0.25 to 25 ml/min, &lt; 3 MPa, 0.8 to 2 cP)</li> <li>ÄKTA avant 150:</li> <li>Conditions: 1.0 to 150 ml/min, &lt; 3 MPa, 0.8 to 2 cP</li> <li>Accuracy: ± 1.5%</li> <li>RSD &lt; 0.5%</li> </ul>

## Sample pump

Parameter	Data
Pump type	Piston pump, metering type
Flow rate range	ÄKTA avant 25: 0.01 to 25 ml/min ÄKTA avant 150: 0.01 to 150 ml/min
Pressure range	ÄKTA avant 25: 0 to 10 MPa (1450 psi) ÄKTA avant 150: 0 to 5 MPa (725 psi)
Viscosity range	0.7 to 10 cP
Flow rate specifications	<ul> <li>ÄKTA avant 25:</li> <li>Conditions: 0.25 to 25 ml/min, &lt; 3 MPa, 0.8 to 3 cP</li> <li>Accuracy: ± 2%</li> <li>RSD &lt; 0.5%</li> <li>ÄKTA avant 150:</li> <li>Conditions: 1.0 to 150 ml/min, &lt; 3 MPa, 0.8 to 2 cP</li> <li>Accuracy: ± 2%</li> <li>RSD &lt; 0.5%</li> </ul>

#### Valves

Parameter	Data
Туре	Rotary valves
Number of valves	Up to 12
Functions	Inlet A, Inlet B, Sample inlet, Injection, Column, pH, Outlet

## Quaternary valve

Parameter	Data
Туре	4-port solenoid actuated membrane valve
Functions	Quaternary gradients or BufferPro

# Number of inlets

Parameter	Data
Inlet A	7, expandable to 14
Inlet B	7, expandable to 14
Sample inlet	7, expandable to 14
Quaternary inlet	4, expandable to 18

#### **Pressure sensors**

Parameter	Data
Placement of sensors	System pump, Sample pump, Pre-Column, Post- Column
Range	ÄKTA avant 25: 0 to 20 MPa (2900 psi) ÄKTA avant 150: 0 to 5 MPa (725 psi)
Accuracy	ÄKTA avant 25: $\pm$ 0.02 MPa or $\pm$ 2% whichever is greater ÄKTA avant 150: $\pm$ 0.015 MPa or $\pm$ 1.5% whichever is greater

## Air sensors

Parameter	Data
Placement of sensors	Inlet A, Inlet B, Sample inlet

Parameter	Data
Optional placement	ÄKTA avant 25: Before Sample inlet valve or after Injection valve ÄKTA avant 150: After Injection valve
Sensing principle	Ultrasonic

## **Outlet valve fractionation**

Parameter	Data
Number of outlets	10, expandable to 32
Fraction volumes	ÄKTA avant 25: 0.01 to 100 000 ml ÄKTA avant 150: 1 to 100 000 ml
Delay volume (UV – Outlet valve)	ÄKTA avant 25: 142 μl ÄKTA avant 150: 535 μl

#### Mixer

Parameter	Data
Mixing principle	Chamber with magnetic stirrer
Mixer volume	ÄKTA avant 25: 0.6, 1.4 or 5 ml ÄKTA avant 150: 1.4, 5 or 15 ml

# **Gradient formation**

Parameter	Data
Gradient flow rate range	ÄKTA avant 25:
	• Binary: 0.25 to 25 ml/min
	• Quaternary: 0.5 to 25 ml/min
	ÄKTA avant 150:
	• Binary: 1 to 150 ml/min
	• Quaternary: 2 to 40 ml/min
Gradient composition accuracy	Binary: ± 0.6%
	Quaternary: ± 1%

# **Pressure monitors**

Parameter	Data
Placement of sensors	System pump
Range	0 to 20 MPa (2900 psi)
Accuracy	$\pm$ 0.02 MPa or $\pm$ 2% whichever is greater

## **UV monitor**

Parameter	Data
Wavelength range	190 to 700 nm in steps of 1 nm, up to 3 wavelengths
Absorbance range	-6 to +6 AU
Linearity	within $\pm$ 2% at 0 to 2 AU
Operating pressure	0 to 2 MPa (290 psi)
Flow cells	• 0.5 mm optical path length, 1 µl cell volume
	• 2 mm optical path length, 2 µl cell volume
	• 10 mm optical path length, 8 µl cell volume

## **Conductivity monitor**

Parameter	Data
Conductivity reading range	0.01 mS/cm to 999.99 mS/cm
Accuracy	$\pm$ 0.01 mS/cm or $\pm$ 2%, whichever is greater, (within 0.3 to 300 mS/cm)
Operating pressure	0 to 5 MPa (725 psi)
Flow cell volume	22 µl

## **Temperature monitor**

Parameter	Data
Reading range	0°C to 99°C
Accuracy	± 1.5°C within 4°C to 45°C

## pH monitor

Parameter	Data
pH reading range	0 to 14
Accuracy	± 0.1 pH unit (within pH 2 to 12, temp. within 3°C from calibra- tion temp.)
Operating pressure	0 to 0.5 MPa (72 psi)
Flow cell volume	76 μl

# **Fraction collector**

Parameter	Data
Number of fractions	up to 576

#### 10 Reference information 10.2 Component specifications

Parameter	Data
Vessel types	3, 8, 15 or 50 ml tubes 250 ml bottles Deep well plates, 96 / 48 / 24
Vessel type selection	Automatic recognition
Fraction volumes	0.1 to 50 ml
Spillage-free modes	ÄKTA avant 25: Drop sync or Accumulator ÄKTA avant 150: Accumulator
Protection of fractions	Covered vessels and temperature control
Organic solvents	Ei
Delay volume (UV – Dispenser head)	ÄKTA avant 25: 518 μl ÄKTA avant 150: 1831 μl
Temperature control	Target temperature: 6°C to 20°C

# 10.3 Wetted materials

#### Introduction

This section specifies the wetted materials of ÄKTA avant.

#### Wetted materials

The following wetted materials are used in ÄKTA avant:

Material	Component
Aluminium oxide	Sample pump
Borosilicate	Fraction collector
ECTFE, EthyleneChloroTriFluoroEthylene	System pumps Sample pump
ETFE, EthyleneTetraFluoroEthylene	Tubing
EPDM, EthylenePropyleneDiene M-class rubber	Quaternary valve Fraction collector
Elgiloy™/UHMWPE, UltraHighMolecularWeightPolyEthylene	System pumps Sample pump
FEP, FluorinatedEthylenePropylene	Tubing
FFPM/FFKM, FullyFluorinatedPropyleneMonomer	pH valve Flow restrictor Pressure monitors
FPM/FKM, FluorinatedPropyleneMonomer	Mixer chamber Sample pump
Hastelloy™ C-276	Pressure monitors
PCTFE, PolyChloroTriFluoroEthylene	Conductivity monitor

Material	Component
PEEK, PolyEtherEtherKetone	Quaternary valve Inlet valves System pumps Pressure monitors Mixer chamber Injection valve Column valve UV flow cell Conductivity monitor pH valve Flow restrictor Sample pump Outlet valve Fraction collector Tubing
PolyImide	UV flow cell
PP, PolyPropylen	Mixer chamber Fraction collector
PPS, PolyPhenyleneSulfide	Fraction collector
PTFE, PolyTetraFluorEthylene	Mixer chamber Injection valve Column valve UV flow cell pH valve Outlet valve
PVDF, PolyVinylideneDiFluoride	System pumps Sample pump
Ruby	System pumps Sample pump

Material	Component	
Sapphire	System pumps	
	Sample pump	
Silica	UV flow cell	
Silicone	Tubing	
Titanium	System pumps	
	Pressure monitors	
	Conductivity monitor	
	Sample pump	

# 10.4 Chemical resistance guide

#### Introduction

This section specifies the chemical resistance of ÄKTA avant to some of the most commonly used chemicals in liquid chromatography.

#### **Biocompatibility**

ÄKTA avant 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 overview**

Strong cleaning works well with 2 M sodium hydroxide, 70% acetic acid or the alcohols methanol, ethanol and isopropyl alcohol. Complete system cleaning with 1 M hydrochloric acid should be avoided due to sensitivity in the pressure sensors. For cleaning separation media with 1 M hydrochloric acid, use loop injections of the acid. Make sure that the column is not mounted on a column valve **V9-C** (which contains a pressure sensor).

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

# Chromatography chemicals overview

Reversed phase chromatography of proteins works well with 100% acetonitrile and additives trifluoroacetic acid (TFA) up to 0.2% or formic acid up to 5%.

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

#### Assumptions made

The ratings 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%.

#### **List of chemicals**

Proposed chemical compatibility for ÄKTA avant. All chemicals used for CIP and cleaning are for short term use only, ambient temperature < 25°C, if not other stated.

**Note:** A user can be exposed to large volumes of chemical substances over a long time period. A Material Safety Data Sheet (MSDS) provides the user with information regarding characteristics, human and environmental risks and preventive measures. Make sure that you have the MSDS available from your chemical distributor and/or databases on the internet.

Chemical	Concen- tration	CAS no/ EC no	Usage
Aqueous buffers, pH 2-12	-	N/A	Separation
Acetic acid	70%	64-19-7/ 200-580-7	Cleaning In Place (CIP)
Acetonitrile <sup>1</sup> <b>Note:</b> Quaternary valve is not resistant. Depending on pres- sure, tubing between pump head and pressure monitor needs to be changed. See User Manual, chapter Prepare the system for a run.	100%	75-05-8/ 200-835-2	Reversed Phase Chromatography (RPC)
Acetonitrile/THF <b>Note:</b> Quaternary valve is not resistant.	85/15	109-99-9/ 203-726-8 (Tetrahydrofuran)	RPC

# 10 Reference information

10.4 Chemical resistance guide

Chemical	Concen- tration	CAS no/ EC no	Usage
Acetone	10%	67-64-1/ 200-662-2	Rare: RPC
Ammonia	30%	7664-41-7/ 231-635-3	Oligonucleotide syn- thesis
Ammonium chloride	2 M	12125-02-9/ 235-186-4	Hydrophobic Interac- tion Chromatogra- phy (HIC)
Ammonium sulphate	3 M	7783-20-2/231-984-1	Cleaning of plas- mids, HIC
Arginine	2 M	74-79-3/ 200-811-1	Wash and elution (pH 4), using protein A media, refolding
Benzyl alcohole	2%	100-51-6/ 202-859-9	Cleaning and stor- age of columns
Decon™ 90	10%	N/A	Cleaning
Dimethyl sulphoxide (DMSO)	5%	67-68-5/ 200-664-3	CIP, RPC, cell separa- tion
Dithiothreitol (DTT)	100 mM	3483-12-3 / 222-468-7	Reducing agent
Dithioerythritol (DTE)	100 mM	6892-68-8/ 229-998-8	Reducing agent
Ethylenediaminete- traacetic acid (EDTA)	100 mM	60-00-4/ 200-449-4	Buffer additive
Ethanol	20%	75-08-1/200-837-3	Storage
			<b>Note:</b> Long term use (1 month)
Ethanol	96%	75-08-1/ 200-837-3	CIP
Ethanol + NaOH	40% + 1 M	N/A	CIP
Ethylene glycol	50%	107-21-1/203-473-3	HIC elution
Formic acid	1%	64-18-6/ 200-579-1	RPC, peptide separa- tion
Glycerol	50%	56-81-5/ 200-289-5	HIC

Chemical	Concen- tration	CAS no/ EC no	Usage
Glycine	2 M	56-40-6/ 200-272-2	Cleaning of MAb binding media
Guanidinium hydrochloride	6 M	50-01-1/ 200-002-3	Denaturizing agent
Hydrochloric acid <sup>2</sup>	max. 0.1 M	7647-01-0/ 231-595-7	CIP
Imidazole	2 M	288-32-4/ 206-019-2	2 M needed for buffer preparation
Isopropanol	100%	67-63-0/ 200-661-7	CIP fatty gels
Methanol	100%	74-93-1/ 200-659-6	RPC, CIP
Mercaptoethanol	20 mM	37482-11-4/ 253-523-3	Reducing agent
n-Propanol	50%	67-63-0/ 200-661-7	RPC
Phosphoric acid	0.1 M	7664-38-2/231-633-2	CIP
Potassium phosphate	1 M	7778-77-0/ 231-913-4	HIC
Potassium chloride	4 M	7447-40-7/231-211-8	4M for pH cell stor- age <b>Note:</b> Long term use (1 month)
Sodium dodecyl sulfate (SDS)	1%	151-21-3/ 205-788-1	Detergent
Sodium chloride	4 M	7647-14-5/ 231-598-3	CIP
Sodium hydroxide	2 M	1310-73-2/215-185-5	CIP
Sodium sulphate	1 M	7757-82-6/231-820-9	HIC
Trichloroacetic acid	1%	76-03-9/ 200-927-2	RPC additive
Trifluoroacetic acid	1%	176-05-1/ 200-929-3	RPC additive, pep- tide separation
Triton™-X	1%	9002-93-1 (Triton X 100)	Detergent
Tween™	1%	9005-64-5/ 500-018-3 (Tween 20)	Detergent

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Chemical	Concen- tration	CAS no/ EC no	Usage
Urea	8 M	57-13-6/ 200-315-5	Buffer additive, de- naturizing agent
Water	100%	7732-18-5/ 231-791-2	<b>Note:</b> Long term use (1 month)

- PEEK tubing is biocompatible and chemically inert to most solvents used in purification of proteins. It has in general very good pressure limits, especially for water based buffers. However, organic solvents penetrates weaknesses in the tubing walls easier than water based buffers. Special care should therefore be taken with prolonged use of organic solvents close to pressure limits.
- <sup>2</sup> If hydrochloric acid, HCl, is used as a cleaning agent when columns are connected to the system, the HCl concentration should not exceed 0.1 M in the pressure sensors. Remember that the ÄKTA avant system has pressure sensors in the Column valve V9-C.

For other parts of the system up to 1 M HCl is acceptable for short time use.

Long time use of 0.2 M HCl connected to the Quaternary valve as part of a *BufferPro* recipe is acceptable. The solution becomes diluted further down in the system.

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