# ÄKTA<sup>™</sup> avant Operating Instructions

## Original instructions







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## 1 Introduction

### About this chapter

This chapter contains important user information, descriptions of safety notices, regulatory information, intended use of the ÄKTA avant instrument, and lists of associated documentation.

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1.1 About this manual	5
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### 1.1 About this manual

#### Purpose of this manual

The *Operating Instructions* provide you with the instructions needed to install, operate and maintain the product in a safe way.

#### **Typographical conventions**

Software items are identified in the text by **bold italic** text. A colon separates items in a group, thus *Flowpath:Injection valve* refers to the *Injection valve* item in the *Flowpath* group.

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

### 1.2 Important user information

## Read this before operating the product



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

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

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

#### Intended use of the product

ÄKTA avant is a liquid chromatography system intended for method and process development in purification of biomolecules. 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 procedures.

#### **Prerequisites**

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

- You have a general understanding of how the computer and Microsoft® Windows® work.
- You understand the concepts of liquid chromatography.
- You have read and understood the Safety instructions chapter in this manual.
- A user account has been created according to the UNICORN™ Administration and Technical Manual.

#### **Safety notices**

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



#### WARNING

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



#### CAUTION

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



#### NOTICE

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

#### Notes and tips

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

### 1.3 Regulatory information

#### Introduction

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

#### **Manufacturing information**

The table below summarizes the required manufacturing information. For further information, see the EU Declaration of Conformity (DoC) document.

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

#### **Conformity with EU Directives**

This product complies with the European directives listed in the table, by fulfilling the corresponding harmonized standards.

A copy of the EU Declaration of Conformity is included in the documentation package.

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

#### **CE marking**



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

- used as a stand-alone unit, or
- connected to other products recommended or described in the user documentation, and
- used in the same state as it was delivered from GE, 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 principles for design. Risk assessment and risk reduction.	EN ISO standard is har- monized with EU direc- tive 2006/42/EC
EN/IEC 61010-1, UL 61010-1, CAN/CSA C22.2 No. 61010-1	Safety requirements for electrical equipment for measurement, control, and laboratory use.	EN standard is harmo- nized with EU directive 2006/95/EC
EN/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
ETSI EN 301 489-3	Electromagnetic compatibility and Radio spectrum Matters (ERM); Elec- troMagnetic Compatibility (EMC) standard for radio equipment and services.	EN standard is harmo- nized with EU directives 1999/5/EC

Standard	Description	Notes
ETSI 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 compliance

This device complies with part 15 of the FCC Rules. Operation is subject to the following two conditions: (1) This device may not cause harmful interference, and (2) this device must accept any interference received, including interference that may cause undesired operation.

## **Note:** The user is cautioned that any changes or modifications not expressly approved by GE could void the user's authority to operate the equipment.

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.

#### **Environmental conformity**

Requirement	Title
2011/65/EU	Restriction of Hazardous Substances (RoHS) Directive
2012/19/EU	Waste Electrical and Electronic Equipment (WEEE) Directive
ACPEIP	Administration on the Control of Pollution Caused by Electronic Information Products, China Restriction of Hazardous Sub- stances (RoHS)
Regulation (EC) No 1907/2006	Registration, Evaluation, Authorization and restriction of CHemicals (REACH)

This product conforms to the following environmental requirements.

## Regulatory compliance of connected equipment

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

### 1.4 Associated Documentation

#### Introduction

This section describes the user documentation that is delivered with the ÄKTA avant instrument.

#### ÄKTA avant user documentation

The user documentation listed in the following table is delivered with the ÄKTA avant instrument.

Document	Main contents
ÄKTA avant Unpacking Instruction	Instructions for unpacking the instrument, and how to lift the instrument onto a bench.
ÄKTA avant Operating Instructions	Instructions needed to install, operate and maintain the system in a safe way.
ÄKTA avant User Manual	Instructions for handling the system. Descrip- tions of components. Information about how to run and maintain the system.
ÄKTA avant 25 Product Documentation OR ÄKTA avant 150 Product Documentation <sup>1</sup>	System specification and declaration of material conformity.

1 The instrument is delivered with the relevant document.

#### **UNICORN user documentation**

The user documentation listed in the following table is available from the *Help* menu in UNICORN or from the *UNICORN Online Help and Documentation* software accessed by pressing the **F1** key in any UNICORN module.

Documentation	Main contents
UNICORN Help	Descriptions of UNICORN dialog boxes (available from the <i>Help</i> menu).

Documentation	Main contents
Getting started with Evaluation	<ul> <li>Video clips showing common workflows in the Evaluation module.</li> </ul>
<b>Note:</b> Available in UNICORN 7.0 and later.	Overview of features of the Evaluation module.
UNICORN Method Manual <sup>1</sup>	<ul> <li>Overview and detailed descriptions of the method creation features in UNICORN.</li> <li>Workflow descriptions for common operations.</li> </ul>
UNICORN Administration and Technical Manual <sup>1</sup>	<ul> <li>Overview and detailed description of network setup and complete software installation.</li> <li>Administration of UNICORN and the UNICORN database.</li> </ul>
UNICORN Evaluation Manual <sup>1</sup>	<ul> <li>Overview and detailed descriptions of the Evaluation Classic module in UNICORN.</li> <li>Description of the evaluation algorithms used in UNICORN.</li> </ul>
UNICORN System Control Manual <sup>1</sup>	<ul> <li>Overview and detailed description of the system control features in UNICORN.</li> <li>Includes general operation, system settings and instructions on how to perform a run.</li> </ul>

1 Current UNICORN version is added to the title of the manual.

## 2 Safety Instructions

#### About this chapter

This chapter describes safety precautions and emergency shutdown procedures for the product. The labels on the system and information regarding recycling are also described.

#### Important



#### WARNING

Before installing, operating or maintaining the product, all users must read and understand the entire contents of this chapter to become aware of the hazards involved.

#### In this chapter

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2.1 Safety Precautions	15
2.2 Labels	24
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2.5 Declaration of Hazardous Substances (DoHS)	31

### 2.1 Safety Precautions

#### Introduction

The safety precautions in this section are grouped in the following categories:

- General precautions, on page 15
- Flammable liquids and explosive environment, on page 16
- Personal protection, on page 17
- Installing and moving, on page 18
- System operation, on page 20
- Maintenance, on page 22

#### **General precautions**



#### WARNING

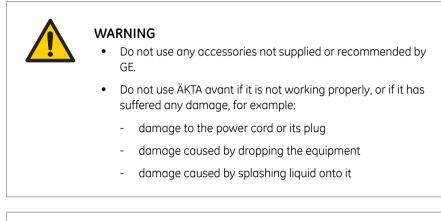
**Risk assessment**. Perform a risk assessment for any risks due to the process or process environment. Evaluate the effects the use of the product and the operational processes may have on the classification of the hazardous area. The process might cause the area to increase or the zone classification to change. Implement the risk reduction measures needed, including use of personal protective equipment.



#### WARNING

Always follow these General precautions to avoid injury when using the ÄKTA avant instrument.

- Do not operate the ÄKTA avant instrument in any other way than described in the ÄKTA avant and UNICORN manuals.
- Only properly trained personnel may perform operation and user maintenance of the product.
- Before connecting to a column, read the instructions for use of the column. To avoid exposing the column to excessive pressure, make sure that the pressure limit is set to the specified maximum pressure for the column.

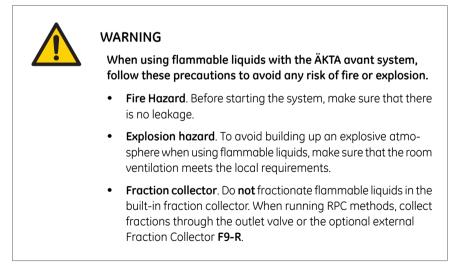




#### NOTICE

**Avoid condensation**. If ÄKTA avant is kept in a cold room, cold cabinet or similar, keep it switched on in order to avoid condensation.

## Flammable liquids and explosive environment





#### WARNING

- RPC runs with 100% acetonitrile and system pressure above
   5 MPa (50 bar) in ÄKTA avant 25. Always replace the green
   PEEK tubing between the used system pump and the pump pressure monitor with orange PEEK tubing, i.d. 0.5 mm, before running RPC with 100% acetonitrile. Set the system pressure alarm to 10 MPa (100 bar).
- RPC runs with 100% acetonitrile in ÄKTA avant 150. Always replace the beige PEEK tubing between the used system pump and the pump pressure monitor before running RPC with 100% acetonitrile. Replace it with green PEEK tubing, i.d. 0.75 mm.

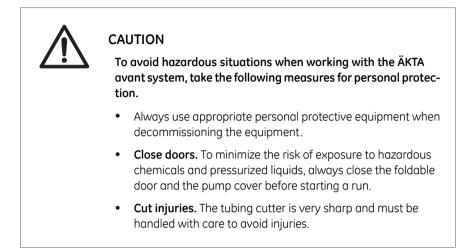
#### **Personal protection**



#### WARNING

To avoid injury when working with the ÄKTA avant system, take the following measures for personal protection.

- Always use appropriate Personal Protective Equipment (PPE) during operation and maintenance of this product.
- Hazardous substances and biological agents. When using hazardous chemical and biological agents, take all suitable protective measures, such as wearing protective glasses and gloves resistant to the substances used. Follow local and/or national regulations for safe operation and maintenance of ÄKTA avant.
- Spread of biological agents. The operator must take all necessary actions to avoid spreading hazardous biological agents. The facility must comply with the national code of practice for biosafety.
- **High pressure**. The product operates under high pressure. Wear protective glasses and other required Personal Protective Equipment (PPE) at all times.



#### Installing and moving

WA	RNING
	avoid injury when installing and moving the ÄKTA avant stem, take the following measures for personal protection.
•	Move transport crates. Make sure that the forklift has the capacity to safely lift the crate weight. Make sure that the crate is properly balanced so that it will not accidentally tip when moved.
•	<b>Heavy object</b> . 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.
•	<b>Moving the product horizontally</b> . Three people are required to move the product horizontally.
•	<b>Supply voltage.</b> Before connecting the power cord, make sure that the supply voltage at the wall outlet corresponds to the marking on the instrument.
•	<b>Protective ground</b> . The product must always be connected to a grounded power outlet.
•	<b>Power cord</b> . Only use power cords with approved plugs delivered or approved by GE.



#### WARNING

- Access to power switch and power cord with plug. 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.
- **Installing the computer**. The computer must be installed and used according to the instructions provided by the manufacturer of the computer.



#### NOTICE

To avoid damage to the instrument when installing and moving the ÄKTA avant system, take the following measures.

- 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.
- The maximum level of the waste vessel for the waste tubing from the valves must be lower than 30 cm above the lab bench.
- 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.
- Vents on the ÄKTA avant instrument. To ensure adequate ventilation, keep papers and other objects away from the vents of the instrument.
- Disconnect power. To prevent equipment damage, always disconnect the power from the product before an instrument module is removed or installed, or a cable is connected or disconnected.
- 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 other than instrument modules designed for ÄKTA avant. See ÄKTA avant User Manual. Do not disconnect or move the UniNet-9 bus cable.

#### System operation



#### WARNING

To avoid personal injury when operating the ÄKTA avant system, follow these instructions.

- 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.
- Fasten bottles and cassettes. Always fasten bottles and cassettes to the rails at the front and side panel. Use appropriate holders for bottles. Shattered glass from falling bottles may cause injury. Spilled liquid may cause fire hazard and personal injury.
- Electrical shock hazard after spillage. If there is a risk that large volumes of spilled liquid may penetrate the casing of the instrument, immediately switch off the instrument, disconnect the power cord, and contact an authorized service engineer.
- Moving parts in fraction collector. Do not open the built-in fraction collector door when the instrument is running.
- Using a Superloop. After loading a Superloop, always plug the Syr port on the injection valve with a stop plug. With a Superloop connected to the valve, an over-pressure may be created during injection.
- **Over-pressure.** Never block the outlet tubing with, for instance, stop plugs, since this will create over-pressure and might result in injury.
- Hazardous chemicals during run. When using hazardous chemicals, run *System CIP* and *Column CIP* to flush the entire system tubing with distilled water, before service and maintenance.



#### WARNING

Hazardous biological agents during run. When using hazardous biological agents, run *System CIP* and *Column CIP* to flush the entire pump with bacteriostatic solution (e.g. 1M NaOH) followed by a neutral buffer and finally distilled water, before service and maintenance.



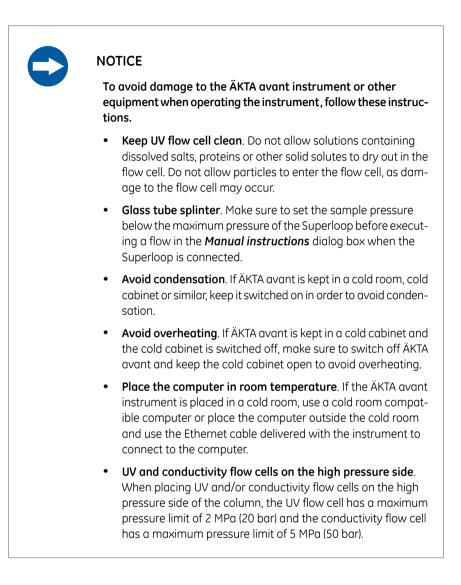
#### CAUTION

To avoid hazardous situations when operating the ÄKTA avant system, follow these instructions.

- **Risk of breaking test vials**. Do not use excessive force to press vials of wrong dimensions into the fraction collector cassettes. Glass vials may break and cause injuries.
- Hazardous chemicals in UV flow cell. Make sure that the entire flow cell has been flushed thoroughly with bacteriostatic solution, for example NaOH, and distilled water, before service and maintenance.
- **pH-electrode**. Handle the pH-electrode with care. The glass tip may break and cause injury.



- Do not fasten bottles with a volume of more than 1 liter in the front panel rails.
- Max. weight on buffer tray. Do not place containers with a volume of more than 10 liters each on the buffer tray. The total allowed weight on the buffer tray is 40 kg.



#### Maintenance



#### WARNING

To avoid personal injury when performing maintenance on the ÄKTA avant instrument, follow these instructions.



#### WARNING

- Electrical shock hazard. All repairs should be done by service personnel authorized by GE. Do not open any covers or replace parts unless specifically stated in the user documentation.
- **Disconnect power**. Always disconnect power from the instrument before replacing any component on the instrument, unless stated otherwise in the user documentation.
- Corrosive chemicals during maintenance. If the system or column is cleaned with a strong base or acid, flush with water afterwards and wash with a weak neutral buffer solution in the last step or phase.



To avoid damage to the ÄKTA avant instrument or other equipment when performing maintenance on the ÄKTA avant instrument, follow these instructions.

- **Cleaning**. Keep the exterior of the instrument dry and clean. Wipe regularly with a soft damp tissue and, if necessary, a mild cleaning agent. Let the instrument dry completely before use.
- Advanced maintenance. Read the instruction carefully before disassembly of the pump head.

#### 2 Safety Instructions 2.2 Labels

### 2.2 Labels

#### Introduction

This section describes the safety labels that are attached to the ÄKTA avant instrument. For information about marking of the computer equipment, refer to the manufacturer's instructions.

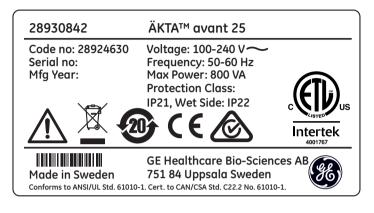
## Labels on the ÄKTA avant instrument

The following illustrations show the labels that are attached to the ÄKTA avant instrument.



#### System label

**Note:** The specific data shown on this system label is only an example. Actual data is specific for each individual system and may vary from system to system.



#### Safety symbols

The following safety symbols are used in the labels:

Label	Meaning
Do NOT fractionate flammable liquids.	Warning! Fraction collector. Do not fractionate flammable liq- uids in the built-in fraction collector. When running RPC methods, collect fractions through the outlet valve or the optional external Fraction Collector F9-R.
$\bigwedge$	<ul> <li>Warning! Read the Operating Instruction before using the system.</li> <li>Electrical shock hazard. All repairs should be done by service personnel authorized by GE. Do not open any covers or replace parts unless specifically stated in the user documentation.</li> </ul>
	<b>Supply voltage.</b> Before connecting the power cord, make sure that the supply voltage at the wall outlet corresponds to the marking on the instrument.

Label	Meaning
20)	This symbol indicates that the product might contain hazardous materials in excess of the limits established by the Chinese standard SJ/T11363-2006 Requirements for Concentration Limits for Certain Hazardous Sub- stances in Electronic Information Products.
CE	The system complies with applicable European direc- tives.
	The system complies with applicable requirements for Australia and New Zealand.
c Listev Intertek	This symbol indicates that ÄKTA avant has been certi- fied by a Nationally Recognized Testing Laboratory (NRTL). NRTL means an organization, which is recog- nized by the US Occupational Safety and Health Ad- ministration (OSHA) as meeting the legal requirements of Title 29 of the Code of Federal Regulations (29 CFR), Part 1910.7.

### 2.3 Emergency procedures

#### Introduction

This section describes how to perform an emergency shutdown of the ÄKTA avant instrument, including connected equipment. This section also describes the results in the event of power failure or network interruption.

#### **Emergency shutdown**

In an emergency situation, stop the run by either pausing the run or switching off the instrument as described in the following table:

If you want to	then
pause the run	<ul> <li>Press the <i>Pause</i> button on the instrument display. This will stop all pumps in the instrument.</li> <li>Image: Image: Image:</li></ul>

lf you want to	then
switch off the instrument	<ul> <li>Push the Power switch to the O position, or</li> <li>disconnect the power cord from the wall socket.</li> <li>Result: The run is interrupted immediately.</li> <li>Note: The sample and data may be lost as a result of switching off the power.</li> </ul>

#### Power failure

The result of a power failure depends on which unit is affected.

Power failure to	will result in		
ÄKTA avant instrument	• The run is interrupted immediately		
	• The data collected up to the time of the power failure is available in UNICORN.		
Computer	The UNICORN computer shuts down		
	The instrument display shows status <i>Not connected</i>		
	• The run is interrupted immediately		
	Data generated up to 10 seconds before the power failure can be recovered		
	Note:		
	The UNICORN client may lose connection to the instrument during a temporary overload of the processor and display an error message. This may appear as a computer failure. The run continues and you can restart the UNICORN client to regain control. No data will be lost.		

## Uninterruptible power supply (UPS)

A UPS can prevent data loss during a power failure, and allow time for a controlled shutdown of the ÄKTA avant instrument.

For UPS power requirements, see *Technical specifications, on page* 174. Remember to also take into account the specifications for the computer and monitor. Refer to the manufacturers' documentation.

#### Restart the instrument after emergency shutdown or power failure

Follow the instructions to restart the instrument after an emergency shutdown or power failure.

Step	Action			
1	Make sure that the condition that caused the emergency shutdown or power failure is corrected.			
2	If power to the instrument has been lost, restart the instrument.			
3	• Press the <i>Continue</i> button on the instrument display. or			
	Click the <i>Continue</i> button in the <i>System Control</i> module.			

### 2.4 Recycling information

#### Introduction

This section describes the procedures for disposal and recycling of the ÄKTA avant instrument.

## Decommissioning and disposal of the equipment

When taking the ÄKTA avant instrument out of service:

- The equipment must be decontaminated.
- The components must be separated and recycled according to national and local environmental regulations



#### CAUTION

Always use appropriate personal protective equipment when decommissioning the equipment.

## Disposal of electrical components

Waste comprising electrical and electronic equipment must not be disposed of as unsorted municipal waste and must be collected separately. Please contact an authorized representative of the manufacturer for information concerning the decommissioning of equipment.



### 2.5 Declaration of Hazardous Substances (DoHS)

#### 根据SJ/T11364-2006《电子信息产品污染控制标识要求》特提供如下有关污染控制为面的信息。

The following product pollution control information is provided according to SJ/T11364-2006 Marking for Control of Pollution caused by Electronic Information Products.

#### 电子信息产品污染控制标志说明 Explanation of Pollution Control Label



该标志表明本产品含有超过SJ/T11363-2006《电子信息产品中有毒有害物质的限 量要求》中限量的有毒有害物质。标志中的数字为本产品的环保使用期,表明本 产品在正常使用的条件下,有毒有害物质不会发生外泄或突变,用户使用本产品 不会对环境造成严重污染或对其人身、财产造成严重损害的期限。单位为年。

为保证所申明的环保使用期限,应按产品手册中所规定的环境条件和方法进行正 常使用,并严格遵守产品维修手册中规定的期维修和保养要求。

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In order to maintain the declared EFUP, the product shall be operated normally according to the instructions and environmental conditions as defined in the product manual, and periodic maintenance schedules specified in Product Maintenance Procedures shall be followed strictly.

Consumables or certain parts may have their own label with an EFUP value less than the product. Periodic replacement of those consumables or parts to maintain the declared EFUP shall be done in accordance with the Product Maintenance Procedures.

This product must not be disposed of as unsorted municipal waste, and must be collected separately and handled properly after decommissioning.

#### 有毒有害物质或元素的名称及含量

#### Name and Concentration of Hazardous Substances

#### 产品中有毒有害物质或元素的名称及含量

Table of Hazardous Substances' Name and Concentration

部件名称 Component name	有毒有害物质或元素 Hazardous substance					
	铅 Pb	汞 Hg	镉 Cd	六价铬 Cr6+	多溴联苯 PBB	多溴二苯醚 PBDE
28-9308-42	Х	0	Х	0	0	0
28-9763-37	Х	0	Х	0	0	0
29-0113-60	Х	0	0	0	0	0
29-0113-61	Х	0	0	0	0	0
29-0113-62	Х	0	0	0	0	0
29-0906-89	Х	0	0	0	0	0
29-0906-91	Х	0	0	0	0	0
29-0113-53	Х	0	0	0	0	0
29-0113-58	Х	0	0	0	0	0

#### 0: 表示该有毒有害物质在该部件所有均质材料中的含量均在SJ/T11363-2006标准规定的限 量要 求以下

X: 表示该有毒有害物质至少在该部件的某一均质材料中的含量超出SJ/T11363-2006标准规 定的限量要求

#### 此表所列数据为发布时所能获得的最佳信息

0: Indicates that this toxic or hazardous substance contained in all of the homogeneous materials for this part is below the limit requirement in SJ/T11363-2006.

X: Indicates that this toxic or hazardous substance contained in at least one of the homogeneous materials used for this part is above the limit requirement in SJ/T11363-2006.

• Data listed in the table represents best information available at the time of publication.

## 3 System description

#### About this chapter

This chapter gives an overview of the ÄKTA avant instrument, software and accessories.

#### In this chapter

This chapter contains the following sections:

Section	See page
3.1 ÄKTA avant instrument overview	34
3.2 UNICORN software	43

#### Illustration of the system

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



### 3.1 ÄKTA avant instrument overview

#### Introduction

This section shows an overview of the ÄKTA avant instrument. Technical details about the instrument and the individual modules are found in *ÄKTA avant User Manual*.

#### **Exterior design**

The ÄKTA avant instrument has a modular design, with all the liquid handling modules placed on the exterior of the instrument. Buffer vessels are placed on the buffer tray on top of the instrument. An instrument display is located on the front. From this side the built-in fraction collector is handled, as well as the sample. The remaining modules are placed on the right-hand side of the instrument. This side can be covered by a foldable door and a pump cover. By rotating the instrument using the swivel foot any side is easily accessed.

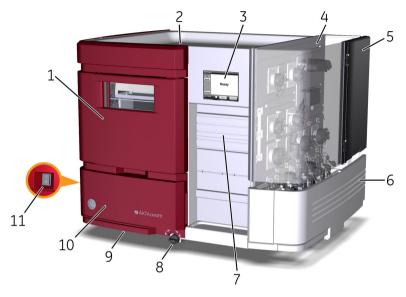
#### **Operating ranges**

The following table show some of the operational limits of  $\ddot{\mathsf{A}}\mathsf{KTA}$  avant 25 and  $\ddot{\mathsf{A}}\mathsf{KTA}$  avant 150

Parameter	Limits		
	ÄKTA avant 25	ÄKTA avant 150	
Flow rate	0.001 to 25 ml/min	0.01 to 150 ml/min	
	Note: When running the Column packing flow instruction, the maximum flow rate is 50 ml/min.	Note: When running the Column packing flow instruction, the maximum flow rate is 300 ml/min.	
Max. operating pressure	20 MPa (200 bar)	5 MPa (50 bar)	
UV monitor wavelength	190 to 700 nm	190 to 700 nm	

## Illustration of the main parts of the instrument

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

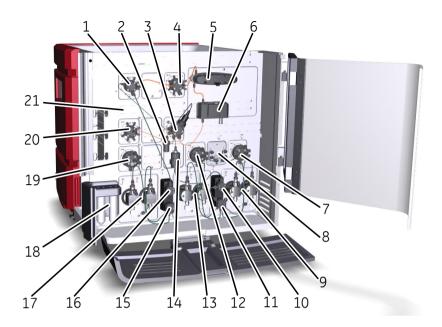


Part	Function	Part	Function
1	Fraction collector	2	Buffer tray
3	Instrument display	4	Wet side
5	Foldable door	6	Pump cover
7	Holder rails	8	Swivel foot lock/unlock knob
9	Swivel foot	10	Swing out toolbox
11	Power switch		

## Illustration of the wet side modules of the instrument

The descriptions of the ÄKTA avant instrument and the work flow in this manual are based on an ÄKTA avant 25 instrument that consists of the modules and parts shown in the following illustration. (ÄKTA avant 150 is delivered in a corresponding setup.)

The following illustration shows the modules of the wet side of the instrument.



Part	Function	Part	Function
1	Injection Valve	2	Flow Restrictor
3	pH Valve	4	Column Valve
5	UV Monitor	6	Conductivity Monitor
7	Inlet Valve B	8	Quaternary Valve
9	System Pump B	10	Pressure monitor of system pumps
11	System pump flow restrictor	12	Inlet Valve A
13	System Pump A	14	Mixer
15	Sample pump flow restrictor	16	Pressure monitor of sample pump
17	Sample Pump	18	Pump rinsing solution tube
19	Sample Inlet Valve	20	Outlet Valve
21	Holder rails		

#### Available modules

The ÄKTA avant instrument is always delivered with the standard modules installed, but one or more optional modules may be added to the flow path.

The following tables contains information on the standard modules and the optional modules of the ÄKTA avant 25 and ÄKTA avant 150 instruments. The sections that follows contain descriptions of the modules.

**Note:** The valves for ÄKTA avant 25 and ÄKTA avant 150 are compatible with both systems but for the best performance the specific valve type should be used. The narrow channels in the valves for ÄKTA avant 25 will give too high back pressure if used above 50 ml/min. The larger volumes in the "H" valves for ÄKTA avant 150 may decrease resolution and increase peak broadening if used in ÄKTA avant 25.

Module	Label in		
	ÄKTA avant 25	ÄKTA avant 150	
System Pump A	P9 A	Р9Н А	
System Pump B	Р9 В	Р9Н В	
Sample Pump	P9-S	Р9Н	
Pressure Monitor	R9	R9	
Mixer	M9	M9	
Injection Valve	V9-Inj	V9H-Inj	
Quarternary Valve	Q9	Q9	
Inlet Valve A	V9-IA	V9H-IA	
Inlet Valve B	V9-IB	V9H-IB	
Sample Inlet Valve	V9-IS	V9H-IS	
Column Valve	V9-C	V9H-C	
pH Valve	V9-рН	V9H-рН	
Outlet Valve	V9-O	V9H-O	
UV Monitor	U9-M	U9-M	
Conductivity Monitor	С9	С9	
Built-in fraction collector	NA	NA	

#### **Standard modules**

### 3 System description

3.1 ÄKTA avant instrument overview

#### **Optional modules**

Module	Label in	
	ÄKTA avant 25	ÄKTA avant 150
Second Inlet Valve A	V9-A2	V9H-A2
Second Inlet Valve B	V9-B2	V9H-B2
Extra Inlet Valve X1	V9-IX	V9H-IX
Extra Inlet Valve X2	V9-IX	V9H-IX
Second Sample Inlet Valve	V9-S2	V9H-S2
Versatile Valve	V9-V	V9H-V
Loop Valve	V9-L	V9H-L
Second Column Valve	V9-C2	V9H-C2
Second Outlet Valve	V9-O2	V9H-O2
Third Outlet Valve	V9-O3	V9H-O3
External Air Sensor L9-1.5	L9-1.5	L9-1.5
External Air Sensor L9-1.2	L9-1.2	L9-1.2
I/O-box	E9	E9
Second UV Monitor	U9-L	U9-L
Second Conductivity Monitor	С9	С9
Second Fraction Collector	F9-R	F9-R

#### **Description of standard modules**

The following modules are installed in the instrument when delivered.

Module	Description
Quaternary Valve ( <b>Q9</b> )	Valve which allows automatic mixing of four different solutions.
System Pump A ( <b>P9 A</b> or <b>P9H A</b> )	A high precision pump, which delivers buffer in purification runs.

## 3 System description 3.1 ÄKTA avant instrument overview

Module	Description		
System Pump B ( <b>P9 B</b> or <b>P9H B</b> )	A high precision pump, which delivers buffer in purification runs.		
Sample Pump ( <b>P9-S</b> or <b>P9H</b> )	A high precision pump which delivers sample or buffer in purification runs.		
Pressure Monitor ( <b>R9</b> )	Pressure monitor which reads the system pressure after System Pump A and System Pump B.		
Pump flow restrictor	Prevents the system from siphoning if the flow path after the pump is open. Gives a small back pressure to the pump in extreme low pressure applications.		
Mixer ( <b>M9</b> )	Mixes the buffers delivered from the system pumps to a homogeneous buffer composition. Three mixer chambers are available for ÄKTA avant 25. Available volumes are: 0.6 ml, 1.4 ml (mounted at delivery) and 5 ml.		
	Three mixer chambers are available for ÄKTA avant 150. Available volumes are: 1.4 ml, 5 ml (mounted at delivery), and 15 ml.		
	CAUTION Risk of explosion. Do not use Mixer chamber 15 ml with an ÄKTA avant 25 sys- tem configuration. The maximum pressure for Mix- er chamber 15 ml is 5 MPa (50 bar).		
Inlet Valve A ( <b>V9-IA</b> or <b>V9H-IA</b> )	Inlet valve for System Pump A with seven inlet ports and integrated air sensor.		
Inlet Valve B ( <b>V9-IB</b> or <b>V9H-IB</b> )	Inlet valve for System Pump B with seven inlet ports and integrated air sensor.		
Sample Inlet Valve ( <b>V9-IS</b> or <b>V9H-IS</b> )	Inlet valve for sample solution, with eight inlet ports (seven sample inlets and one buffer inlet) and integrated air sensor.		

Module	Description
Injection Valve ( <b>V9-Inj</b> or <b>V9H-Inj</b> )	Valve which directs sample onto the column.
Column Valve ( <b>V9-C</b> or <b>V9H-C)</b>	Column valve which connects up to five columns to the instrument, and directs the flow to one column at a time. The column valve features two integrated pressure sensors. Allows the user to choose flow direction through the column, or to bypass the column.
рН Valve ( <b>V9-рН</b> or <b>V9H-рН</b> )	Valve which enables the pH electrode to be includ ed in the flow path or bypassed during a run. The pH electrode may be calibrated when installed in the pH Valve. It also enables the flow restrictor to be included in the flow path (default position) or bypassed during a run.
Outlet Valve ( <b>V9-O</b> or <b>V9H-O</b> )	Valve which directs the flow to the fraction collector, any of the ten outlet ports or waste.
UV Monitor ( <b>U9-M</b> )	Monitor which measures the UV/Vis absorbance at up to three wavelengths simultaneously in the range 190 to 700 nm.
Conductivity Monitor ( <b>C9</b> )	Monitor which continuously measures the conduc tivity of buffers and sample solutions.
Built-in fraction collector	Built-in fraction collector. A cooling function pro- tects the fractions from heat degradation.

#### **Core modules**

Core modules need to be installed for the system to run. They are mandatory in the software.

All standard modules except the built-in fraction collector are considered core modules.

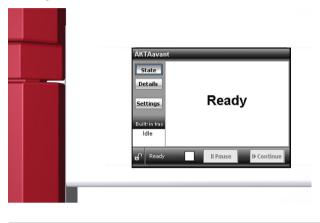
#### **Description of optional modules**

Module	Description
Second Inlet Valve A and Inlet Valve B ( <b>V9-A2</b> and <b>V9-B2</b> or <b>V9H-A2</b> and <b>V9H-B2</b> )	Second inlet valve for System Pump A, or System Pump B, to extend the number of inlets up to 14.
Inlet Valve X1 and Inlet Valve X2 ( <b>V9-IX</b> or <b>V9H-IX</b> )	Inlet valve with eight inlet ports. No integrated air senso
Second Sample Inlet Valve ( <b>V9-S2</b> or <b>V9H-S2</b> )	Second inlet valve for the Sample Pump to extend the number of sample inlets up to 14.
Versatile Valve ( <b>V9-V</b> or <b>V9H-V</b> )	A 4-port, 4-position valve, which can be used to cus- tomize the flow path.
Loop Valve ( <b>V9-L</b> or <b>V9H-L</b> )	Valve which enables automatic sample application from up to five sample loops, or to collect intermediate frac tions in automated two-step purification.
Second Column Valve ( <b>V9-C2</b> or <b>V9H-C2</b> )	Valve which connects five additional columns to the in strument. The valve allows the user to choose flow di- rection through the column, or to bypass the column.
Second Outlet Valve ( <b>V9-O2</b> or <b>V9H-O2</b> )	Valve which adds 12 outlet ports to the system, giving a total of 21 outlets.
Third Outlet Valve ( <b>V9-O3</b> or <b>V9H-O3</b> )	Valve which adds 12 outlet ports to the system, giving a total of 32 outlets
External Air Sensor ( <b>L9-1.5</b> or <b>L9-1.2</b> )	Sensor which prevents air from being introduced into the flow path.
I/O-box ( <b>E9</b> )	Module which receives analog or digital signals from, or transfers analog or digital signals to, external equip ment that has been incorporated in the system.
Second UV Monitor ( <b>U9-L</b> )	Monitor which measures the UV absorbance at a fixed wavelength of 280 nm.
Second Conductivity Mon- itor ( <b>C9</b> )	Monitor which measures the conductivity of buffers and sample solutions.
Second Fraction Collector ( <b>F9-R</b> )	Round fraction collector that can collect up to 175 fractions.

The following modules may be added to the flow path.

# Illustration of the instrument display

The following illustration shows the instrument display with the system state *Ready* showing.



## Instrument display indicators and buttons

The instrument display is a touchscreen that shows the current system status. The instrument display includes the following indicators and buttons

Indicator/Button	Description
ഴി	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.
I▶ Continue	<ul> <li>Resumes instrument operation from the following states:</li> <li>Wash</li> <li>Pause</li> <li>Hold</li> </ul>

## 3.2 UNICORN software

#### Introduction

This section gives an overview of the UNICORN software. It also describes the *System Control* module.

To learn more about *System Control* and the other three modules *Administration*, *Method Editor* and *Evaluation*, see the UNICORN documentation package.

#### In this section

This chapter contains the following sections:

Section	See page
3.2.1 UNICORN software overview	44
3.2.2 The System Control module	46

## 3.2.1 UNICORN software overview

#### Introduction

This section gives a brief overview of the UNICORN software: a complete package for control, supervision and evaluation of chromatography instruments and purification runs.

From hereon, UNICORN refers to compatible versions of the software. The examples given in this manual are from UNICORN 6.4.

#### **UNICORN** modules overview

UNICORN consists of four modules: *Administration*, *Method Editor*, *System Control* and *Evaluation*. The main functions of each module are described in the following table.

Module	Main functions
Administration	Perform user and system setup, system log and database administration.
Method Editor	Create and edit methods using one or a combination of:
	Predefined methods with built-in application support
	Drag-and-drop function to build methods with relevant steps
	Line-by-line text editing
	The interface provides easy viewing and editing of run properties.
System Control	Start, monitor and control runs. The current flow path is illustrated in the <i>Process Picture</i> , which allows manual interactions with the system and provides feedback on run parameters.
Evaluation	Open results, evaluate runs and create reports.
	• The default <i>Evaluation</i> module includes a user inter- face optimized for workflows like quick evaluation, compare results and work with peaks and fractions.
	• To perform operations like Design of Experiments, users can easily switch to <i>Evaluation Classic</i> .

When working with the modules *Administration*, *Method Editor*, *System Control* and *Evaluation Classic* it is possible to access descriptions of the active window by pressing the **F1** key. This can be especially helpful when editing methods

## 3.2.2 The System Control module

#### Introduction

The System Control module is used to start, view, and control a manual or method run.

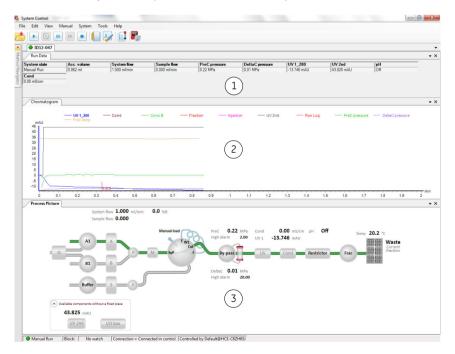
#### System Control panes

As seen in the following illustration, three panes are shown in the **System Control** module by default.

The Run Data pane (1) presents current data in numerical values.

The *Chromatogram* pane (2) illustrates data as curves during the entire run.

The current flow path is illustrated in the *Process Picture* (3), which allows manual interactions with the system and provides feedback on run parameters.



## **Note:** On the **View** menu, click **Run Log** to open the **Run Log** pane which presents all registered actions.

#### System Control toolbar buttons

The following table shows the System Control toolbar buttons that are referred to in this manual.

Button	Function	Button	Function
	<b>Open Method Navigator</b> . Opens the <b>Method Naviga-</b> <b>tor</b> where available meth- ods are listed.		<i>Run</i> . Starts a method run.
0	<i>Hold</i> . Suspends the method run, while current flow rate and valve positions are sustained.	Ш	<b>Pause</b> . Suspends the method run and stops all pumps.
	<b>Continue</b> . Resumes for example a held or paused method run.		<i>End</i> . Permanently ends the method run.
<u>~</u>	<i>Customize</i> . Opens the <i>Cus-tomize</i> dialog box where curve settings, run data groups and run log contents can be set.	Ps	<b>Connect to Systems</b> . Opens the <b>Connect to Systems</b> di- alog box where systems can be connected, and cur- rently connected users are displayed.

## 4 Installation

#### About this section

This section provides the instructions necessary to enable users and service personnel to: install the instrument, install the computer, and install the software.

Read the entire Installation chapter before starting to install the ÄKTA avant instrument.

**Note:** For information on how to how to unpack the ÄKTA avant instrument and how to lift the instrument onto a laboratory bench see ÄKTA avant Unpacking Instructions.

#### In this section

This sections contains the following subsections:

Section	See page
4.1 Site preparation	49
4.2 Hardware installation	64
4.3 Software installation	78
4.4 Start UNICORN and connect to system	79
4.5 Prime inlets and purge pump heads	82
4.6 Performance tests	100

## 4.1 Site preparation

#### Introduction

This subsection 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.

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.

#### In this subsection

Section	See page
4.1.1 Delivery and storage	50
4.1.2 Room requirements	52
4.1.3 Site environment	56
4.1.4 Power requirements	57
4.1.5 Computer requirements	59
4.1.6 Required materials	61

## 4.1.1 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 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	$1000 \times 900 \times 800$ (width × height × depth)	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%, noncondensing

#### **Equipment for transportation**

Equipment	Specifications
Pallet mover	Suitable for a lightweight pallet 80 × 100 cm
Cart for transporting the instru- ment to the lab	Dimensioned to accommodate the size and weight of the instrument

The following equipment is recommended for handling the delivery boxes:

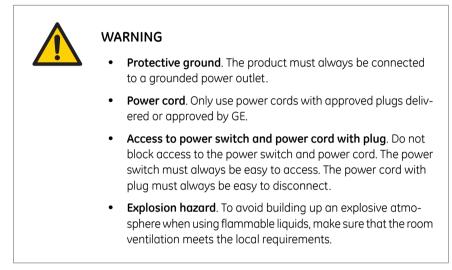
# Unpacking the ÄKTA avant instrument

For information on how to how to unpack the ÄKTA avant instrument and how to lift the instrument onto a laboratory bench see ÄKTA avant Unpacking Instructions.

## 4.1.2 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 75 cm to allow for transporting the instrument. Allow additional space for moving around corners.

#### **Space requirements**



The following illustration 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 the following topic)

4 Installation4.1 Site preparation4.1.2 Room requirements

#### 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 the following table *Equipment weight*.

4 Installation 4.1 Site preparation 4.1.2 Room requirements

#### **Equipment dimensions**



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

#### **Equipment weight**

Item	Weight
ÄKTA avant instrument	116 kg
Computer	approximately 9 kg
Monitor	approximately 3 kg
Total	approximately 130 kg

### 4.1.3 Site environment

#### Introduction

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

#### Room climate

The following requirements must be fulfilled:

- The instrument is intended for indoor use only.
- 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 following table.

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%, noncondensing
Altitude	Maximum 2000 m
Pollution degree	2

#### Heat output

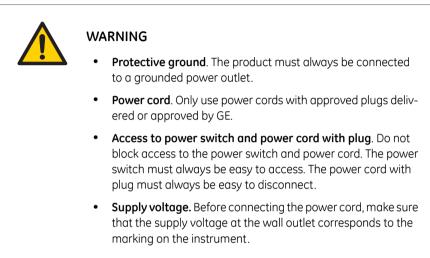
The heat output data is listed in the following table.

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

### 4.1.4 **Power requirements**

#### Introduction

This section describes the power supply requirements for the ÄKTA avant instrument.



#### Requirements

The following table specifies the power requirements.

Parameter	Requirement
Supply voltage	100-240 VAC
Frequency	50-60 Hz
Transient level	Overvoltage category II
Max power consump- tion	800 VA
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.

4 Installation4.1 Site preparation4.1.4 Power requirements

### Quality of power

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

### 4.1.5 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.

#### **General computer specifications**

The table below describes the recommended computer specifications for a UNICORN system operating with ÄKTA instruments. Installation is supported for Windows 7 Professional, 32-bit or 64-bit, with Service Pack 1.

	UNICORN Client	Database Server	Workstation installation	E-License Server
Min. free disk space	6 GB	6 GB	12 GB	500 MB
Min. available RAM	3 GB	3 GB	3 GB	2 GB
Disc format	NTFS	NTFS	NTFS	NTFS
OS	Windows 7 Professional SP1 32/64 bit	Windows 7 Professional SP1 32/64 bit Windows Server 2008/R2 64 bit	Windows 7 Professional SP1 32/64 bit	Windows 7 Professional SP1 32/64 bit Windows Server 2008/R2 64 bit
OS language	English (U.S.) Code 1033	English (U.S.) Code 1033	English (U.S.) Code 1033	English (U.S.) Code 1033
Architecture	Intel Dual Core (or faster)	Intel Dual Core (or faster)	Intel Dual Core (or faster)	Intel Dual Core (or faster)

Note:

• UNICORN is tested using an English operating system version. Using other language versions of the operating system may cause errors.

- A screen resolution of 1280x1024 or higher is recommended. Parts of the UNICORN user interface may not be displayed properly using a lower resolution.
- Changing the default font and font size in Windows may cause problems in the UNICORN user interface.

4 Installation4.1 Site preparation4.1.5 Computer requirements

- The Windows basic color scheme is recommended<sup>1</sup>.
- Using the Windows 7 Aero color scheme is not recommended.
- Windows power save features should be turned off to avoid conflicts with system operations.
- UNICORN is not compatible with the Windows 7 feature High DPI Awareness, which allows the graphic user interface to be scaled. The interface scale must remain at 100% to avoid issues with clipping and misaligning of parts of the UNICORN user interface. Normally, the scale is set at 100% by default.

<sup>1</sup> UNICORN must be closed when the color scheme is changed.

### 4.1.6 Required materials

#### Introduction

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

#### **Buffers and solutions**

The buffers and solutions listed in the following table 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, Quaternary Valve test and system test
1% acetone in distilled water	0.5 liter	Quaternary 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 in the following table 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

4 Installation4.1 Site preparation4.1.6 Required materials

### **Fraction collector tubes**

The tubes used in the built-in fraction collector must fulfill the requirements listed in the following table. Examples of manufacturers are also listed in the table.

Tube size	Diamet	er (mm)	Heigh	t (mm)	Max.	Examples of
(ml)	Min.	Max.	Min.	Max.	volume (ml)	manufacturers
3	10.5	11.5	50	56	3	NUNC™
5	10.5	11.5	70	76	5	NUNC, SARSTEDT™, Thermo Scientific™
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

#### **Deep well plates**

#### Requirements

The deep well plates used in the built-in fraction collector 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 to be used with the built-in fraction collector.

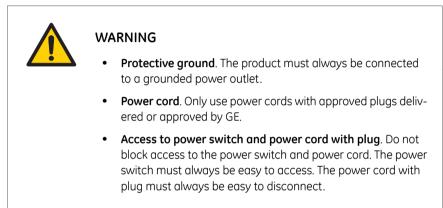
Plate type	Manufacturer	Part no.
96 deep well plate	GE	7701-5200
	BD Biosciences	353966
	Greiner Bio-One	780270
	Porvair Sciences	219009
	Seahorse Bioscience™	S30009
	Eppendorf™	951033405/0030 501.306
48 deep well plate	GE	7701-5500
	Seahorse Bioscience	S30004
24 deep well plate	GE	7701-5102
	Seahorse Bioscience	\$30024

## 4.2 Hardware installation

#### About this chapter

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

**Note:** For information on how to how to unpack the ÄKTA avant instrument and how to lift the instrument onto a laboratory bench see ÄKTA avant Unpacking Instructions.



#### In this section

This section contains the following subsections:

Section	See page
4.2.1 Install the computer equipment	65
4.2.2 Connect the system units	66
4.2.3 Prepare waste tubing	70
4.2.4 Install the Barcode Scanner 2-D and the pH electrode	73
4.2.5 Prepare the pump rinsing system	74
4.2.6 Start the instrument and the computer	77

## 4.2.1 Install the computer equipment

#### Introduction

The computer is supplied as a part of the ÄKTA avant delivery, or supplied locally.

#### **Unpacking and installing**

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



#### NOTICE

Any computer used with the equipment must comply with IEC 60950 and be installed and used according to the manufacturer's instructions.

## 4.2.2 Connect the 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

- **Power cord**. Only use power cords with approved plugs delivered or approved by GE.
- **Supply voltage.** Before connecting the power cord, make sure that the supply voltage at the wall outlet corresponds to the marking on the instrument.

#### Illustration

The following illustration shows the location of the connectors.



Part	Function
1	Power input connector
2	Network connector (Ethernet)

#### 4 Installation 4.2 Hardware installation 4.2.2 Connect the system units

Part	Function
3	UniNet-9 connectors
	<b>Note:</b> 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.

# Connect power to the ÄKTA avant instrument

Follow the instructions 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 to 240 VAC , 50 to 60 Hz.
3	Attach 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 and local printer (if used).

#### **Connect to network**

Follow the instructions to make network connections.

information on network settings.

Step	Action
1	Connect a network cable between the network connector (Ethernet) on the back of the instrument and the computer network card dedicated to ÄKTA.
	The illustration shows the symbol of the Ethernet connector.
	머미
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 and if network configuration is to be used, see UNICORN Administration and Technical Manual for further

ÄKTA avant Operating Instructions 29-1015-56 AA

## 4.2.3 Prepare waste tubing

#### Location of waste tubing

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



Part	Description
1	Waste tubing from the injection valve, the pH valve and the outlet valve (pieces of tubing marked <b>W</b> , <b>W1</b> , <b>W2</b> and <b>W3</b> ).
2	Waste tubing from the fraction collector and the buffer tray.

#### Prepare the waste tubing

2

Follow the instructions to prepare the waste tubing.

1 Place the four pieces of waste tubing from the injection valve, the pH valve and the outlet valve (pieces of tubing marked **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 fraction collector 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.

## 4.2.4 Install the Barcode Scanner 2-D and the pH electrode

#### Introduction

This section describes how to install the Barcode Scanner 2-D and the pH electrode.

#### Install the barcode scanner

Connect the cable of the Barcode Scanner 2-D to the scanner head 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 instructions to install the pH electrode.

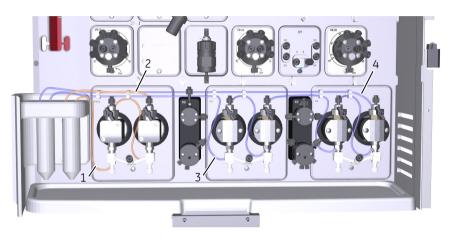
Step	Action
------	--------

- 1 Unpack the pH electrode. Make sure that the electrode is not broken or dry.
- 2 Unscrew the dummy electrode from the flow cell.
- <sup>3</sup> 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.

## 4.2.5 Prepare the pump rinsing system

# Illustration of the pump piston rinsing systems

The following illustration 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

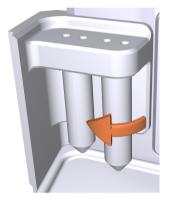
# Prime the pump piston rinsing system

1

Follow the instructions 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* 74.



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 close to the 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 close to the bottom of the rinsing solution tube.

#### 4 Installation

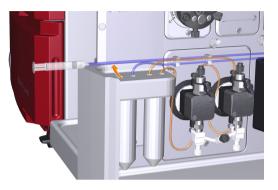
4.2 Hardware installation

4.2.5 Prepare the pump rinsing system

#### Step Action

6

Connect a 25 to 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 to 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.

## 4.2.6 Start the instrument and the computer

### Introduction

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

#### Instruction

Follow the instructions to start the instrument and the computer.

#### Step Action

1

Switch on the instrument by pressing the **Power** switch to the I position.



*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.

## 4.3 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

You can install UNICORN in one of the following configurations:

- as a complete UNICORN installation on a stand-alone workstation (full installation)
- as a UNICORN database and license server (custom installation)
- as a UNICORN software client and instrument server software on a network client station (custom installation)

You can also do the following when installing UNICORN:

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

## 4.4 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 to start UNICORN and log on to the program. A valid e-license must be available for the workstation. See UNICORN Administration and Technical Manual for more information about e-licenses.

#### Step Action

1 Double-click the UNICORN icon on the desktop.

Result: The Log On dialog box 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 box will give the option to start **System Control** without a database. Click **Start System Control** to proceed to the next **Log On** dialog box.

Step	Action
2	In the <i>Log On</i> dialog box:
	• select <i>User Name</i> and
	• enter <i>Password</i> .

#### Note:

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

Use <u>W</u> ind	ows Authentication	
<u>U</u> ser Name:	Default	
<u>D</u> omain:		
Access Group:	AccessToEverything	, ,
Start:	<u>A</u> dministration <u>M</u> ethod Editor	System Control
	ОК	Cancel Options <<

• click OK.

Result: The selected UNICORN modules open.

#### Connect to system

Follow the instructions to connect the instrument to UNICORN.

Step	Action
1	In the <b>System Control</b> module, click the <b>Connect to Systems</b> button.



Result: The Connect to Systems dialog box opens.

ystem name	Control	View
🗌 🗐 System1		۲
🗌 📕 System2		۲
📉 🔳 System3	۲	0
🖥 🔳 System4		
📄 📕 System5		
🗌 📕 System6		۲
🔄 🔳 System7		(0)

In the **Connect to Systems** dialog box:

- Select a system check box.
- Click Control for that system.
- Click OK.

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

#### Tip:

If UNICORN is unable to connect to the selected instrument, see Chapter Troubleshooting in ÄKTA avant User Manual.

2

## 4.5 Prime inlets and purge pump heads

## About this section

Before using the sample pump or system pumps it is important to do the following:

- Prime the inlets (fill the inlets with liquid).
- Purge the pumps (remove air from the pump heads).

This section 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 section

This section contains the following subsections:

Section	See page
4.5.1 Prime buffer inlets and purge system pumps	83
4.5.2 Prime sample inlets and purge Sample Pump	90
4.5.3 Prime Q inlets	95

## 4.5.1 Prime buffer inlets and purge system pumps

#### Overview

The procedure consists of the following s	stages:
---	---------

Stage	Description
1	Prime all inlet tubing to be used during the run.
2	Validate priming of inlet tubing.
3	Purge System Pump B if pressure signal indicates air bubbles.
4	Validate purge of System Pump B.
5	Purge System Pump A if pressure signal indicates air bubbles.
6	Validate purge of System Pump A.
7	End the run.
Note:	To increase life length of the pump sealing rings, make sure that the pump rinsing system is filled with fresh rinsing solution.
Tip:	The procedures for purging the pump heads and priming the inlets using the <b>Process Picture</b> , are described in the following topic. It is also possible to perform the procedures from the <b>Manual instructions</b> dialog box.

## **Prime inlet tubing**

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

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

2 Open the **System Control** module.

#### 4 Installation

4.5 Prime inlets and purge pump heads

4

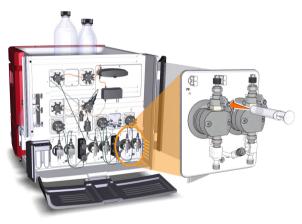
4.5.1 Prime buffer inlets and purge system pumps

Step	Action
3	In the <b>Process Picture</b> :
	• Click the inlet valve icons. (Click both the <i>Inlet A</i> and <i>Inlet B</i> icons if both inlets are to be primed.)
	• Click the position of the inlet to be filled. Fill the positions in reverse al- phabetical order and start with the highest number. For example, if all the seven inlets in Inlet Valve B are to be filled, click them in the following order: B7, B6B1, assuming that B1 is the starting buffer.



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

Connect a 25 to 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.



- 5 Open the purge valve by turning it counter-clockwise about three quarters of a turn. Draw liquid slowly into the syringe until the liquid reaches the pump.
- 6 Close the purge valve by turning it clockwise. Disconnect the syringe and discard its contents.
- 7 Repeat steps 3 to 6 for each piece of inlet tubing that is to be used during the run. In the final inlet position, draw liquid into the syringe through both purge valves.

Step	Action
8	Check that there is no air left in the pump by following the instructions in <i>Validate prime or purge of System Pump A or B or Sample Pump, on page</i> 89. If air bubbles are indicated, follow the instructions in <i>Purge System Pump B, on page</i> 85

#### Purge System Pump B

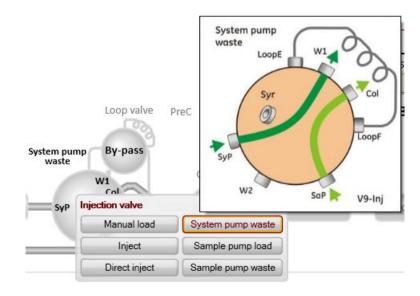
If the priming was done thoroughly and the final buffer was drawn all the way into the syringe and the validation of the priming showed that there was no air left in the pump it is not necessary to purge System Pump B.

However, if the pressure signal indicated air bubbles left in the pump, follow these instructions to purge both pump heads of System Pump B:

#### Step Action

- 1 Make sure that the piece of waste tubing connected to the injection valve port **W1** is placed in a waste vessel.
- 2 In the **Process Picture**:
  - Click the *Injection valve* icon and then click *System pump waste*.

*Result:* The injection valve switches to waste position. This is necessary to achieve a low back pressure during the purge procedure.



#### 4 Installation

4.5 Prime inlets and purge pump heads

4.5.1 Prime buffer inlets and purge system pumps

Step	Action
3	In the <b>Process Picture</b> :
	• Click the <i>Inlet valve B</i> icon.
	• Click the position of one of the inlets that will be used at the beginning of the run.

Inlet valve	B					94
-------------	---	--	--	--	--	----

Result: The inlet valve switches to the selected port.

- 4 In the **Process Picture**:
  - Click the **System pumps** icon.
  - Set *Conc % B* to 100% B and click *Set % B*.

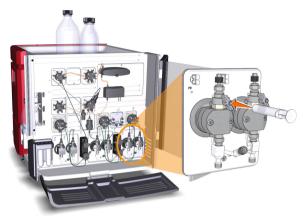
System flow				1
		1.000	ml/min	Set flow rate
0	25			High Martin
Conc % B				
_	IVI SVM	100.0	% B	Set % B
0	100	)		
Pump wash	А			в
A1 •	Start pump A wash	B1 •	Star	t pump B wash
Q1 -	Start Q inlet wash			
System wash				
	ut (W) 🔘 Injection val	ve (W1)		
<b>•</b> • • • • • • • •				
15 ml	Start system wash			
10 - 999				

- Set the *System flow* to 1.0 ml/min for ÄKTA avant 25 or 5.0 ml/min for ÄKTA avant 150.
- Click Set flow rate.

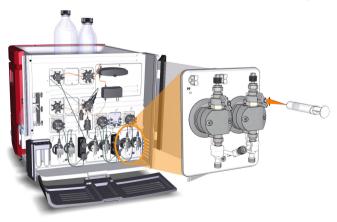
*Result:* Only System Pump B is active, and a system flow through injection valve waste starts.

#### Step Action

5 Connect a 25 to 30 ml syringe to the purge valve of the left pump head of System Pump B. Make sure that the syringe fits tightly into the purge connector.



- 6 Open the purge valve by turning it counter-clockwise about three quarters of a turn. Draw 5 to 10 ml of liquid slowly into the syringe with a rate of about 1 ml/s.
- 7 Close the purge valve by turning it clockwise. Disconnect the syringe and discard its contents.
- 8 Connect the syringe to the purge valve on the right pump head of System Pump B, and repeat steps 6 to 8. Keep the system flow running.



9

Check that there is no air left in the pump by following the instructions in Validate prime or purge of System Pump A or B or Sample Pump, on page 89.

#### 4 Installation

4.5 Prime inlets and purge pump heads

4.5.1 Prime buffer inlets and purge system pumps

#### Purge System Pump A

Purge both pump heads of System Pump A by following the same procedure as in *Purge System Pump B, on page 85*, but replace step 3 and 4 with the following:

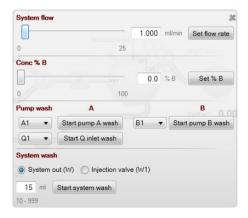
Step	Action
3	In the <b>Process Picture</b> :

- Click the *Inlet valve A* icon.
- Click the position of one of the inlets that will be used at the beginning of the run.



Result: The inlet valve switches to the selected port.

- 4 In the **Process Picture**:
  - Click the **System pumps** icon.
  - Set Conc % B to 0% B and click Set % B.



Result: Only System Pump A is active.

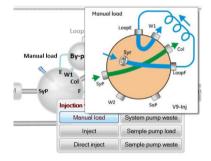
### Validate prime or purge of System Pump A or B or Sample Pump

Follow these instructions to check that there is no air left in the pump after performing a prime or a purge.

Step	Action
1	In the <b>Process Picture</b> :

• Click on the Injection valve and select Manual load.

Result: The injection valve switches to manual load position.



- 2 Make sure that the pump flow is on.
- 3 In the *Chromatogram* pane:
  - Check the **PreC pressure** curve.
  - If the *PreC pressure* do not stabilize within a few minutes there may be air left in the pump. See *ÄKTA avant User Manual*.

### End the run

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



#### 4 Installation

4.5 Prime inlets and purge pump heads

4.5.2 Prime sample inlets and purge Sample Pump

## 4.5.2 Prime sample inlets and purge Sample Pump

#### Overview

The procedure consists of the following stages:

Stage	Description
1	Prime all sample inlet tubing to be used during the run.
2	Validate priming of inlet tubing.
3	Purge the sample pump if pressure signal indicates air bubbles.
4	Validate purge of the samle pump.
5	End the run.
Note:	To increase life length of the pump sealing rings, make sure that the pump rinsing system is filled with fresh rinsing solution.

#### **Prime sample inlets**

Follow the instructions below to fill all sample inlet tubing, to be used in the run, with appropriate buffer or sample 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 samples.
2	Make sure that the waste tubing connected to injection valve port <b>W2</b> is immersed in a waste vessel.
3	Open the <b>System Control</b> module.

#### Step Action

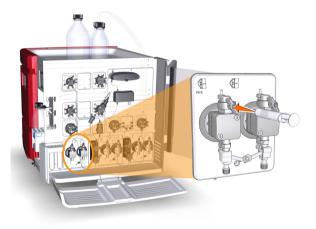
#### 4 In the **Process Picture**

- Click the Sample inlet valve icon.
- Select the position of the inlet to be filled. Start at the inlet position with the highest number and end at the position with the lowest number or the buffer position (assuming that the first sample to run is connected to inlet 1 etc.).



Result: The sample inlet valve switches to the selected port.

Connect a 25 to 30 ml syringe to one of the purge valves of the pump heads in the sample pump. Make sure that the syringe fits tightly into the purge connector.



- 6 Open the purge valve by turning it counter-clockwise about three-quarters of a turn. Draw slowly with the syringe until the sample just passes the Sample inlet valve.
- 7 Close the purge valve by turning it clockwise. Disconnect the syringe and discard its contents.
- 8 Repeat steps 2 to 5 for each sample inlet that is to be used in the method run. The final sample or the buffer from the buffer position should be drawn all the way through both pump heads into the syringe.

5

#### 4 Installation

4.5 Prime inlets and purge pump heads

4.5.2 Prime sample inlets and purge Sample Pump

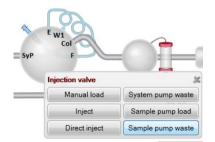
Step	Action
9	Check that there is no air left in the pump by following the instructions in <i>Validate prime or purge of System Pump A or B or Sample Pump, on page</i> 89. If air bubbles are indicated, follow the instructions in <i>Purge Sample Pump, on page</i> 92.

#### **Purge Sample Pump**

If the priming was done thoroughly and the final buffer was drawn all the way into the syringe and the validation of the priming showed that there was no air left in the pump it is not necessary to purge the sample pump.

However, if the pressure signal indicated air bubbles left in the pump, follow the instruction below to purge both the pump heads of the sample pump.

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	Make sure that the waste tubing connected to injection valve port <b>W2</b> is immersed in a waste vessel.
3	Open the <b>System Control</b> module.
4	In the <b>Process Picture</b> :
	• Click the Injection valve icon, and then click Sample pump waste.

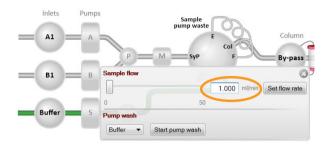


*Result*: The injection valve switches to waste position. This is necessary to achieve a low back pressure during the purge procedure.

## Step Action

#### 5 In the **Process Picture**:

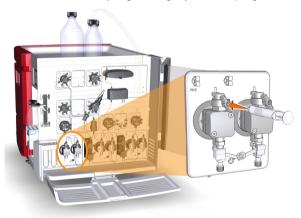
- Click the *Sample inlet* icon, then click *Buffer*.
- Click the *Sample pump* icon: Set the *Sample flow* to 1.0 ml/min for ÄKTA avant 25 or 5.0 ml/min for ÄKTA avant 150.



• Click Set flow rate.

Result: The sample pump flow starts.

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



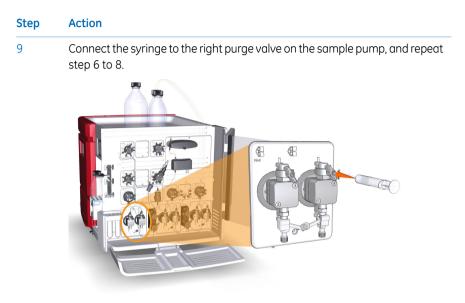
- 7 Open the purge valve by turning it counter-clockwise about three-quarters of a turn. Draw 5 to 10 ml of liquid slowly into the syringe with a rate of about 1 ml/s.
- 8 Close the purge valve by turning it clockwise. Disconnect the syringe and discard its contents.

6

#### 4 Installation

4.5 Prime inlets and purge pump heads

4.5.2 Prime sample inlets and purge Sample Pump



10 Check that there is no air left in the pump by following the instructions in *Validate prime or purge of System Pump A or B or Sample Pump, on page 89.* 

### End the run

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



## 4.5.3 Prime Q inlets

### Overview

The procedure consists of the following stages:

Stage	Description
1	Prime all Q inlet tubing.
2	Validate priming of Q inlet tubing.
3	Purge Quaternary Valve and the system pumps if pressure signal indicates air bubbles.
4	Validate purge of Quarternary Valve and system pumps.
5	End the run.

## Prime the Q inlets

Follow the instructions 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. The <b>A1</b> and <b>B1</b> positions are used for pump synchronization and these lines should already be primed.

# 4 Installation4.5 Prime inlets and purge pump heads4.5.3 Prime Q inlets

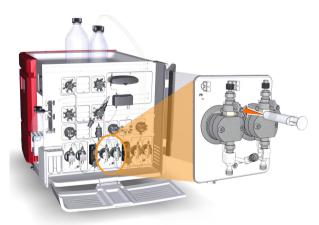
3

Step	Action
2	In the <b>Manual instructions</b> dialog box:

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

tructions:	Select	ed column type:	Selec
Pumps and pressures	Param	eters for Quaternary start concentrations Start concentration Q1 [0.0 - 100.0]	
System flow Sample flow		100.0 🚍 %	
Gradient		Start concentration Q2 [0.0 - 100.0]	
Pump wash System wash		0.0 🚆 %	
Quaternary start concentrations		Start concentration Q3 [0.0 - 100.0]	
Quaternary gradient BufferPropH		0.0 🚍 %	
Column packing flow		Start concentration Q4 [0.0 - 100.0]	
∃ Flow path ∎ Monitors	~	0.0 🔹 %	
ive result as:			Brows

- Select *Pumps and pressures:System flow* and set *Flow rate* to 0.01 ml/min.
- Click *Execute*.
- Connect a 25 to 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.



Step	Action
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 <b>Q1</b> 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 to 5 for <b>Q2</b> , <b>Q3</b> and <b>Q4</b> respectively by setting the respective <i>Quaternary start concentration</i> to 100%.
	<b>Tip:</b> The inlet tubing that is immersed in distilled water should be the last piece of inlet tubing to be primed.
	<b>Tip:</b> If you will perform a BufferPro run, end with either <b>Q1</b> or <b>Q2</b> .
7	Check that there is no air left in the pump by following the instructions in <i>Validate prime or purge of System Pump A or B or Sample Pump, on page</i> 89. If air bubbles are indicated, follow the instructions in <i>Purge Quaternary Valve and the system pumps, on page</i> 97.

# Purge Quaternary Valve and the system pumps

If the priming was done thoroughly and the final buffer was drawn all the way into the syringe and the validation of the priming showed that there was no air left in the pump it is not necessary to purge Quaternary Valve and the system pumps.

However, if the pressure signal indicated air bubbles left in the valve or the pump, follow these instructions to purge Quaternary Valve, System Pump A and System Pump B. Note that both pump heads of each system pump have to be purged.

# 4 Installation4.5 Prime inlets and purge pump heads

4.5.3 Prime Q inlets

#### Step Action

#### 1 In the *Manual instructions* dialog box:

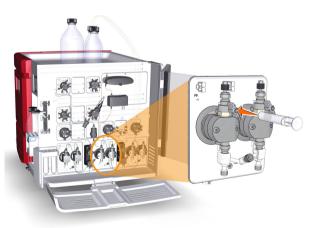
• Select *Pumps and pressures:Pump wash*, and click *All* on the *BufferPro / Q inlets* menu.

istructions:		Selected column type:	Selec
Pumps and pressures	^	Parameters for Pump wash Inlet A	
System flow Sample flow		Off	~
Gradient		Inlet B	
Pump wash System wash Quaternary start concentrations		Off	~
		BufferPro / Q inlets	
Quaternary gradient BufferPro pH		All	~
Column packing flow	~	Sample inlet	
<ul> <li></li></ul>		Off	~
ave result as:			Brows

• Click Execute.

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

- 2 Wait until the pump wash is completed.
- 3 Select **Pumps and pressures:System flow** and set **Flow rate** to 0.01 ml/min.
- 4 Connect a 25 to 30 ml syringe to the left purge valve of the selected system pump. Make sure that the syringe fits tightly into the purge connector.



Step	Action
5	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.
6	Close the purge valve by turning it clockwise. Disconnect the syringe and discard its contents.
7	Repeat steps 3 to 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 during this procedure.
8	Check that there is no air left in the pump by following the instructions in <i>Validate prime or purge of System Pump A or B or Sample Pump, on page</i> 89.

## End the run

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



## 4.6 Performance tests

## Introduction

Before taking the ÄKTA avant instrument into use, run performance tests to check the function of the equipment. See ÄKTA avant User Manual for further instructions.

# 5 Prepare the system for a run

## About this chapter

This chapter describes the preparations necessary before starting a run.

## In this chapter

This chapter contains the following sections:

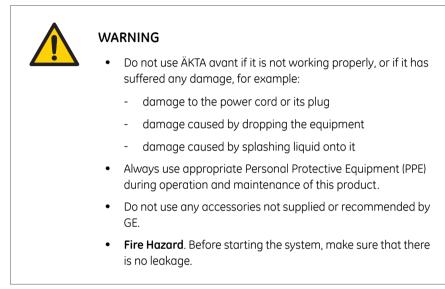
Section	See page
5.1 Before you prepare the system	102
5.2 Prepare the flowpath	104
5.3 Prime buffer inlets and purge system pumps	109
5.4 Connect a column	110
5.5 Set pressure alarms	115
5.6 Calibrate the pH monitor	117
5.7 Prepare the built-in fraction collector	119
5.8 Prepare for a run at cold temperature	125

5.1 Before you prepare the system

# 5.1 Before you prepare the system

## Introduction

It is important to prepare the system in accordance with the settings in the method to be run. Before preparing the system, check the settings in the *Method Editor* and make sure that all accessories to be used are available.



## Checklist

Remember to check the following:

- which valve ports to use for inlets and outlets
- which column type to use
- which column position to use
- which buffers and samples to prepare
- which sample application technique to use
- that the pH electrode is connected, if applicable
- which cassettes with corresponding deep well plates and/or tubes to use in the fraction collector, if applicable
- if it is a reversed phase chromatography (RPC) run



#### WARNING

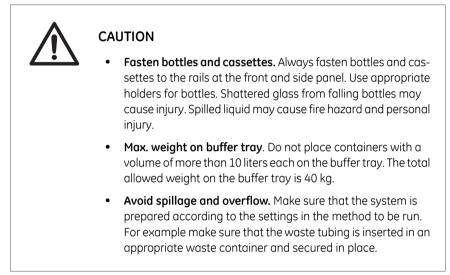
When using flammable liquids with the ÄKTA avant instrument, follow these precautions to avoid any risk of fire or explosion.

- Fraction collector. Do not fractionate flammable liquids in the built-in fraction collector. When running RPC methods, collect fractions through the outlet valve or the optional external Fraction Collector **F9-R**.
- RPC runs with 100% acetonitrile and system pressure above 5 MPa (50 bar) in ÄKTA avant 25. Always replace the green PEEK tubing between the used system pump and the pump pressure monitor with orange PEEK tubing, i.d. 0.5 mm, before running RPC with 100% acetonitrile. Set the system pressure alarm to 10 MPa (100 bar).
- RPC runs with 100% acetonitrile in ÄKTA avant 150. Always replace the beige PEEK tubing between the used system pump and the pump pressure monitor before running RPC with 100% acetonitrile. Replace it with green PEEK tubing, i.d. 0.75 mm.

# 5.2 Prepare the flowpath

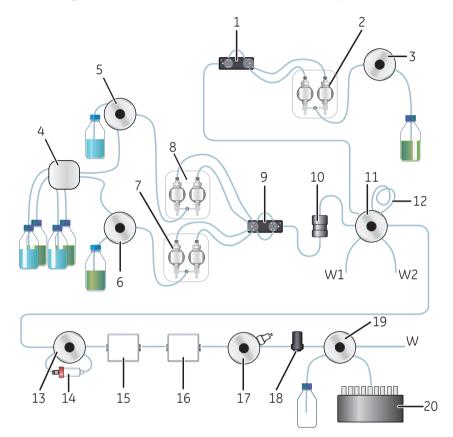
## Introduction

The flow path contains tubing, valves, pumps and monitors. This section gives an overview of the flow path and describes how to prepare the flow path before a run.



## Illustration of the flow path

The following illustration shows an overview of the standard flow path.



Part	Description
1	Pressure Monitor
2	Sample Pump
3	Sample Inlet Valve
4	Quaternary Valve
5	Inlet Valve A
6	Inlet Valve B
7	System Pump A

#### 5 Prepare the system for a run

5.2 Prepare the flowpath

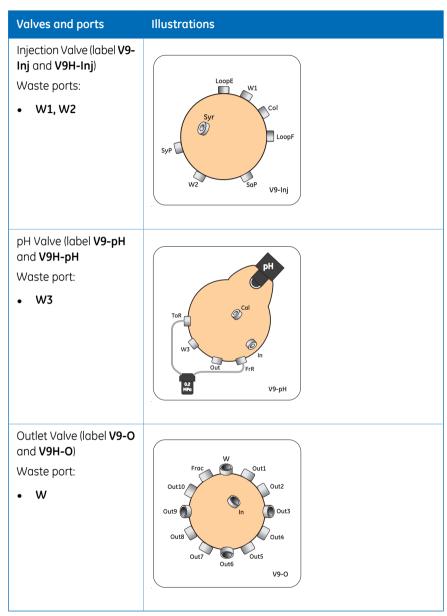
Part	Description
8	System Pump B
9	Pressure Monitor
10	Mixer
11	Injection Valve
12	Sample loop or Superloop
13	Column Valve
14	Column
15	UV Monitor
16	Conductivity Monitor
17	pH valve with pH monitor
18	Flow Restrictor
19	Outlet Valve
20	Fraction collector

## Prepare the inlet tubing

Connect inlet tubing to the inlet ports that are to be used, and immerse all inlet tubing that is to be used during the method run in the correct buffers.

#### Waste ports

The following table shows the waste ports of Injection Valve, pH Valve and Outlet Valve.



## Prepare the waste tubing

Make sure that the waste tubing is prepared according to the instructions in Section 4.2.3 *Prepare waste tubing, on page* 70.

#### Prepare the outlet tubing

Connect outlet tubing to the outlet ports of the outlet valve that are to be used during the run. If a fraction collector is to be used, make sure that tubing is connected between the outlet valve **Frac** port and the fraction collector, and prepare the fraction collector. Otherwise, immerse the outlet tubing in suitable tubes or flasks.

## Plug unused valve ports

It is recommended to plug all unused valve ports with stop plugs before starting a run. See *ÄKTA avant User Manual* for information about connectors.

# 5.3 Prime buffer inlets and purge system pumps

## Introduction

Before you start the system pumps, it is important to do the following:

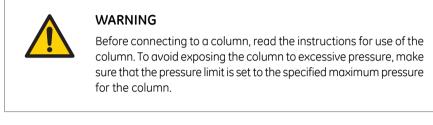
- Prime the inlets (fill the buffer inlets with liquid).
- Purge the system pumps (remove air from the pump heads).

For instructions on how to prime the inlets and purge the system pumps, see Section 4.5 *Prime inlets and purge pump heads, on page 82.* 

# 5.4 Connect a column

# Introduction

This section describes how to connect a column to the instrument using a column holder and without introducing air into the flow path. Several types of column holders are available for the ÄKTA avant instrument.



Methods automatically include a pressure alarm based on the specifications of the chosen column type. However, when running manual runs you have to set the pressure limits yourself. Also, to protect the column media, special settings are needed. See *Section 5.5 Set pressure alarms, on page 115* for more information on pressure alarms.

**Note:** Do not overtighten when connecting columns. Overtightening might rupture the connectors or squeeze the tubing and thereby result in high back pressure.

# Attach a column holder and connect a column

2

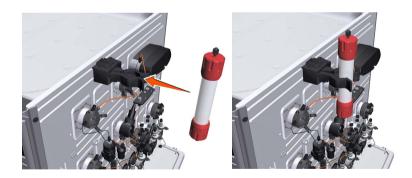
Follow the instructions to connect a column to the instrument. Always use a column holder. The column is connected to two opposite parts of the column valve, using appropriate tubing and connectors.

#### Step Action

1 Attach an appropriate column holder to the rail on the instrument.



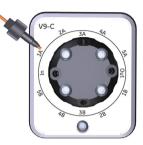
Attach the column to the column holder.



3

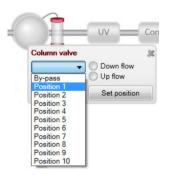
#### Step Action

Connect a suitable tubing to a column valve port, for example port **1A** if column position 1 was chosen in the method to be run.



4 In the **Process Picture**:

- Click the Column valve icon.
- Click, e.g., *Position 1* and *Down flow*.



*Result:* The column valve switches to position **1**.

# Step Action

#### 5 In the **Process Picture**:

- Click the **System pumps** icon.
- Enter a low System flow (e.g., 0.2 ml/min).
- Click Set flow rate.

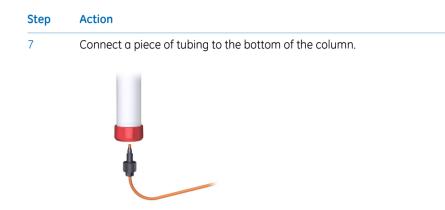
System flow				6
<b></b>	Manual Inad	0.200	ml/min	Set flow rate
0	25			
Conc % B		W1		
	SYP	0.0	% B	Set % B
0	100			
Dumn wach	٨	11		R

Result: A system flow of 0.2 ml/min starts.

6

When buffer leaves the tubing on port **1A** (if port **1A** was chosen in the method to be run) in a continuous mode and the top part of the column is filled with buffer, connect the tubing to the top of the column.





When buffer leaves the tubing at the bottom of the column in a continuous mode, connect this piece of tubing to the column valve. Use the port opposite to the one already connected to the column, in this example port **2B**.



9

8

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



# 5.5 Set pressure alarms

## Introduction

The columns can be protected by two different types of pressure alarms:

- The precolumn pressure alarm protects the column hardware.
- The delta-column pressure alarm protects the column media.

Column Valve (label **V9-C** and **V9H-C**) have built-in pressure sensors that automatically measure the precolumn and delta-column pressure.

See the instructions in the next topic to set the pressure alarm for the column to be used in the run and, if applicable, to set the parameters for the tubing dimensions.

Note: Remember to lower the system pressure alarm and sample pressure alarm if the optional UV Monitor U9-L and/or the optional second Conductivity Monitor C9 is used on the high pressure side in the system (before the column[s]). The UV Monitor U9-L flow cells has a maximum pressure limit of 2 MPa (20 bar) and the second Conductivity Monitor C9 flow cell has a maximum pressure limit of 5 MPa (50 bar).



### NOTICE

**UV and conductivity flow cells on the high pressure side**. When placing UV and/or conductivity flow cells on the high pressure side of the column, the UV flow cell has a maximum pressure limit of 2 MPa (20 bar) and the conductivity flow cell has a maximum pressure limit of 5 MPa (50 bar).



#### NOTICE

Remember to lower the system pressure alarm and sample pressure alarm if the optional UV Monitor **U9-L** and/or the optional second Conductivity Monitor **C9** is used on the high pressure side in the system (before the column[s]). The UV Monitor **U9-L** flow cells has a maximum pressure limit of 2.0 MPa (20 bar) and the second Conductivity Monitor **C9** flow cell has a maximum pressure limit of 5.0 MPa (50 bar).

## Precolumn pressure alarms

It is important that the precolumn pressure alarm is set during all runs where a column is used. The pressure alarm can be set in: the method to be run, the **System Settings** dialog box, or during a manual run.

Precolumn pressure alarm limits are automatically set in the method when a column from the column list is selected in the method. Refer to *UNICORN Method Manual* for more information on pressure alarms.

#### Set pressure alarms

Pressure alarm limits may be set manually in **System Control**. The example below describes how to set the high pressure limit for the column. Other alarms are set in a corresponding way.

#### Step Action

4

1 In the System Control module, on the Manual menu, click Execute Manual Instructions .

Result: The Manual instructions dialog box opens.

2 In the *Instructions* box, select *Alarms:Alarm pre column pressure*.

Instructions:	Selected column type:Instruction execution list:	
Pumps     Flow path     For path     Forior collection     Arms     Adam system pressure     Adam cystem pressure     Advectem pres	Personales for Alamo pre source         Intent         Debits           O De-added         © E-nabled         Intent         Debits           U Do-added         0.00 - 20.001         Intent         Debits           Low starm         0.00 - 20.001         Intent         Debits           0.30 ± MPa         0.00 ± 0.001         Intent         Debits	
Save result as:	Bowse	

- 3 Click *Enabled* in the *Mode* field.
  - Enter the high pressure limit in the *High alarm* box.
    - Click Execute.

# 5.6 Calibrate the pH monitor

## Introduction

If pH will be measured during the chromatographic run, the pH monitor should be calibrated before the run is started. Use two pH calibration buffers with a difference of at least one pH unit. Preferably use a pH standard buffer pH 4 or 7 as the first calibration point, and a pH standard buffer close to the lowest or highest pH you need to measure as your second point. Allow the buffers to reach the operating temperature before use.

**Note:** Do not run a system flow during pH calibration.

# Calibrate the pH monitor

1



#### CAUTION

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

Follow the instructions to perform the calibration.

#### Step Action

Open the **System Control** module. On the **System** menu, click **Calibration**. *Result*: The **Calibration** dialog box opens.

nitor to calibrate: pH Calibration procedure		V Curren	it value	6.027
Prepare for calibration				
Calibration for pH electrode buffer 1.		[0-14]		Description
pH for buffer 1	7	[0 (4)	Calibrate	pH' calibrates the pH electrode with two point calibration, i.e., using two pH calibration solutions. It
		[0-14]		can also be used to fill the pH cell with storage solution since the pH valve is in calibration position.
pH for buffer 2	9		Calibrate	For calibration, preferably use pH standard buffers pH 4 or 7 as the first calibration point and a pH
ast calibrated on: 2009-01-13 10:52:07 +01:00				standard buffer close to the lowest or highest pH you need to measure as your second point.
Calibrated electrode slope; %	92.391			Parameters
Assymetry potential at pH 7; mV	-19.05			'pH for buffer 1'
				'pH for buffer 2'

- 2
- Set the pH monitor as the monitor to calibrate by clicking **pH** on the **Monitor to calibrate** menu.

# 5 Prepare the system for a run 5.6 Calibrate the pH monitor

Step	Action
3	Click <b>Prepare for calibration</b> .
	<i>Result</i> : The pH valve switches to the calibration position.
4	Enter the pH of the first pH standard buffer in the <b>pH for buffer 1</b> box.
5	Fill a syringe with approximately 10 ml of the first pH standard buffer. Con- nect the syringe to the Luer connector in pH valve port <b>Cal</b> , and inject the buffer.
6	When the <i>Current value</i> is stable, click <i>Calibrate</i> .
7	Wash the pH flow cell by injecting water into pH valve port <b>Cal</b> using a new syringe.
8	Enter the pH of the second pH standard buffer in the <b>pH for buffer 2</b> box.
9	Repeat steps 5 to 6 using the second pH standard buffer. <i>Result:</i> The calibration date and time are displayed in the dialog box, and also values for <i>Calibrated electrode slope</i> and <i>Asymmetry potential at pH</i> <b>7</b> .
10	Is the <b>Calibrated electrode slope</b> $\ge$ 80% and the <b>Asymmetry potential at <i>pH</i> <b>7</b> inside the interval ±60 mV?</b>
	<ul> <li>If Yes: Click <i>Close</i> to switch the pH valve back to the default position, and to close the <i>Calibration</i> dialog box.</li> </ul>
	• If No: Clean the pH electrode, and repeat the calibration procedure. If this does not help, replace the electrode. For information about cleaning and replacing the pH electrode, see ÄKTA avant User Manual Chapter Maintenance.

# 5.7 Prepare the built-in fraction collector

## Introduction

This section describes how to prepare the built-in raction collector. For information regarding the types of deep well plates, tubes and cassettes, see ÄKTA avant User Manual.



#### WARNING

**Fraction collector**. Do **not** fractionate flammable liquids in the built-in fraction collector. When running RPC methods, collect fractions through the outlet valve or the optional external Fraction Collector **F9-R**.

### Prepare the fraction collector

Before starting to prepare the built-in fraction collector, check the fractionation settings in the method to be run. Perform the steps described below according to the settings in the method.

- Insert the cassette tray or a rack for tubes or bottles.
- Change the *System Settings* in UNICORN to set the fractionation mode and other settings for fraction collection.

How to insert a tray or a rack is shown in the following topic.

For information on how to change the **System Settings** before a run, see UNICORN System Control Manual. The available **System Settings** are described in ÄKTA avant User Manual. 5.7 Prepare the built-in fraction collector

# Prepare and insert the cassette tray

Follow the instructions to prepare the fraction collector before a run.

#### Cassettes and cassette tray

#### Step Action

1

2

If you are to use cassettes with the QuickRelease function, first open the cassettes.



Place the tubes and deep well plates in the cassettes. Make sure that the deep well plates are rotated so that the well marked **A1** is positioned above the **A1** marking on the cassette.

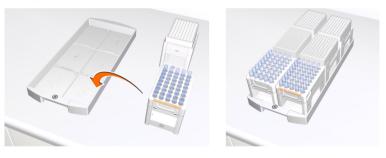




3 Close the cassettes that have the QuickRelease function.



Place the cassettes on the cassette tray. Make sure that the cassette type code (see the illustration) faces the front of the tray marked with the GE monogram.



5

4

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



5.7 Prepare the built-in fraction collector

6

#### Step Action

Place the cassette tray on the tray support of the fraction collector drawer. Make sure that the front of the tray (marked with the GE monogram) faces the front of the drawer and is hooked onto the two pins.



7 Close the drawer. Make sure that it snaps into closed position.

*Result*: After the door has been closed, the fraction collector arm scans the cassette type code of each cassette to identify the cassette types. If deep well plates are used, the instrument also identifies the types of deep well plates.

#### Rack for 50 ml tubes and rack for 250 ml bottles

Step	Action
1	Place 50 ml tubes or 250 ml bottles in the corresponding rack.
2	Open the fraction collector drawer by pressing the handle upwards, and pulling out the drawer.



#### Step Action

<sup>3</sup> Place the rack on the tray support of the fraction collector drawer. Make sure that the front of the rack (marked with the GE monogram) faces the front of the drawer and is hooked onto the two pins.



#### Note:

The cassette tray shall not be used when the rack for 50 ml tubes or the rack for 250 ml bottles is placed in the fractionation collector drawer.

4 Close the drawer. Make sure that it snaps into closed position.

#### **Cassette and tray identification**

After the fraction collector drawer has been closed, the fraction collector arm scans the cassette type code of each cassette or tray to identify the cassette types. If deep well plates are used, the instrument also identifies the types of deep well plates.



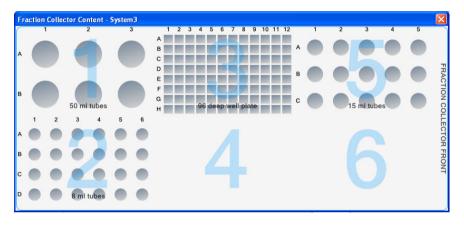
#### WARNING

Moving parts in fraction collector. Do not open the fraction collector drawer when the fraction collector is active. If you need to access the fraction collector, press *Pause*, and make sure that the movement has stopped before opening the drawer.

#### 5.7 Prepare the built-in fraction collector

## View fraction collector content

To view the content of the Fraction collector, open the *System control* module. On the *View* menu, click *Fraction Collector Content*.



# 5.8 Prepare for a run at cold temperature

## Introduction

To fit the ÄKTA avant instrument in a cold cabinet, the foldable door and pump cover can be removed. For instructions, see *ÄKTA avant User Manual*. When using the instrument in a cold room or cold cabinet, make sure to follow the precautions listed in the next topic.

# Precautions concerning runs in a cold temperature

	NOTICE	
	<ul> <li>Avoid condensation. If ÄKTA avant is kept in a cold room, cold cabinet or similar, keep it switched on in order to avoid conden- sation.</li> </ul>	
	<ul> <li>Avoid overheating. If ÄKTA avant is kept in a cold cabinet and the cold cabinet is switched off, make sure to switch off ÄKTA avant and keep the cold cabinet open to avoid overheating.</li> </ul>	
	• Place the computer in room temperature. If the ÄKTA avant instrument is placed in a cold room, use a cold room compat- ible computer or place the computer outside the cold room and use the Ethernet cable delivered with the instrument to connect to the computer.	
Note:	When the instrument is kept in a cold room, it is important to tighten all tubing connectors, also the inlet manifold connectors. Otherwise air might get into the flow path.	
Note:	Make sure that the instrument, buffers and sample have had time to reach the ambient temperature. When the instrument has reached the ambient temperature, calibrate all pressure sensors.	
Tip:	When runs are performed in a cold cabinet, make sure to adjust the target temperature of the built-in fraction collector temperature control function. The target temperature is 20°C by default. Settings for the temperature control function can be edited in the <b>System Settings</b> dialog box of <b>System Control</b> or in the <b>Text Instructions</b> pane in <b>Method Editor</b> .	

# 6 Run a method

## About this chapter

This chapter describes how to start up and run a method, and also how to handle the system after the run.

## In this chapter

This chapter contains the following sections:

Section	See page
6.1 Before you start	127
6.2 Applying the sample	130
6.3 Start a method run	133
6.4 Monitor the run	139
6.5 After run procedures	142

# 6.1 Before you start

## Introduction

Before starting a run, it is necessary to read and understand the information in this section and to perform the checks listed in the next topic.



#### WARNING

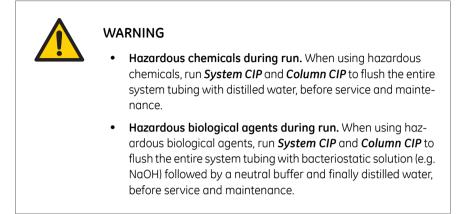
- Always use appropriate Personal Protective Equipment (PPE) during operation and maintenance of this product.
- Hazardous substances. When using hazardous chemicals, take all suitable protective measures, such as wearing protective glasses and gloves resistant to the substances used. Follow local and/or national regulations for safe operation and maintenance of the product.
- **High pressure**. The product operates under high pressure. Wear protective glasses and other required Personal Protective Equipment (PPE) at all times.

### Checklist

Make sure that the system is correctly prepared:

- Prepared the system according to the settings in the method to be run.
- Select a suitable column for the application.
- Immerse the buffer inlet tubing in correct buffer vessels.
- Immerse all waste tubing in appropriate waste vessels (consider vessel size, placement and material).
- Make sure no tubing is twisted and that the flow path is free from leakage.

# Warnings concerning use of hazardous substances



### Hold, pause or stop the run

At the end of a method the run stops automatically. All pumps stop and an acoustic end signal sounds and *End* is displayed in the *Run Log*.

To interrupt a method during a run you may click the *Hold*, *Pause* or *End* buttons in *System Control*. A held or paused method run can be resumed by clicking the *Continue* button. See the instructions in the following table.

If you want to	then
temporarily hold the method, with current flow rate and valve positions sustained	click the <i>Hold</i> button.
temporarily pause the method, and stop all pumps	click the <b>Pause</b> button.

If you want to	then
resume, for example, a held or paused method run.	click the <b>Continue</b> button.
	Note:
	An ended method cannot be continued.
permanently end the run	click the <b>End</b> button.

*Note:* When ending a method run in advance, it is possible to save the partial result.

# 6.2 Applying the sample

## Introduction

A number of different sample application techniques are available. The sample can be applied either directly onto the column using the sample pump, or via a loop. A loop can be filled either manually or by use of the sample pump. This section describes sample application using a syringe to manually fill a sample loop. The two stages of the sample application are described in the following table. For detailed instructions and information regarding the different sample applications techniques, see *ÄKTA avant User Manual*.

Stage	Description
Load	The sample loop is filled with sample.
Inject	The sample is injected onto the column.

### How to fill a sample loop

Follow the instructions to fill the sample loop with sample.

Ste	D	Action
Sec	P	/////

1 Connect a suitable sample loop to the Injection Valve ports **LoopF** (fill) and **LoopE** (empty).



2 Fill a syringe with sample.

#### Step Action

- 3
- Connect the syringe to the Injection Valve port Syr.



4 Load sample into the sample loop. To avoid sample loss due to siphoning, leave the syringe in the port until the sample has been injected onto the column during the run.

#### Tip:

It is recommended to overload the loop to make sure that the loop is completely filled. Excess of sample will leave the valve through port **W1**.

# Sample application through a sample loop

A sample loop is manually filled with sample using a syringe connected to the Injection Valve port **Syr**. During the method run, the sample is automatically injected onto the column. The loop is emptied and washed out using buffer from the system pumps. The total buffer volume to be used for emptying and washing the sample loop is set in the **Phase Properties** tab of the **Sample Application** phase in the **Empty loop with** box.

Phase Properties	Text Instructions iT		
Sample Application Use the same flow rate Flow rate 10.000 ml/	e as in Method Settings /min [0.000 - 25.000]		
<ul> <li>Inject sample from loop</li> </ul>	p Fill the loop using	Manual load 🛛 🗸	Wash sample pump with buffer
O Inject sample directly of	Loop type	Capillary loop 🛛 🗸	Prime sample inlet with 6.00 ml
O mject sample directly on	Sample inlet	S1 💌	Wash sample pump with buffer after sample application.
	Fill loop with	0.60 ml	Note: The system will be paused during wash
	Empty loop with	1.00 ml	pouses during wash
	Sample volume	0.00 ml	
	Use the same	e inlets as in Method Settings	
	Inlet A A1	*	
	Inlet B B1	▼ 0.0	%
	Fill the system	n with the selected buffer	



Empty the sample loop with a buffer volume that exceeds the volume of the loop. This will ensure that the loop is completely emptied.

# 6.3 Start a method run

### Introduction

This section describes how to start a run using a previously created method. If **Column Logbook** was enabled during installation of the software, registration and selection of individual columns is possible at method start. For further information on method creation, please refer to UNICORN Method Manual.

#### Choose and start a method

The following instructions describe how to open a method and start a run.

Step	Action
1	Open the <b>System Control</b> module and click the <b>Open Method Navigator</b> button.



Result: The Method Navigator pane opens.

Method Navig	ator				Φ×
b 🖨	Methods, Method	• 📰 •			
Folder name		System	Last modified	Created by	*
· · 😑	oeMethod		2014-10-06 14:3	Default	
😑 📄	Elvis		2012-11-27 14:5	Default	
	AutoTests 2013-0	5-31	2013-05-31 12:0	Default	
	Elvis1		2012-11-27 14:5	Default	

2

Select the method to run, and click the **Run** button.



Result: The Start Protocol dialog box opens.

3 Step through the displayed pages in the *Start Protocol*, add requested input and make appropriate changes if necessary. Click *Next*.

#### 6 Run a method 6.3 Start a method run

Step	Action
4	Click <b>Start</b> on the last page of the <b>Start Protocol</b> .

Result:

• If column logging was chosen at installation of UNICORN and a column type was selected at method creation, the *Select Columns* dialog box opens. Continue with the steps outlined in the next topic.

lect Columns - System:	1		
Enter or select a column indivi	dual: Code lot	exp. ID	
Enter ID:	[	and the second	ear
Select ID: 26	3-9288-13 12345678 0000-00	0005, HiPrep 261 💉 🚺 Ne	w
Disable column logging for	this run		
Apply to all methods with	the same column type		
Apply to all methods with Methods	the same column type Remark	Column Barcode	Column type
Methods		Column Barcode	Column type HiPrep 26/18 Desalting
Methods Column Handling	Remark	Column Barcode	
	Remark Scouting run 1	Column Barcode	HiPrep 26/10 Desalting
Methods Column Handling Column Handling	Remark Scouting run 1 Scouting run 2	Column Barcode	HiPrep 26/10 Desalting HiPrep 26/10 Desalting
Methods Column Handling Column Handling	Remark Scouting run 1 Scouting run 2	Column Barcode	HiPrep 26/10 Desalting HiPrep 26/10 Desalting
Methods Column Handling Column Handling	Remark Scouting run 1 Scouting run 2	Column Barcode	HiPrep 26/10 Desalting HiPrep 26/10 Desalting
Methods Column Handling Column Handling	Remark Scouting run 1 Scouting run 2	Column Barcode	HiPrep 26/10 Desalting HiPrep 26/10 Desalting

• If column logging was *not* chosen at installation of UNICORN and/or *no* column type was selected at method creation, the run starts directly.

# Register a column and start a run

The following instructions describe how to register a column and start a run.

Step	Action
1	Is the column to be used already registered?
	• If No, continue to step 2.
	• If Yes, continue to step 5.
	Select Columns - System3
	Enterorselect a column individual: Code lot exp. ID
	Enter ID: Clear
	O Select ID: 28-9288-13 12345678 0000-00 0005, HiPrep 261 ✓ New

Methods	Remark	Column Barcode	Column type
Column Handling	Scouting run 1		HiPrep 26/10 Desalting
Column Handling	Scouting run 2		HiPrep 26/10 Desalting
Column Handling	Scouting run 3		HiPrep 26/10 Desalting

In the Select Columns dialog box, click New.

O Disable column logging for this run

Result: The first New Column dialog box opens.

New Column					×
Column ID:	Code	lot	exp.		Clear
columnity.	The Column H	nas a uniTag	(has a fixed C	ode and exp	
			Ca	Intinue	Cancel

2

Step	Action
3	Register the column using the Barcode Scanner 2-D as follows:
	• Make sure that the pointer is placed in the first position of the <i>Code</i> box.

- Point the Barcode Scanner 2-D towards the data matrix tag on the column.
- Press and hold the trigger to create a beam.
- When the scanner beeps, the column ID is registered and displayed in the dialog box.



- Alternatively, manually enter the column ID, that you find on the column label, in the dialog box using your keyboard.
- Click Continue.

Result: The expanded New Column dialog box opens.

New Column					
Column ID:	Code	lot	exp.	ID	
columnity.				Code and exp.)	
Alias (optional):					
Technique:	Desalting				~
Column type:	HiPrep 26/10 D	esalting			~
🔲 Use medium	batch ID:		🔽 Set med	ium expiration date	
			den 18 fe	bruari 2009	~
Notes			C	ОК	Cancel

Step	Action
4	In the expanded <b>New Column</b> dialog box:
	• Enter an column alias in the <i>Alias</i> box (optional).
	Click a chromatography technique on the <i>Technique</i> menu.
	Click a column type on the <i>Column type</i> menu.
	<ul> <li>Select the Set medium expiration date check box and click a date on the menu.</li> </ul>
	Click OK.
	Tip:
	Alias can be used for easy identification of a column.
	Result: The entered information is saved and the dialog box closes.

#### 6 Run a method 6.3 Start a method run

#### Step Action

5 In the **Select Columns** dialog box:

- Click Enter ID.
- Use the Barcode Scanner 2-D (see step 3) to enter the column ID.

Enter ID:	Code lot 9-9288-13 12345678 0	exp. ID 0000-00 0005 Clear	
) Select ID: 28	3-9288-13 12345678 0000-00 0	0005, HiPrep 261 🗸 New	
) Disable column logging for	this run		
	he same column tune		
Apply to all methods with t			
Apply to all methods with t Methods	Remark	Column Barcode	Column type
Methods		Column Barcode 28-9298-13 12345678 0000-00 0005	Column type HiPrep 26/10 Desalting
Methods Column Handling	Remark		the second second second second
	Remark Scouting run 1		HiPrep 26/10 Desalting
Methods Column Handling Column Handling	Remark Scouting run 1 Scouting run 2		HiPrep 26/10 Desalting HiPrep 26/10 Desalting

• Alternatively, click *Select ID* and click the column individual to be used in the run from the menu.

Enter or select a column individ	lual: Code lot	exp. ID	
Enter ID:		- Clear	
	-9288-13 28928813 0000-00 1		
Disable column logging 28 28 28 28	9288-13 12345678 0000-00 0 9288-13 28928813 0000-00 1	1005, HiPrep 2610 Desalting E 1006, HiPrep Desalting 2610 E 234, HiPrep 2610 Desalting E	
5	Bemark		
Methods	Hemark	Column Barcode	Column type
	Scouting run 1	28-9288-13 28928813 0000-00 1234	Column type HiPrep 26/10 Desalting
Column Handling			
Methods Column Handling Column Handling Column Handling	Scouting run 1		HiPrep 26/10 Desalting

• Click OK.

*Result:* The run starts. All necessary actions occur automatically according to the method, including ending of the run.

# 6.4 Monitor the run

#### Introduction

You may follow the on-going method run in the **System Control** module. The current system status is shown in the **System state** panel in the **Run Data** pane. For example, it may state **Run**, **Wash** or **Hold**. The same information is also shown on the Instrument display.

- Selected curves are shown in the Chromatogram pane.
- All registered actions during the run are displayed in the *Run Log* pane.
- The current flow path is shown in the *Flow Scheme* pane.

For an overview of the **System Control** interface, see Section 3.2.2 The System Control module, on page 46.

#### Monitor the run

To interrupt a method during a run you may click the *Hold*, *Pause* or *End* buttons in *System Control*. A held or paused method run can be resumed by clicking the *Continue* button. See the following table.

If you want to	then
temporarily hold the method, with current flow rate and valve positions sustained	click the button.
temporarily pause the method, and stop all pumps	click the button.
resume, for example, a held or paused method run.	click the button.
	Note:
	An ended method cannot be resumed.
permanently end the run	click the button.

**Note:** When ending a method run in advance, it is possible to save the partial result.

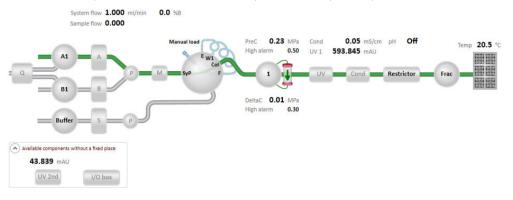
More information regarding UNICORN capabilities during the method run is available in the UNICORN System Control Manual.

### **Process Picture**

The *Process Picture* displays the current flow path, run parameters and real-time data from monitors during a run. It also allows manual interactions with the system.

Tubing colors indicate flow path states, as shown in the following illustration and described in the following table.

Modules without a fixed place in the system are shown in a panel below the process picture (modules are called components in the process picture).

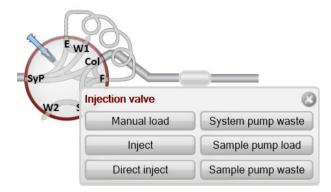


Color	Indication
Green	Open flow path with flow.
Grey	Closed flow path or an open path without flow.
Blue	Syringe port in loop open for manual injection.

# Actions in the Process Picture pane

It is possible to interact with the *Process Picture* pane.

• To open a related instruction, click the component icon. The example below shows the pop-up toolbar for the *Injection valve* icon. Instructions can be given from the pop-up toolbar of each component icon.



• To display a detailed picture with explanations, for example for a valve, right-click the component icon and click *Detailed picture*.

# 6.5 After run procedures

## Introduction

This section describes how to clean the instrument and columns after a chromatographic run, and how to prepare the system for storage.

The instrument and the columns should be cleaned between the runs. This will prevent, for example, sample contamination, protein precipitation and column clogging. If the instrument is not going to be used for a couple of days or longer, the instrument, columns and the pH flow cell should be filled with storage solution. For further information about cleaning and maintenance procedures, see *Chapter 7 Maintenance, on page 145*.

To clean and fill the instrument and columns with storage solution, use the **System CIP** and **Column CIP** methods. Either as separate, predefined methods or as phases included in a chromatographic method.



Tip:

#### WARNING

**Corrosive chemicals during maintenance.** If the system or column is cleaned with a strong base or acid, flush with water afterwards and wash with a weak neutral buffer solution in the last step or phase.

## System cleaning

After a method run is completed, perform the following:

- Rinse the instrument with one or several cleaning solution(s) (e.g., NaOH, buffer solution or distilled water) using the **System CIP** method.
- If applicable, empty the fraction collector.
- Clean all spills on the instrument and on the bench using a moist tissue.
- Empty the waste vessel.
- Clean the manual injection port of the injection valve, see ÄKTA avant User Manual for detailed instructions.
- If applicable, clean the pH electrode manually and make sure to leave it in an appropriate buffer. See ÄKTA avant User Manual for detailed instructions.

#### System storage

If the instrument is not going to be used for a couple of days or longer, also perform the following:

• Fill the system and inlets with storage solution (e.g., 20% ethanol) using the *System CIP* method.

#### **Column cleaning**

After a method run is completed, perform the following:

• Clean the column with one or several cleaning solution(s) using the **Column CIP** method.

#### **Column storage**

If the column is not going to be used for a couple of days or longer, also perform the following:

• Fill the column with storage solution (e.g., 20% ethanol) using the Column CIP method.

### pH electrode storage

If pH monitoring will not be used for a week or longer, perform one of the following actions:

- Inject new storage solution into the pH flow cell.
- Replace the pH electrode with the dummy electrode that is installed in the pH valve on delivery.

In the following situations, in order to increase the lifetime of the pH electrode, use the *By-pass* position and store the electrode in storage solution inside the pH flow cell:

- pH monitoring is not needed during the run.
- Organic solutions are used.
- Extremely acidic or extremely basic solutions are used.

For further information on how to prepare the pH electrode for storage, refer to ÄKTA avant User Manual.

## Log off or exit UNICORN

Follow the instructions to log off or exit UNICORN. This can be performed from any of the UNICORN modules.

If you want to	then
log off UNICORN	on the <i>File</i> menu, click <i>Log off</i> .
exit UNICORN	on the <i>File</i> menu, click <i>Exit UNICORN</i> .
Note: If an edited method or result is open and not saved when you try to exit or log	

Note: If an edited method or result is open and not saved when you try to exit or log off UNICORN, you will see a warning. Click **Yes** to save, **No** to exit without saving, or **Cancel** to stay logged on.

# Shut down the instrument

Switch off the instrument by pressing the **Power** switch to the **O** position.



# 7 Maintenance

## About this chapter

This chapter provides schedules for preventive maintenance that should be performed by the user of the ÄKTA avant instrument. Regular maintenance is essential for reliable function and results. Refer to the *ÄKTA avant User Manual* for detailed instructions. Procedures that require special attention are also described in this chapter.



#### WARNING

Always use appropriate Personal Protective Equipment (PPE) during operation and maintenance of this product.

## In this chapter

This chapter contains the following sections:

Section	See page
7.1 Maintenance program	146
7.2 Replace pump piston seal, O-rings, and rinse membrane of Pump P9 or Pump P9H	149
7.3 Replace pump piston seal, O-ring, and rinse membrane of Pump P9-S	160
7.4 Replace pump pistons	168
7.5 Clean pump head check valves	170

# 7.1 Maintenance program

# Introduction

An overview of the preventive maintenance to be performed on the ÄKTA avant instrument is outlined in the following list. See *ÄKTA avant User Manual* for detailed information about the maintenance procedures.

Maintenance is divided into:

- Daily maintenance
- Weekly maintenance
- Monthly maintenance
- Bi-annual maintenance
- Maintenance when required



## WARNING

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

## Periodic maintenance program

The following periodic maintenance should be performed by the user of the ÄKTA avant instrument.

Interval	Maintenance action
Daily	Calibrate the pH monitor
Weekly	Change pump rinsing solution
Weekly	Replace inline filter in the mixer
Weekly	Clean the fraction collector
Monthly	Check the flow restrictor
Bi-annual	Clean the UV flow cell
Bi-annual	Replace the pH electrode

# Maintenance when required

The following maintenance should be performed by the user of the ÄKTA avant instrument when required. Refer to the ÄKTA avant User Manual for detailed instructions.

Maintenance action
Clean the instrument externally
Run System CIP (System cleaning-in-place)
Run Column CIP (Column cleaning-in-place)
Clean the fraction collector
Replace tubing and connectors
Storage of pH electrode
Clean the pH electrode
Clean the conductivity flow cell
Calibrate the conductivity monitor
Calibrate the UV monitor
Calibrate pressure monitors
Replace the mixer
Replace O-ring in mixer
Replace the UV flow cell
Replace the flow restrictor
Replace inlet filters
Wipe off excess oil from pump heads
Clean the check valves. See Section 7.5 Clean pump head check valves, on page 170
Replace check valves
Replace pump piston seal, O-rings, and rinse membrane. See Sec- tion 7.2 Replace pump piston seal, O-rings, and rinse membrane of Pump P9 or Pump P9H, on page 149 and Section 7.3 Replace pump piston seal, O-ring, and rinse membrane of Pump P9-S, on page 160.
Replace pump pistons. See <i>Section 7.4 Replace pump pistons, on page 168</i> .

Maintenance action

Replace pump rinsing system tubing

Replace valve modules

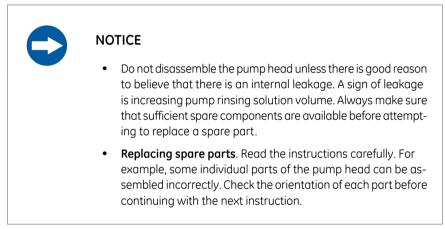
# 7.2 Replace pump piston seal, O-rings, and rinse membrane of Pump P9 or Pump P9H

# Introduction

Follow the instructions to replace the O-rings, piston seal, and rinse membrane of pumps **P9, P9H A**, **P9H B**, or **P9H S**.

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

*Tip:* A sign of internal leakage is that the pump rinsing solution volume starts to increase.



## Maintenance interval

Replace the O-rings, piston seals, and rinse membranes the pumps **P9**, **P9H A**, **P9H B**, and **P9H S** if they are damaged. After replacement, perform a run to break in the new piston seals.



## NOTICE

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

## 7 Maintenance

7.2 Replace pump piston seal, O-rings, and rinse membrane of Pump P9 or Pump P9H

# **Required material**

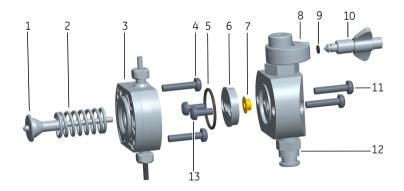
The following materials are required:

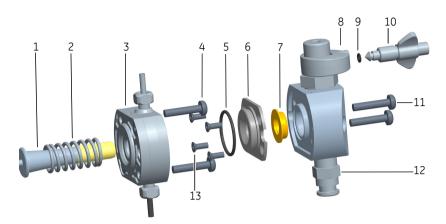
- Adjustable wrench
- For Pump **P9**: Star screwdriver, T20
- For Pump **P9H**: Star screwdriver, T10 and T20
- Ultrasonic bath
- Ethanol, 20%
- For Pump **P9**: Tubing giving a back pressure of 6 to 8 MPa (60 to 80 bar).
- For Pump **P9H**: Tubing giving a back pressure of 2 to 3 MPa (20 to 30 bar)
- For Pump P9: P9 Seal kit, 25 ml
- For Pump **P9H**: P9H Seal kit, 150 ml

## Illustrations

The illustrations below show the parts of the pump heads of the pumps P9 and P9H.

#### Pump P9





## Pump P9H

Part	Description	Part	Description
1	Piston	7	Piston seal
2	Return spring	8	Outlet check valve
3	Pump membrane housing	9	O-ring
4	Star screws	10	Purge valve
5	O-ring	11	Star screws
6	Support washer	12	Inlet check valve
13	Star screws		

# Disassemble the pump head

Step	Action
1	Make sure that no run is in progress on the instrument.
2	Disconnect the tubing from the pump head, and disconnect the pump inlet tubing.

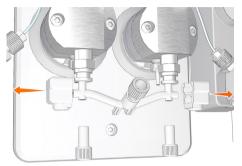
## 7 Maintenance

7.2 Replace pump piston seal, O-rings, and rinse membrane of Pump P9 or Pump P9H

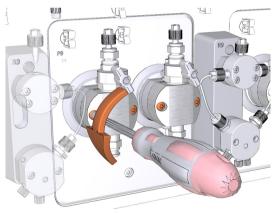
## Step Action

3

Unscrew the two white plastic screws located below each pump head by hand. Pull the plastic connectors to the sides to release the inlet manifold.



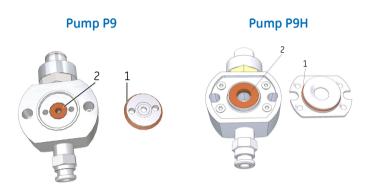
- 4 Disconnect the tubing of the pump piston rinsing system.
- 5
- Unscrew the two screws of the front section of the pump head using a star screwdriver T20, and pull off the front section.



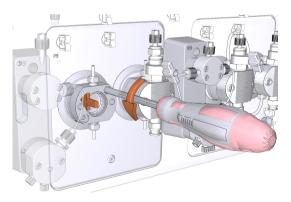
Step	Action
6	Place the front section of the pump head face down on the bench. For Pump
	<b>P9</b> , unscrew the two screws of the support washer using a star screwdriver,
	T20. For Pump <b>P9H</b> , unscrew the four screws of the support washer using
	a star screwdriver, T10. Discard the O-ring (1) on the support washer, and
	the discard the piston seal (2) located in the front section of the pump head.

#### Note:

Be careful not to scratch the metal surfaces.



Unscrew *one* of the two screws securing the pump membrane housing using a star screwdriver, T20. Unscrew the second screw, and at the same time push firmly on the front of the pump membrane housing to compensate for the pressure of the piston return spring.



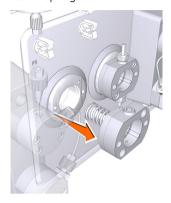
7

## 7 Maintenance

7.2 Replace pump piston seal, O-rings, and rinse membrane of Pump P9 or Pump P9H



return spring.



- 9 Inspect the piston and return spring for signs of damage. If damaged, discard the piston and return spring and use a new piston and return spring when assembling the pump head.
- 10 Clean the pump head and pump membrane housing in an ultrasonic bath. If there are particles on any surfaces, the check valves should be removed and cleaned separately, see *Section 7.5 Clean pump head check valves, on page* 170.

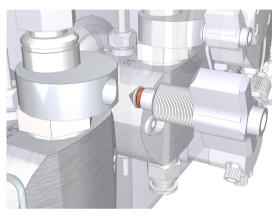
# Replace O-rings, piston seal and pump membrane housing

1

2

#### Step Action

Unscrew the purge valve of the pump head. Replace the O-ring on the purge valve with a new O-ring, , and screw the purge valve back into the pump head.



#### Note:

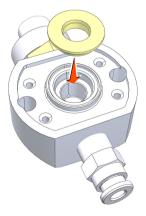
Always use Lubricant 56686700 when exchanging the O-ring 3 x 1 mm.

Wet a new seal with 20% ethanol. Place the new seal in the hole in the front section of the pump head and press it into position.

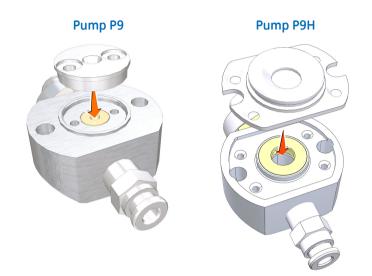
#### Pump P9

Pump P9H

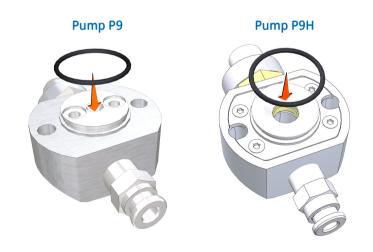




Step	Action
3	Place the support washer on top of the new seal in the front section of the pump head. Screw the two or four screws of the support washer. Make sure to tighten the screws fully.



4 Wet a new O-ring, 21.4 x 1.6 mm, with 20% ethanol. Fit the O-ring around the support washer.



# Assemble the pump head

Make sure to assemble the pump head correctly. Refer to *Illustrations, on page 150*.

Step	Action			
1	Insert the piston into the return spring. Insert piston and return spring into hole in the pump module.			
	Note:			
	Do no touch the ceramic or glass part of the pump piston.			
2	Wet the membrane in the hole with 20% ethanol before mounting.			
3	Place the pump membrane housing onto the locating pins on the front of the pump module.			
4	Screw one of the two screws securing the pump membrane housing using a star screwdriver, T20. Push firmly on the front of the pump membrane housing to compensate for the pressure of the piston and then screw the second screw.			
5	Make sure that the new seal is wetted with 20% ethanol and then tighten both screws fully.			
6	Reconnect the tubing of the pump piston rinsing system.			
7	Reconnect the inlet manifold.			
8	Reconnect the tubing to the pump head, and reconnect the pump inlet tubing.			

# Break in the new pump piston seal

Follow the instruction below to break in the new pump piston seal of Pump P9 or P9H.

Step	Action
1	Fill a buffer vessel with 20% ethanol in water. Immerse the inlet tubing, for example <b>A1</b> for System Pump A, <b>B1</b> for System Pump B, or <b>S1</b> for the Sample Pump in the buffer vessel. Place the buffer vessel on the buffer tray.
2	Prime the inlets and purge the pump, see Section 4.5.1 Prime buffer inlets and purge system pumps, on page 83.

Step	Ac	tion		
3	•	For Pump <b>P9</b> : Connect the reference capillary <b>Ref 2</b> (or an equivalent capillary that gives a backpressure of 6 to 8 MPa [60 to 80 bar]) to one of the column positions of the column valve (e.g., ports <b>1A</b> and <b>1B</b> ).		
	•	For Pump <b>P9H</b> : Connect the reference capillary <b>Ref 1</b> (or an equivalent capillary that gives a backpressure of 2 to 3 MPa [20 to 30 bar] to one of the column positions of the column valve (e.g., ports <b>1A</b> and <b>1B</b> ).		
4	Im	nmerse the waste tubing in the buffer vessel to recirculate the liquid.		
5	•	If breaking in a pump piston seal of a system pump, follow the instruction below:		
		In the <b>Manual instructions</b> dialog box:		
		- Select <i>Flow path:Column valve</i> , and select the position of the capil- lary connected to the column valve. Click <i>Insert</i> .		
		<ul> <li>Select Flow path:Inlet A (for System Pump A) or Flow path:Inlet B (for System Pump B) and select a Position. Click Insert.</li> </ul>		
		- Select <b>Pumps and Pressures:Gradient</b> and set <b>Target</b> to 0% B (for System Pump A) or 100% B (for system pump B).		
		- For Pump <b>P9</b> : Select <b>Pumps and Pressures:System flow</b> and set the <b>Flow rate</b> to 5.0 to ml/min. Click <b>Insert</b> .		
		For Pump <b>P9H</b> : Select <b>Pumps and Pressures:System flow</b> and set the <b>Flow rate</b> to 25.0 to ml/min. Click <b>Insert</b> .		
		- Click <b>Execute</b> .		
		Result: A system flow starts.		
	•	If breaking in a pump piston seal of a sample pump, follow the instruction below:		
		In the <b>Manual instructions</b> dialog box:		
		- Select <i>Flow path:Column valve</i> , and select the position of the capil- lary connected to the column valve. Click <i>Insert</i> .		
		- Select <i>Flow path:Sample inlet</i> and select a <i>Position</i> . Click <i>Insert</i> .		
		- Select <i>Flow path:Injection valve</i> and click <i>Direct inject</i> on the <i>Posi-</i> <i>tion</i> menu. Click <i>Insert</i> .		
		<ul> <li>Select Pumps and Pressures:Sample flow and set the Flow rate to 25.0 ml/min. Click Insert.</li> </ul>		
		- Click <b>Execute</b> .		

*Result*: A sample flow starts.

Step	Action
6	Run the flow for 2 hours.
7	Discard the used buffer.

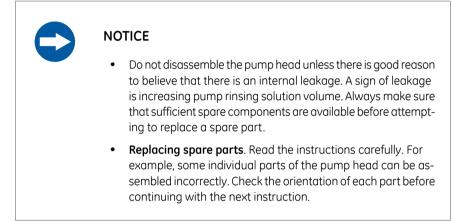
# 7.3 Replace pump piston seal, O-ring, and rinse membrane of Pump P9-S

# Introduction

Follow the instructions to replace the O-ring, piston seal, and rinse membrane of Pump **P9-S**.

Note:

Always replace the O-rings, piston seals, and rinse membranes of both pump heads of a pump at the same time.



## **Maintenance interval**

Replace the O-ring, piston seal, and rinse membrane of Pump **P9-S** if they are damaged. After replacement, perform a run to break in the new piston seal.



## NOTICE

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

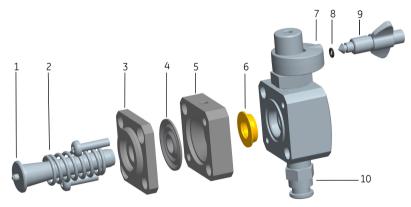
# **Required material**

The following material is required:

- Adjustable wrench
- Star screwdriver, T20
- Cross-headed screwdriver
- Hex wrench
- Ultrasonic bath
- Ethanol, 20%
- Reference capillary **Ref 1**
- P9-S Seal kit, 65 ml

## Illustration

The illustration below shows the parts of the pump heads of Pump P9-S.

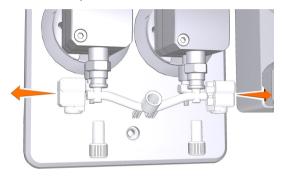


Part	Description	Part	Description
1	Piston	6	Piston seal
2	Return spring	7	Outlet check valve
3	Drain plate	8	O-ring
4	Rinse membrane	9	Purge valve
5	Rinse chamber	10	Inlet check valve

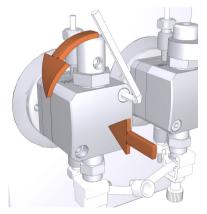
# Disassemble the pump head

Step	Action
1	Make sure that no run is in progress on the instrument.
2	Disconnect the tubing from the pump head, and disconnect the pump inlet tubing.

3 Unscrew the two white plastic screws located below each pump head by hand. Pull the plastic connectors to the sides to release the inlet manifold.



- 4 Disconnect the tubing of the pump piston rinsing system.
- 5 Unscrew *one* of the two screws of the pump head using a hex wrench. Unscrew the second screw, and at the same time push firmly on the front of the rinse chamber to compensate for the pressure of the piston return spring.





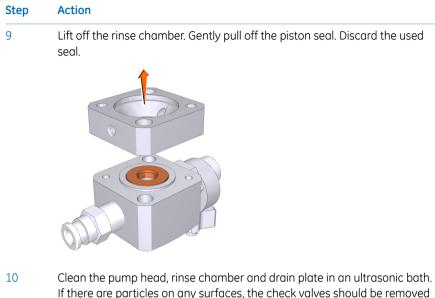
- 7 Inspect the piston and return spring for sign of damage. If damaged, discard the piston and return spring and use a new piston and return spring when assembling the pump head.
  - Unscrew the two screws securing the drain plate and the rinse chamber. Lift off the drain plate, and discard the membrane located between the drain plate and the rinse chamber.



8

## 7 Maintenance

7.3 Replace pump piston seal, O-ring, and rinse membrane of Pump P9-S



10 Clean the pump head, rinse chamber and drain plate in an ultrasonic bath. If there are particles on any surfaces, the check valves should be removed and cleaned separately, see *Section 7.5 Clean pump head check valves, on page 170.* 

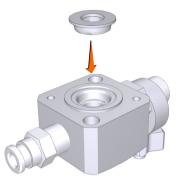
# Replace O-ring, piston seal, and rinse membrane

# Step Action 1 Unscrew the purge valve of the pump head. Replace the O-ring on the purge valve with a new O-ring, 3x1 mm, and screw the purge valve back into the pump head. Image: Comparison of the pump head of thead of thead of the pump head of thead of the pump head

## Note:

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

StepAction2Wet a new seal with 20% ethanol. Place the new seal in the hole in the front<br/>section of the pump head and press it into position.



With the pump head facing downwards on the bench, place the rinse chamber onto the front section of the pump head with the rinse ports in line with the check valves. The conical depression in the rinse chamber shall be facing upwards. Wet a new membrane with 20% ethanol, and place the membrane into the rinse chamber with the conical face upwards.



# Assemble the pump head

3

Make sure to assemble the pump head correctly. Refer to Illustrations, on page 150.

Step	Action
1	Place the drain plate on top of the assembly. Screw the two screws through the drain plate and the rinse chamber using a cross-headed screwdriver.

Step	Action
2	Wipe clean the piston and remove all finger prints. Wet the piston with 20% ethanol, and insert the piston into the return spring. With the pump head facing downwards on the bench, insert the piston into the pump head by pushing it gently but firmly vertically downwards into the seal.
3	Place the complete pump head over the locating pins on the front panel of the sample pump module. Turn the pump head so that the text <b>UP</b> on the drain plate is facing upwards. Push firmly on the front of the pump head, and at the same time screw one of the screws to fasten the pump head onto the front of the module using a hex wrench. Screw the second screw of the pump head. Make sure to tighten both screws fully.
4	Reconnect the tubing of the pump piston rinsing system.
5	Reconnect the inlet manifold.
6	Reconnect the tubing to the pump head, and reconnect the pump inlet tubing.
7	Break in the new pump piston seal, see instruction below.

# Break in the new pump piston seal

Follow the instruction to break in the new pump piston seal of Pump P9-S.

Step	Action
1	Fill a buffer vessel with 20% ethanol in water. Immerse a piece of sample inlet tubing, for example <b>S1</b> , in the buffer vessel. Place the buffer vessel on the buffer tray.
2	Prime the inlets and purge the pump, see Section 4.5.2 Prime sample inlets and purge Sample Pump, on page 90.
3	Connect the reference capillary <b>Ref 1</b> (or an equivalent capillary that gives a backpressure of 2 to 3 MPa [20 to 30 bar]) to one of the column positions of the column valve (e.g., ports <b>1A</b> and <b>1B</b> ).
4	Immerse the waste tubing in the buffer vessel to recirculate the liquid.

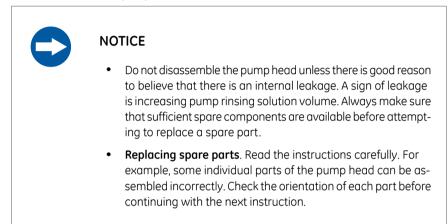
Step	Action	
5	In the <b>Manual instructions</b> dialog:	
	<ul> <li>Select Flow path:Column position, and select the Position of the capillary connected to the column valve. Click Insert.</li> </ul>	
	• Select Flow path:Sample inlet and select a Position. Click Insert.	
	<ul> <li>Select Flow path:Injection valve and click Direct inject on the Position menu. Click Insert.</li> </ul>	
	<ul> <li>Select Pumps:Sample flow and set the Flow rate to 25.0 ml/min. Click Insert.</li> </ul>	
	Click <i>Execute</i> .	
	<i>Result:</i> A sample flow of 25.0 ml/min starts. Run the flow for 2 hours. Discard the used buffer.	
6		
7		

# 7.4 Replace pump pistons

# Introduction

Follow the instructions to replace the pump pistons of the pumps P9, P9H and P9-S.

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



## Maintenance interval

Replace the pump pistons if they are damaged.

# **Required material**

The following materials are required:

- Adjustable wrench
- Star screwdriver, T20
- Piston kit

## Replace pump pistons of Pump P9 and P9H

If a damaged piston has been in operation, the piston seal will be destroyed and should also be replaced. To replace the piston and the seal of a system pump, see Section 7.2 *Replace pump piston seal, O-rings, and rinse membrane of Pump P9 or Pump P9H, on page 149. Section 7.2 Replace pump piston seal, O-rings, and rinse membrane of Pump P9 or Pump P9 or Pump P9H, on page 149.* 

# Replace pump pistons of Pump P9-S

If a damaged piston has been in operation, the piston seal will be destroyed and should also be replaced. To replace the piston and the seal of Pump **P9-S**, see Section 7.3 Replace pump piston seal, O-ring, and rinse membrane of Pump P9-S, on page 160.

# 7.5 Clean pump head check valves

# Introduction

Clean the check valves when required, for example if particles like dust or salt crystals in the check valve cause irregular or low flow. The cleaning procedure is the same for the system pumps and the sample pump.

# **Required material**

The following materials are required:

- Adjustable wrench
- 100% Methanol
- Distilled water
- Ultrasonic bath

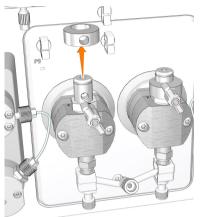
# Instruction

Follow the instructions to remove and clean the pump head check valves.

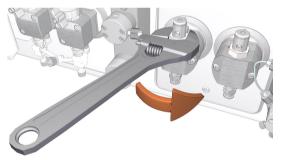
Step	Action	
1	Before taking the check valve apart, always try to clean the check valves by priming the pump heads first with distilled water, then with 100% Methanol and then with distilled water again.	
2	Switch off the instrument.	
3	Disconnect the tubing from the pump head and disconnect the pump inlet tubing. Disconnect the tubing of the pump rinsing system.	

## Step Action

4 Unscrew the purge valve by turning it counter-clockwise, and lift off the metal ring.



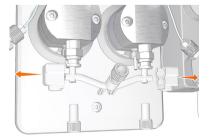
Unscrew the plastic nut of the upper check valve using an adjustable wrench, and gently lift off the upper check valve.



6

5

Unscrew the two white plastic screws located below each pump head. Pull the plastic connectors to the sides to release the inlet manifold.



## 7 Maintenance 7.5 Clean pump head check valves





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

Immerse the valves completely in methanol and place them in an ultrasonic bath for a few minutes. Repeat the ultrasonic bath with deionized water.

- 9 Refit the check valves.
- 10 Tighten the nut until fully finger-tight and then use the adjustable wrench to tighten a further 90 degrees.
- Refit the inlet manifold and reconnect the tubing to the pump head. 11

# 8 Reference Information

## About this chapter

This chapter lists the technical specifications of the ÄKTA avant instrument. The chapter also includes a chemical resistance guide. See also ÄKTA avant 25 Product Documentation and ÄKTA avant 150 Product Documentation for detailed technical specifications.

## In this chapter

This chapter contains the following sections:

Section	See page
8.1 System specifications	174
8.2 Chemical resistance guide	176
8.3 Check and change the Node ID of a module	181

# 8.1 System specifications

# **Technical specifications**

Parameter	Data
System configuration	Benchtop system, external computer
Control system	UNICORN™ 6.0 or later version
Connection between PC and instrument	Ethernet
Dimensions (Length × Depth × Height)	860 × 710 × 660 mm
Weight (excluding computer)	116 kg
Power supply	100°C to 240°C VAC, 50°C to 60°C Hz
Power consumption	800 VA
Enclosure protective class	IP 21, wet side IP 22
Tubing and connectors	• Inlet: FEP tubing, inner diameter (i.d.) 1.6 mm, Tubing connector 5/16" + Ferrule (yellow), 1/8"
	<ul> <li>Pump to Injection valve: PEEK tubing, i.d.</li> <li>0.75 mm, Fingertight connector, 1/16"</li> </ul>
	• After Injection valve: PEEK tubing, i.d. 0.50 mm, Fingertight connector, 1/16"
	• Outlet and waste: ETFE tubing, i.d. 1.0 mm, Fingertight connector, 1/16"

# **Environmental ranges**

Parameter	Data
Storage and transport temperature range	-25°C to 60°C
Chemical environment	See Section 8.2 Chemical resistance guide, on page 176.

# **Operating range**

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

# Equipment noise level

Equipment	Acoustic noise level
ÄKTA avant instrument	< 70 dBA

# 8.2 Chemical resistance guide

# Introduction

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

## **Biocompatibility**

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

# **Cleaning chemicals**

Strong cleaning works well with 2 M sodium hydroxide, 70% acetic acid or the alcohols methanol, ethanol and isopropyl alcohol. Complete system cleaning using 1 M hydrochloric acid should be avoided in order to not damage the pressure sensors. If you are cleaning separation media using 1 M hydrochloric acid, use loop injections of the acid and make sure that the column is not mounted on the Column Valve **V9-C**. The Column Valve **V9-C** contains a pressure sensor which can be damaged by 1 M hydrochloric acid.

Long time use of 0.2 M HCl connected to the Quaternary Valve **Q9** as part of a **BufferPro** recipe is acceptable. The solution becomes diluted further down in the system.

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

## **Organic solvents**

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 chromatography 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

**Note:** A user can be exposed to large volumes of chemical substances over a long time period. Material Safety Data Sheets (MSDS) provide 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.

## **Aqueous buffers**

Continuous use.

Chemical	Concentra- tion	CAS no/EC no
Aqueous buffers pH 2-12	N/A	N/A

## Strong chemicals and salts for CIP

Up to 2 h contact time at room temperature.

Chemical	Concentra- tion	CAS no/EC no
Acetic acid	70%	75-05-8/ 200-835-2
Decon™ 90	10%	N/A
Ethanol	100%	75-08-1/ 200-837-3
Methanol	100%	67-56-1/ 200-659-6
Hydrochloric acid <sup>1</sup>	0.1 M	7647-01-0/ 231-595-7
Isopropanol	100%	67-63-0/ 200-661-7
Sodium hydroxide	2 M	1310-73-2/ 215-185-5
Sodium hydroxide/ethanol	1 M/40%	N/A

# 8 Reference Information

8.2 Chemical resistance guide

Chemical	Concentra- tion	CAS no/EC no
Sodium chloride	4 M	7647-14-5/ 231-598-3
Sodium hypochlorite	10%	7681-52-9/231-668-3

1 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 periods of use. See *Cleaning chemicals, on page 176* 

## Solubilization and denaturing agents

Chemical	Concentra- tion	CAS no/EC no
Guanidinium hydrochloride	6 M	50-01-1/ 200-002-3
Sodium dodecyl sulfate (SDS)	1%	151-21-3/ 205-788-1
TRITON™ X-100	1%	9002-93-1
Tween™ 20	1%	9005-64-5/ 500-018-3
Urea	8 M	57-13-6/ 200-315-5

Continuous use, as additives in separation and purification methods

## Chemicals used in reversed phase chromatography (RPC)

Chemical	Concentra- tion	CAS no/EC no
Acetonitrile <sup>1</sup>	100%	75-05-8/ 200-835-2
Acetonitrile/Tetrahydrofu- ran <sup>1</sup>	85%/15%	109-99-9/ 203-726-8
Acetonitrile/water/Trifluo- roacetic acid (TFA) <sup>2</sup>	Max 0.2% TFA	N/A
Ethanol	100%	75-08-1/ 200-837-3
Isopropanol	100%	67-63-0/ 200-661-7
Methanol	100%	74-93-1/ 200-659-6
Water/organic mobile phase/formic acid	Max 5% formic acid	N/A

Continuous use.

1 Organic solvents can penetrate weaknesses in PEEK tubing walls more easily than water based buffers. Special care should therefore be taken with prolonged use of organic solvents close to pressure limits.

Note: Quaternary valve is not resistant.

Depending on pressure, tubing between pump head and pressure monitor needs to be changed. See ÄKTA avant User Manual for more information.

- <sup>2</sup> Mobile phase system
- **Note:** It is recommended to replace the mixer sealing ring with the highly resistant O-ring (product code 29-0113-26) if the system is to be exposed to organic solvents or high concentrations of organic acids, such as acetic acid and formic acid, for a longer period of time.

## Salts and additives for hydrophobic interaction chromatography (HIC)

Continuous use.

Chemical	Concentra- tion	CAS no/EC no
Ammonium chloride	2 M	12125-02-9/ 235-186-4
Ammonium sulfate	3 M	7783-20-2/ 231-984-1
Ethylene glycol	50%	107-21-1/ 203-473-3
Glycerol	50%	56-81-5/ 200-289-5

## Reducing agents and other additives

Continuous use.

Chemical	Concentra- tion	CAS no/EC no
Arginine	2 M	74-79-3/ 200-811-1
Benzyl alcohol	2%	100-51-6/ 202-859-9
Dithioerythritol (DTE)	100 mM	3483-12-3 / 222-468-7
Dithiothreitol (DTT)	100 mM	3483-12-3 / 222-468-7
Ethylenediaminetetraacetic acid (EDTA)	100 mM	60-00-4/ 200-449-4
Mercaptoethanol	20 mM	37482-11-4/ 253-523-3
Potassium chloride	4 M	7447-40-7/ 231-211-8

## **Other substances**

Chemical	Concentra- tion	CAS no/EC no
Acetone	10%	67-64-1/ 200-662-2
Ammonia	30%	7664-41-7/ 231-635-3
Dimethyl sulphoxide (DMSO)	5%	67-68-5/ 200-664-3
Ethanol for long-term stor- age	20%	75-08-1/ 200-837-3
Phosphoric acid	0.1 M	7664-38-2/231-633-2

# 8.3 Check and change the Node ID of a module

## Introduction

Node ID is a unit number designation that is used by the instrument to distinguish between several units of the same type. All standard valves and available optional modules are pre-configured to the default function. However, the function of a valve or module can be changed by changing the Node ID. Also, in a troubleshooting situation it may be useful to check a valve's or module's Node ID.

**Note:** The function of a valve or module is defined by its Node ID, not by its physical position.

## Node ID for standard modules

The following table lists the Node ID for the standard modules.

Module	Label	Node ID
System Pump A	P9 A or P9H A	0
System Pump B	P9 B or P9H B	1
Sample Pump	P9-S or P9H S	2
Pressure Monitor, system pressure	R9	0
Pressure Monitor, sample pressure	R9	1
Mixer	М9	0
Injection Valve	V9-Inj or V9H-Inj	4
Quaternary Valve	Q9	0
Inlet Valve A	V9-IA or V9H-IA	0
Inlet Valve B	V9-IB or V9H-IB	1
Sample Inlet Valve	V9-IS or V9H-IS	2
Column Valve	V9-C or V9H-C	5
Precolumn pressure monitor in Column Valve	N/A	2
Post-column pressure monitor in Column Valve	N/A	3

## 8 Reference Information

8.3 Check and change the Node ID of a module

Module	Label	Node ID
pH Valve	V9-pH or V9H-pH	11
pH Monitor	Н9	0
<b>Note:</b> The pH monitor is included in the pH valve module box.		
Outlet Valve	<b>V9-O</b> or <b>V9H-O</b>	8
UV Monitor	U9-M	0
UV detector	U9-D	0
Conductivity Monitor	C9	0
Built-in fraction collector	N/A	Not settable by the user.

# Node ID for optional modules

The following table lists the Node ID for the optional modules.

Module	Label	Node ID
Second Inlet Valve A	V9-A2 or V9H-A2	12
Second Inlet Valve B	V9-B2 or V9H-B2	13
Extra Inlet Valve X1	V9-IX or V9H-IX	15
Extra Inlet Valve X2	V9-IX or V9H-IX	16
Second Sample Inlet Valve	V9-S2 or V9H-S2	14
Versatile Valve	<b>V9-V</b> or <b>V9H-V</b>	20
Second Versatile Valve	<b>V9-V</b> or <b>V9H-V</b>	21
Third Versatile Valve	<b>V9-V</b> or <b>V9H-V</b>	23
Tertiary Versatile Valve	<b>V9-V</b> or <b>V9H-V</b>	24
Loop Valve	V9-L or V9H-L	17
Second Column Valve	V9-C2 or V9H-C2	6
Unused precolumn pressure monitor in second Column Valve	N/A	4

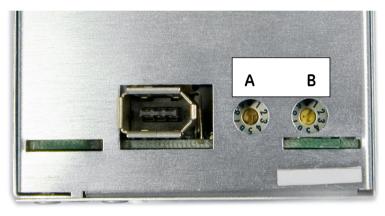
Module	Label	Node ID
Unused post-column pressure monitor in second Column Valve	N/A	5
Second Outlet Valve	V9-O2 or V9H-O2	9
Third Outlet Valve	V9-O3 or V9H-O3	10
External Air Sensor	L9-1.2 or L9-1.5	0
I/O-box	E9	0
Second I/O-box	E9	1
Second UV Monitor	U9-L	1
Second Conductivity Monitor	С9	0
Second Fraction Collector	F9-R	1

# Check and change the Node ID

The Node ID of a module is set by the positions of an arrow of two rotating switches at the back of the module. Follow the instructions to check or change the Node ID.

Step	Action
1	If applicable, remove the module according to the instruction in ÄKTA avant User Manual.
2	The Node ID is set by the positions of an arrow of two rotating switches at

- 2 The Node ID is set by the positions of an arrow of two rotating switches at the back of the module.
  - The first rotating switch, labeled **A** sets the tens.
  - The second switch, labeled **B** sets the units.
  - For example for Node ID **13**, the **A** switch is set to **1** and the **B** switch to **3**.



- 3 Check the Node ID and compare it with the listed Node IDs in the tables above.
- 4 To change the Node ID, use a screwdriver to set the arrows of the switches to the desired number.
- 5 Re-install the module in the instrument, if applicable.

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GE Healthcare Bio-Sciences AB Björkgatan 30 751 84 Uppsala Sweden

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GE Healthcare Europe GmbH Munzinger Strasse 5, D-79111 Freiburg, Germany

GE Healthcare UK Limited Amersham Place, Little Chalfont, Buckinghamshire, HP7 9NA, UK

GE Healthcare Bio-Sciences Corp. 800 Centennial Avenue, P.O. Box 1327, Piscataway, NJ 08855-1327, USA

GE Healthcare Japan Corporation Sanken Bldg. 3-25-1, Hyakunincho Shinjuku-ku, Tokyo 169-0073, Japan

