# ÄKTA<sup>™</sup> start Operating Instructions Original instructions





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# 1 Introduction

### About this chapter

This chapter contains important user information, description of safety notices, regulatory information, intended use of ÄKTA start, and list of associated documentation.

### In this chapter

Section	See page
1.1 About this manual	6
1.2 Important user information	7
1.3 Regulatory information	9
1.4 Associated documentation	12

# 1.1 About this manual

## Purpose of this document

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

#### Nomenclature conventions

The nomenclature used in this manual are explained in the table below.

Concept	Explanation
ÄKTA start	The instrument.
Frac30	The Fraction collector.
UNICORN™ start	The software installed on a computer.
ÄKTA start System	The entire liquid chromatography system, including instrument, Fraction collector and software.

### **Typographical conventions**

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

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

## 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

ÄKTA start is a liquid chromatography system used for preparative purification of proteins at laboratory-scale. The system can be used for a variety of research purposes to fulfill the needs of the users in the academia and in the life sciences industry.

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

#### **Prerequisites**

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

- you understand the concepts of liquid chromatography
- you have read and understood the Safety instructions chapter in the ÄKTA start Operating Instructions.

### 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 describes the directives and standards that are fulfilled by ÄKTA start.

#### **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.

Directive	Title
2006/42/EC	Machinery Directive (MD)
2004/108/EC	Electromagnetic Compatibility (EMC) Directive
2006/95/EC	Low Voltage Directive (LVD)

### **CE marking**

CE

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/IEC 61010-1, UL 61010-1, CAN/CSA C22.2 No. 61010-1	Safety requirements for electrical equipment for mea- surement, control, and laborato- ry use.	EN standard is harmonized with EU directive 2006/95/EC
EN/IEC 61326-1 (Emission according to CISPR 11, Group 1, class A)	Electrical equipment for measurement, control and laboratory use - EMC require- ments	EN standard is harmonized with EU directive 2004/108/EC
EN ISO 12100	Safety of machinery. General principles for design. Risk assessment and risk reduction.	EN ISO standard is harmonized with EU directive 2006/42/EC

# Regulatory compliance of connected equipment

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

## **Environmental conformity**

This product conforms to the following environmental requirements.

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)

# 1.4 Associated documentation

## Introduction

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

#### User documentation on the CD

The user documentation listed in the table below is available on the ÄKTA start User Documentation CD. A display help is available from the Instrument Display.

Documentation	Main contents
ÄKTA start Operating Instructions	Instructions needed to install, operate and maintain ÄKTA start in a safe way.
ÄKTA start Instrument Display Help	Dialog descriptions of the functionality menu for ÄKTA start (only accessible from the Instrument Display).
ÄKTA start Maintenance Manual	Detailed instrument and module descrip- tions, including instructions needed to maintain and troubleshoot ÄKTA start.
ÄKTA start System Cue Card	A condensed guide to prepare and run chromatographic techniques on ÄKTA start.
ÄKTA start Maintenance Cue Card	A condensed guide to handling routine maintenance operations and trou- bleshooting ÄKTA start.

From the Help menu in UNICORN start or on the UNICORN start DVD, the following user documentation is available.

Documentation	Main contents	
UNICORN start 1.0 User Manual	Overview and detailed descriptions of the system control software designed for ÄKTA start, which includes process pic- ture map for real time monitoring, method editor, evaluation and adminis- tration modules.	
UNICORN start Online Help	Dialog descriptions for UNICORN start (only accessible from the Help menu).	

# Data files, application notes and user documentation on the web

To order or download data files, application notes or user documentation, see the instruction below.

Step	Action
1	Go to www.gelifesciences.com/AKTA.
2	Click <b>ÄKTA Lab-Scale Systems</b> .
3	Select <b>ÄKTA start</b> from the list.
4	Click <b>Related Documents</b> .
5	Select to download the chosen literature.

# Access documentation from mobile units

Scan the code using your mobile phone or tablet computer to access the product page for ÄKTA start. Select documents to download under the *Related Documents* tab.



# 2 Safety instructions

### About this chapter

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

#### In this chapter

This chapter contains the following sections:

Section	See page
2.1 Safety precautions	16
2.2 Labels	24
2.3 Emergency procedures	28
2.4 Recycling procedures	30
2.5 Declaration of Hazardous Substances (DoHS)	31

# 2.1 Safety precautions

### Introduction

ÄKTA start is powered by mains voltage and handles liquids that may be hazardous. Before installing, operating or maintaining the system, you must be aware of the hazards described in this manual. *Follow the instructions provided to avoid personal injuries or damage to the equipment*.

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

- General precautions
- Using flammable liquids
- Personal protection
- Installing and moving the instrument
- System operation
- Maintenance

### **General precautions**



### Using flammable liquids



#### WARNING

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

- **Fire Hazard**. Before starting the system, make sure that there is no unintentional leakage in the instrument or tubing.
- **Explosion hazard**. To avoid building up an explosive atmosphere when using flammable liquids, make sure that the room ventilation meets the local requirements.



#### CAUTION

To avoid hazardous situations when unpacking, installing or moving ÄKTA start, follow the precautions below.

ÄKTA start is filled with denaturated alcohol (50% C2H5OH (ethanol)) at delivery. **The denatured alcohol mixture can be hazardous to humans if consumed.** Flush out the denaturated alcohol before assembling, testing or integrating ÄKTA start into the intended process context.

#### **Personal protection**



#### WARNING

To avoid hazardous situations when working with ÄKTA start, take the following measures for personal protection.

**Spread of biological agents**. The operator has to take all necessary actions to avoid spreading hazardous biological agents in the vicinity of the equipment. The facility should comply with the national code of practice for biosafety.



# Installing and moving the instrument



#### WARNING

To avoid damage to person when installing or moving ÄKTA start, follow the instructions below.

- Moving the instrument horizontally. One person is recommended when moving the instrument horizontally.
- **Supply voltage**. Make sure that the supply voltage at the wall outlet corresponds to the marking on the instrument, before connecting the power cord.
- **Power cord**. Only use grounded power cords delivered or approved by GE.
- 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 (optional)**. The computer should be installed and used according to the instructions provided by the manufacturer of the computer.
- **Disconnect power**. Always switch off power to ÄKTA start before an instrument module is removed or installed, or a cable is connected or disconnected.



#### CAUTION

To avoid damage to person when installing or moving ÄKTA start, follow the instructions below.

**Protective ground**. ÄKTA start must always be connected to a grounded power outlet.



### System operation



#### WARNING

To avoid personal injury when operating ÄKTA start, follow the instructions below.

- Rotating the instrument. Make sure that there is always at least 20 cm of free space around ÄKTA start to allow for sufficient ventilation. When turning or moving 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 turn or move carefully.
- Hazardous chemicals during run. When using hazardous chemicals, run the *System cleaning* template to clean and flush the entire system tubing with distilled water, before service and maintenance.
- Setting. Check that the correct outlet size settings are used. Make sure that tubing and fittings are properly connected and secured. Make sure that the pressure limit settings are correct before starting the run.



#### CAUTION

To avoid personal injury when operating ÄKTA start, follow the instructions below.

- Max weight on Buffer tray. Do not place containers with a volume of more than 1 liter each on the Buffer tray. The maximum allowed weight on the Buffer tray is 5 kg.
- Large spillage. Switch off ÄKTA start and unplug the power cord, if large spillage occurs.



#### NOTICE

To avoid damage to ÄKTA start or other equipment when operating the instrument, follow the instructions below.

- Keep UV flow cell clean. Do not allow solutions containing dissolved salts, proteins or other solid solutes to dry out in the flow cell. Do not allow particles to enter the flow cell, as damage to the flow cell may occur.
- **Prefill UV flow cell**. Make sure that the **UV flow cell** is prefilled with liquid before starting the system.
- Avoid condensation. If ÄKTA start is kept in a cold room, cold cabinet or similar, keep the instrument switched on in order to avoid condensation.
- Avoid overheating. If ÄKTA start is kept in a cold cabinet and the cold cabinet is switched off, make sure to switch off the instrument and keep the cold cabinet open to avoid overheating.
- Place the computer in room temperature. If ÄKTA start is
  placed in a cold room, place the computer outside the cold
  room and use the PC Connectivity cable delivered with the instrument to connect to the computer.
- Keep the pump cover open when not using the system. Open the peristaltic pump cover after you switch off the equipment. This will enhance the life time of the pump tubing.

#### Maintenance



#### WARNING

To avoid damage to person when performing maintenance on ÄKTA start, follow the instructions below.

- Electrical shock hazard. Do not open any covers or parts unless specified in the user documentation. Except for the maintenance and service described in the user documentation, all other repairs should be done by service personnel authorized by GE.
- Only spare parts and accessories that are approved or supplied by GE may be used for maintaining or servicing ÄKTA start.
- **Disconnect power**. Always switch off power to the instrument before replacing any component on the instrument or cleaning the instrument, unless stated otherwise in the user documentation.
- **Spillage Hazard**. Avoid spillage of fluids on the surfaces of the instrument which have cables, plugs and other wirings. Be careful if there is spillage of fluids on the tray while trying to remove the tray from ÄKTA start.
- NaOH is corrosive and therefore dangerous to health. When using hazardous chemicals, avoid spillage and wear protective glasses and other suitable Personal Protective Equipment (PPE).



#### CAUTION

To avoid damage to person when performing maintenance on ÄKTA start, follow the instructions below.

- Hazardous UV light. Always switch off power to the instrument before replacing the UV flow cell.
- If hazardous chemicals are used for system or column cleaning, wash the system or columns with a neutral solution in the last phase or step before maintenance.



#### NOTICE

**Cleaning**. Keep 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.

#### 2 Safety instructions 2.2 Labels

## 2.2 Labels

## Introduction

This section describes the safety labels and labels concerning hazardous substances that are attached to ÄKTA start. The instrument serial number is also visible from the instrument product label which is illustrated here.

## Labels on ÄKTA start

The illustrations below show the labels that are attached to ÄKTA start.

Label	Description	Placement
2022000       KATA <sup>TM</sup> start         Cade no. 2022031       Victors: 100-N0 V~r         See of no. 122203       Victors: 100-N0 V~r         Market of the construction Classifier       Protection Classifier         Market of the construction Classifier       Protection Classifier	Instrument label including safety symbols and speci- fication.	
La rupture du sceau annule la garantie Warranty void if seal is broken	Do not open any covers on the instrument. This will void the warranty.	
	Keep the pump cover open when not using the system. Open the pump cover after you switch off the instrument.	

Label	Description	Placement
	Caution! Consult the Operating Instructions before using the system.	
	Pinch hazard. Switch off the Pump before tubing loading.	

## Label on Frac30

The illustration below shows the labels that are attached to Frac30.

Label	Description	Placement
29023051     Frac30       Code no: 2003775     Voltops: 24 V ==:       May Power: 1904     Max Power: 1904       Max Power: 1904     Production Class (22)       Max Po	Instrument label including safety symbols and speci- fication.	
La rupture du sceau annule la garantie Warranty void if seal is broken	Do not open any covers on the instrument. This will void the warranty.	

## Safety symbols

The following safety symbols are used in the labels:

Label	Meaning
	Warning! Do not use ÄKTA start before reading the <i>ÄKTA start</i> <i>Operating Instructions</i> . Do not open any covers or re- place parts unless specifically stated in the user docu- mentation.
$\bigotimes$	The system complies with the requirements for electro- magnetic compliance (EMC) in Australia and New Zealand.
CE	The system complies with applicable European direc- tives.
c C Lister Us Intertek	This symbol indicates that ÄKTA start has been certified by a Nationally Recognized Testing Laboratory (NRTL). NRTL means an organization that is recognized by the US Occupational Safety and Health Administration (OS- HA) as meeting the legal requirements of Title 29 of the Code of Federal Regulations (29 CFR), Part 1910.7.

# Labels concerning hazardous substances

The following symbols on the labels concern hazardous substances:

Label	Meaning
	This symbol indicates that electrical and electronic equipment must not be disposed of as unsorted munic- ipal waste and must be collected separately. Please contact an authorized representative of the manufac- turer for information concerning the decommissioning of equipment.

Label	Meaning
20	This symbol indicates that the product contains haz- ardous 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.

# 2.3 Emergency procedures

## Introduction

This section describes how to do an emergency shutdown of ÄKTA start. The section also describes the result in the event of power failure.

#### **Emergency shutdown**

In an emergency situation:

Switch off power to the instrument by pressing the power switch to the **O** position or by disconnecting the power cord from the instrument. The run is interrupted immediately.





#### 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.

### **Power failure**

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

Power failure to	will result in
ÄKTA start	• The run is interrupted immediately.
	• The data collected up to the time of the power fail- ure is available on the USB memory stick <i>or</i> , if the system is connected to a computer, UNICORN start.
UNICORN start on a computer	The computer with UNICORN start installed shuts down.
	• On the ÄKTA start Instrument Display, all four touch buttons will be highlighted.
	• The run is interrupted immediately.
	• Data generated up to 10 seconds before the power failure can be recovered.
	Note:
	The UNICORN start client may close down during a temporary overload of the processor. This may appear as a computer failure. The run continues and you can restart the UNICORN start client to regain control.

# 2.4 Recycling procedures

### Introduction

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

# Decommissioning of the equipment

ÄKTA start must be decontaminated before decommissioning. Follow local regulations for scrapping of the equipment.

#### **Disposal, general instructions**

When taking ÄKTA start out of service, the different materials must be separated and recycled according to national and local environmental regulations.

# Recycling of hazardous substances

ÄKTA start contains hazardous substances. Detailed information is available from your GE representative.

# 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 控制方面的信息。

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。

产品中的消耗件和某些零部件可能有其单独的环保使用期限标志,并且其环保使 用期限有可能比整个产品本身的环保使用期限短。应到期按产品维修程序更换那 些消耗件和零部件,以保证所申明的整个产品的环保使用期限。

本产品在使用寿命结束时不可作为普通生活垃圾处理,应被单独收集妥善处理。

This symbol indicates the product contains hazardous materials in excess of the limits established by the Chinese standard SJ/T11363-2006 Requirements for Concentration Limits for Certain Hazardous Substances in Electronic Information Products. The number in the symbol is the Environment-friendly Use Period (EFUP), which indicates the period during which the toxic or hazardous substances or elements contained in electronic information products will not leak or mutate under normal operating conditions so that the use of such electronic information products will not result in any severe environmental pollution, any bodily injury or damage to any assets. The unit of the period is "Year".

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
29-0220-94	Х	0	0	0	0	0
29-0230-51	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 provides an overview of ÄKTA start, the Instrument Display, that allows the user to operate and control the system, and Frac30 (Fraction collector).

#### In this chapter

This chapter contains the following sections:

Section	See page
3.1 System overview	34
3.2 Instrument	38
3.3 Instrument Display	41

# 3.1 System overview

### Introduction

ÄKTA start is the main instrument included in a liquid chromatography system intended primarily for preparative purification of proteins at laboratory-scale. The system can be used for a variety of research purposes to fulfill the needs of the users in the academia and in life sciences industry.

ÄKTA start is operated and controlled from the Instrument Display. In addition, the UNICORN start software can be used to control ÄKTA start and to analyze the data acquired during chromatography runs. UNICORN start offers several additional features that are described in detail in UNICORN start 1.0 User Manual.

This section gives an overview of the ÄKTA start System.

#### Illustration of the system

The illustration below shows the ÄKTA start System with UNICORN start installed on a computer.



Part	Description
1	ÄKTA start (instrument).
2	Frac30 (Fraction collector).
3	UNICORN start (software installed on a computer).

### Illustration of the instrument

The illustration below shows the main parts of the instrument.



Part	Description	Function
1	Instrument Display	User interface for controlling the system and visualization of the runtime data.
2	Wet side	The modules interconnected by tubing have the following functions:
		• to deliver the liquid in a specified flow path and divert the flow as required,
		• to monitor the UV absorbance and conductivity of the liquid.
3	Buffer tray	Location intended for the placement of buffer bottles used during chromatography runs.
4	Power switch	Connects or disconnects the power.
5	USB port	To connect a USB memory stick for storage of results and transfer of files.

# Illustration of the Fraction collector

The illustration below shows the Fraction collector; Frac30.



Part	Description	Function
1	Dispenser arm assembly	Holds and positions the tubing holder for dispens- ing the liquid into fractions.
2	Tubing holder	Holds the tubing used for dispensing the liquid fractions into the collection tubes.
3	Collection tubes	10 to 18 mm diameter tubes used to collect the fractions.
4	Bowl assembly	Holder for collection tubes, which supports tubes of four sizes.
5	Base unit	Case for electromechanical assembly and holder for the Bowl assembly.
6	LED	Power on indicator.
## Main features of ÄKTA start

The main features of ÄKTA start are listed below:

- ÄKTA start is a compact and one step purification solution for quick and reliable purification of proteins.
- A simple and modern system offered to automate the protein purification workflow by providing features like automated sample injection, fraction collection, real-time monitoring.
- Method templates are available for all common chromatography techniques such as Affinity Chromatography, Ion Exchange Chromatography, Gel filtration, and Desalting.
- Quick start methods are available for purifying several common proteins.
- Predefined system methods are available for cleaning the flow path.
- ÄKTA start is operated using a touch screen on the instrument.
- In addition, the system can be operated from a computer connected to the instrument using the UNICORN start software.
- ÄKTA start is offered with a dedicated Fraction collector, Frac30, allowing to collect the fractions in four different tube sizes.

#### 3 System description 3.2 Instrument

# 3.2 Instrument

# Introduction

This section provides an overview of ÄKTA start.

# Illustration of the instrument modules

The illustration below shows the locations and gives brief descriptions of the modules placed on the wet side of the instrument.



Part	Function	Description
1	Buffer valve	A 3-port valve that is used as a switching valve for gradient formation. It enables the use of two buffers, which are required for forming a gradient during runs.

Part	Function	Description
2	Mixer	A static mixer that is used for mixing of buffers A and B.
3	Sample valve	A 3-port valve that allows either the buffer or the sample to enter the flow path. The <b>Sample valve</b> enables direct application of the sample onto the column using the <b>Pump</b> .
4	Pump	A peristaltic pump, which delivers buffer or sample to the flow path with a flow rate of up to 5 ml/min. For cleaning procedures, the <b>Pump</b> can flush the flow path at a flow rate of 10 ml/min.
5	Pressure sensor	The <b>Pressure sensor</b> reads the pressure in the flow path and senses overpressure.
6	Wash valve	A 3-port valve that is used to divert the flow path to waste. The <b>Wash valve</b> switches automatically during the predefined cleaning procedure, <i>Pump</i> <i>wash</i> . In a manual run, the valve can be set to the intended position by configuring the run parameters.
7	Injection valve	<ul> <li>A 6-port manually operated valve that is used to transfer the sample loaded in the sample loop on to the column.</li> <li>A sample loop is connected to the appropriate ports of the valve. The valve is switched manually to positions:</li> <li>Load sample (default): to allow the loading of the sample into the sample loop.</li> <li>Inject to column: to transfer the sample from the loop on to the column during a chromatography run.</li> </ul>
8	UV	The <b>UV</b> Monitor continuously measures the ab- sorbance of the liquid in the <b>UV flow cell</b> at a set wavelength of 280 nm. The <b>UV flow cell</b> has a path length of 2 mm.

Part	Function	Description
9	Conductivity	The <b>Conductivity</b> Monitor continuously reads the conductivity of the liquid in the <b>Conductivity flow cell</b> .
		The conductivity is automatically calculated by multiplying the measured conductance by the cell constant of the flow cell. The cell constant is factory-calibrated.
		The <b>Conductivity flow cell</b> is provided with a temperature sensor that measures the tempera- ture of the liquid in the <b>Conductivity flow cell</b> .
		Note:
		The buffers used should be within the conductivity range of the instrument (0 to 300 mS/cm).
10	Outlet valve	A 3-port valve that is used to direct the flow to the Fraction collector, or to Waste.

# 3.3 Instrument Display

## Introduction

This section provides a description of the ÄKTA start Instrument Display and the functions that are accessible from the display.

#### In this section

This section contains the following sub-sections:

Section	See page
3.3.1 Overview of the Instrument Display	42
3.3.2 Description of Method run	47
3.3.3 Description of Create method	50
3.3.4 Description of Settings and service	51

# 3.3.1 Overview of the Instrument Display

## Introduction

The Instrument Display is located on the front side of ÄKTA start. The Instrument Display enables the user to control the system by selecting the intended operation:

- Start a run and control an ongoing run.
- View the progress of the ongoing run.
- Manage user defined methods.
- Perform maintenance and service.

This section provides a brief description of the functions of the Instrument Display.

**Note:** Do not operate the Instrument Display with a sharp and hard object.

# Illustration of the Instrument Display

The illustration below shows the location and detailed view of the Instrument Display.



Part	Description	
1	Screen name area.	
2	Information area. The example shows the ÄKTA start home screen.	
3	Help button.	

### Instrument software

ÄKTA start offers the following functionality menu, displayed on the **ÄKTA start** home screen as presented in the table below. For detailed workflows on the different options, see *Chapter 6 Operations from the Instrument Display, on page 155.* 

Option	Description
Method run	Use a quick start or a method template to perform a run.
Manual run	Perform a run by providing parameters manually.
Create method	Create, edit, import and delete user methods.
Settings and service	Configure settings and calibrate modules, perform diagnostics tests.

#### Description of buttons on the Instrument Display

The Instrument Display includes the following touch buttons:

Button	Name	Description
?	Help	Opens a new dialog screen, providing information about the content of the current screen or indi- cates where further information or instructions can be found.
	Home	Opens the <b>ÄKTA start</b> home screen.
>	Forward	Opens the next screen in the current workflow.
<	Return	Opens the previous screen in the current workflow.

#### 3 System description

3.3 Instrument Display

3.3.1 Overview of the Instrument Display

Button	Name	Description	
× 1.0 ^	Increment (up arrow) Decrement (down arrow)	The value in the text field can be increased or de- creased by tapping up or down arrow. Tapping the value opens the numpad and a new value can be typed in.	
		2.1         1       2       3       DEL       Close         4       5       6       .       C         7       8       9       0       Ok         Note:         After typing in a value on the numpad, tap OK to confirm the new value.	
Next	Next	Opens the next screen.	
Back	Back	Returns to the previous screen.	
Run	Run	Starts a run.	
Pause	Pause	Pauses the ongoing run by stopping the <b>Pump</b> . The flow rate settings and the gradient values are retained.	
Continue	Continue	Continues a run that has been paused.	
Hold	Hold	Holds an ongoing run, with current flow rate, valve positions, and with set %B concentration. The gradient is held at the value displayed.	
Resume	Resume	Resumes a run that is put on hold.	
Edit run	Edit run	Opens a new screen for editing the current run parameters.	

#### 3 System description 3.3 Instrument Display 3.3.1 Overview of the Instrument Display

Button	Name	Description	
Execute	Execute	Executes the edited run parameters during a run.	
Ok	ОК	Confirms a selection or an action.	
Cancel	Cancel	Cancels a selection or an action.	
End	End	Terminates the ongoing run; always followed by a screen that requires to confirm the action.	
Exit	Exit	When a run is completed, it closes the application and returns to the <b>ÄKTA start</b> home screen.	
Graph	Graph icon	Opens the graphic view of the run in progress, displaying the plot of the UV absorbance (mAU) versus Time (min).	
Save	Save	Saves a user method that was either created or edited.	
Select	Select	Confirms and initiates a run from a specific tem- plate or user method.	
Create	Create	Creates a user method based on selected tem- plate. The run parameters have to be edited as needed.	
Yes	Yes	Confirms an action.	
No	No	Rejects an action.	

#### 3 System description

3.3 Instrument Display3.3.1 Overview of the Instrument Display

## Description of Instrument Display Help

The *ÄKTA start Instrument Display Help* is accessible from every screen on the Instrument Display, by tapping the question mark in the upper right corner. The *Help text* provides information about the content of the current screen or refers to more detailed documentation.



# 3.3.2 Description of Method run

#### Method run options

The display option *Method run* allows the user to run methods based on *Quick start* techniques or predefined method templates, user created methods, and run predefined methods such as *Pump Wash*, and *System cleaning*. Detailed instructions to run methods are presented in *Section 6.4 Perform a method run, on page 170*.

When *Method run* is selected, further options are displayed in the *Method run* screen.

Method run	?
Quick start	Templates
User defined	Prepare system
1	

The method types available under *Method run* are briefly described below.



#### 3 System description

3.3 Instrument Display

3.3.2 Description of Method run

Method	Description
Quick start (3/3)	
Quick start ?	
GF 16/60 HiPrep	
<	
3/3	
Back Select	
Templates	Displays the method templates for
Templates ?	chromatography techniques avail- able with ÄKTA start.
Affinity (AC)	For a description of the templates,
Desalting/buffer exchange (DS)	see Section 6.4.3 Templates, on
Gel filtration (GF)	
Back Select	
User defined	Displays the methods created by the
Select method ?	user.
AC01	For detailed instructions on creating
O DS06	ods, see Section 6.6 Manage methods
	and files, on page 195.
Back Select	
Prepare system (1/2)	Displays the predefined system
Select system method ?	system flow path.
Pump wash A     Bump wash B	For detailed instructions on running
Washout fractionation tubing	system methods, see Section 6.4.5 Prepare system methods, on page 189.
Column preparation 1/2	, , , , , , , , , , , , , , , , , , , ,
Back Select	

3 System description 3.3 Instrument Display 3.3.2 Description of Method run

Method	Description
Prepare system (2/2)	
Select system method ?	
System cleaning	
System performance method	
2/2	
Back Select	

# 3.3.3 Description of Create method

# Create method options

The display option *Create method* allows the user to create new methods, edit or delete existing user methods and also import methods stored on a USB memory stick connected to the instrument.

When *Create method* is selected, further options are displayed in the *Create method* screen.

Create method	?
Create	Edit
USB import	Delete

The operations available under Create method are briefly described below.

Option	Description
Create	Displays the method templates that can be used to create a new method.
Edit	Displays the user methods stored on the instrument that can be edited, if required.
USB Import	Displays a list of user methods stored on a USB memory stick that can be imported to the instrument.
Delete	Displays the user methods stored on the instrument that can be deleted, if required.

# 3.3.4 Description of Settings and service

## Settings and service options

The display option *Settings and service* allows the user to perform maintenance, calibration, setting the delay volume, diagnostics and troubleshooting of the modules located on the wet side of the instrument. For a brief description of the modules, see *Section 3.2 Instrument, on page 38.* Detailed instructions for calibrating the **Pump**, the monitors, and the Instrument Display are presented in *Section 5.3 Calibrations, on page 88.* 

The options available under Settings and service are listed in the table below.



3.3 Instrument Display

3.3.4 Description of Settings and service

# Settings and service - Screen 1

The options available for the modules displayed on *Settings and service* Screen 1 are briefly described below.

Module	Function
Fraction collector (Fraction collector)       Fraction collector	<b>Enable Frac/Disable Frac</b> : Activates/Deactivates the Fraction collector connection.
Disable Frac Diagnostics Run log	<b>Diagnostics</b> : Performs a feed tube test that checks if the Bowl assembly rotates correctly. Performs a Home test to verify that the Bowl assembly returns to Home position.
Back	<i>Run log</i> : To see the number of hours the Frac drive has been active and to reset the number of hours of drive usage.
Pressure sensor ? User operations P set 0.00 MPa Zero offset	Zero offset: Allows to set the pres- sure to zero. The <b>Pump</b> has to be off and the flow path connection to the <b>Pressure sensor</b> has to be open.
GEHC service only Stop flow Calibrate	<i>P set</i> : Shows the pressure in the flow path. <i>Note:</i>
Back	Pressure Calibration is performed by a GE Service Engineer only, after the Pressure sensor is replaced.

# 3 System description3.3 Instrument Display3.3.4 Description of Settings and service

Module	Function
Pump ?	<i>Calibration</i> : To calibrate the <b>Pump</b> , so that it delivers liquid with an accurate flow rate.
Calibration Pump tubing log	Pump tubing log:
Diagnostics Back	• To note the number of hours the pump tubing has been in use ( <i>Tubing run</i> ) and to reset the count of tubing usage (after the pump tubing is replaced).
	• To note the number of hours the <b>Pump</b> has been in use ( <b>Pump</b> <b>run</b> ) and to reset the count of <b>Pump</b> usage (after the <b>Pump</b> is replaced).
	<i>Diagnostics</i> : To adjust the flow rate and start/stop the flow ( <b>Pump</b> ).

#### 3 System description

3.3 Instrument Display

3.3.4 Description of Settings and service

#### Module

System Serial # 00000000000000000000	)1 ?
Delay volume setting	Firmware update
Switch valve timing	Export system report to USB

#### Function

**Delay volume setting**: To set the delay volume. The delay volume represents the volume of the liquid found in the flow path between the **UV** and the collection tubes.

*Firmware update*: To update the system firmware.

Switch valve timing: To set the Switch valve timing. It is recommended to optimize the timing of switch valve (**Buffer valve**) when wavy gradients are obtained or when fluctuations in the step gradient are observed during either system performance tests or chromatography runs.

#### Export system report to USB:

Exports system error logs to a USB memory stick in a text format. A system error report is useful when trying to understand a possible error that requires remote service support.

#### Note:

The serial number displayed on the top left of the display screen is the unique identifier for the instrument. The number is also seen as instrument ID when connecting to UNICORN start.

# Settings and service - Screen 2

The options available for the modules displayed on *Settings and service* Screen 2 are briefly described below.

Module	Function
Buffer valve ? Ualve position Buffer A Turn valve Valve switches O Counts Reset Back	<ul> <li>Valve position: Shows which inlet connection in the valve is open,</li> <li>Buffer A (Buffer A) or Buffer B (Buffer B).</li> <li>Turn valve: Switches the valve between its two positions i.e., the A and B inlet ports.</li> <li>Valve switches: The text box displays the number of counts the valve has switched.</li> <li>Reset: Resets the counts to zero when a new valve has been replaced.</li> </ul>
Sample valve          Sample valve       ?         Valve position       Buffer       Turn valve         Valve switches       O       Counts       Reset         Back       Immediate       Immediate       Immediate	<ul> <li>Valve position: Shows which inlet connection in the valve is open, Buffer (Buffer) or Sample (Sample).</li> <li>Turn valve: Switches the valve between its two positions i.e., the Buffer and Sample inlet ports.</li> <li>Valve switches: The text box displays the number of counts the valve has switched.</li> <li>Reset: Resets the counts to zero when a new valve has been replaced.</li> </ul>

#### 3 System description

3.3 Instrument Display

3.3.4 Description of Settings and service

Module	Function
Wash valve     Wash valve	Valve position: Shows which outlet connection in the valve is open, Waste (Waste) or Column (Column).
Value position Waste Turn value	<i>Turn valve</i> : Switches the valve be- tween its two positions i.e., the Waste and Column outlet ports.
Valve switches 0 Counts Reset	<i>Valve switches</i> : The text box displays the number of counts the valve has switched.
	<b>Reset</b> : Resets the counts to zero when a new valve has been replaced.
Outlet valve          Outlet valve       ?         Valve position       Waste       Turn valve         Valve switches       0       Counts       Reset         Back <ul> <li>D</li> <lid< li=""> <li>D</li> <li>D</li></lid<></ul>	Valve position: Shows which outlet connection in the valve is open, Waste (Waste) or Collection (Collec- tion). Turn valve: Switches the valve be- tween its two positions i.e., the Waste and Collection/Frac outlet ports. Valve switches: The text box displays the number of counts the valve has switched. Reset: Resets the counts to zero
	when a new valve has been re- placed.

# Settings and service - Screen 3

The options available for the modules displayed on **Settings and service** Screen 3 are briefly described below.

Module	Function
Main board (Main board)          Main board       ?         Power supply       0.00       V         Memory test       -         Temperature       0.0       *C         FPGA test       -         Back	<ul> <li>Power supply: Shows the actual voltage.</li> <li>Memory test: Performs a memory test.</li> <li>Temperature: Shows the temperature of the main board.</li> <li>FPGA test: Performs a test of the FPGA hardware.</li> </ul>
UV ? UV LED calibration Flow cell path length Diagnostics Configuration Back	<ul> <li>UV LED calibration: To calibrate theUV LED intensity.</li> <li>Flow cell path length: To derive the actual path length of the flow cell.</li> <li>Diagnostics: To troubleshoot the UV module.</li> <li>Configuration: To configure the new UV monitor and flow cell.</li> <li>Note:</li> <li>The C amb and C drft values should not be altered by the user. UV Configuration is performed by a GE Service Engineer only.</li> </ul>
Display (Display)          Display       ?         Touch screen       Color test         Calibration       Log book         Back       fmt	<ul> <li>Touch screen calibration: To calibrate the touch screen.</li> <li>Color test: To perform a diagnostics test for the colors displayed on the touch screen.</li> <li>Diagnostics: To perform a diagnostics test to check the backlight performance of the display.</li> <li>Log book: To read the number of hours the instrument display has been in use.</li> </ul>

#### 3 System description

3.3 Instrument Display

3.3.4 Description of Settings and service

Module	Function
Conductivity Conductivity ?	<i>Calibration</i> : To calibrate the <b>Conduc-</b> <b>tivity flow cell</b> and the temperature sensor.
Calibration Configuration Advanced calibration	<b>Configuration</b> : To set a new cell constant value or reference temper- ature value, usually after the <b>Conduc-</b> <b>tivity flow cell</b> replacement.
Back	Advanced calibration: To calibrate the temperature of the Conductivity flow cell.
	<b>Note:</b> Calibration of the <b>Sine Wave Gener-</b> <b>ator</b> should be performed by a GE Service Engineer only, after the Main board has been replaced.

# 4 Installation

#### About this chapter

This chapter provides the necessary instructions to enable users to unpack and install ÄKTA start and Frac30. Read the entire *Installation* chapter before starting to install ÄKTA start.

# In this chapter

This chapter contains the following sections:

Section	See page
4.1 Space requirements	60
4.2 Transport ÄKTA start and Frac30	62
4.3 Unpack ÄKTA start and Frac30	64
4.4 Accessories package	74
4.5 Install ÄKTA start	75

# 4.1 Space requirements

# **Benchtop setup**

The illustration below shows the space requirements recommended for ÄKTA start.



# Equipment dimensions ÄKTA start



Frac30



# 4.2 Transport ÄKTA start and Frac30

# **Equipment weight**

Item	Weight
ÄKTA start (with packaging)	12 kg
Frac30 (with packaging)	6 kg

## Handling the delivery boxes

ÄKTA start and Frac30 are packed in two separate boxes.



To transport the delivery boxes containing the instrument and Fraction collector, use a hand truck suitable for light weight packages. However, each box can be lifted by 1 person without the help of any lifting equipment.



# 4.3 Unpack ÄKTA start and Frac30

# Introduction

This section describes how to unpack ÄKTA start and Frac30.

**Note:** Save all the original packing material. If the system has to be repacked, for transportation or otherwise, it is important that the system can be safely packed using the original packing material.

# Unpack ÄKTA start

Follow the instructions below to unpack the instrument.

CAUTION ÄKTA start is filled with denaturated alcohol (50% C2 at delivery. The denatured alcohol mixture can b humans if consumed. Flush out the denaturated assembling, testing or integrating ÄKTA start into process context.	2H5OH (ethanol)) <b>He hazardous to</b> alcohol before the intended
---	--

**Note:** ÄKTA start with packaging weighs about 12 kg. No lifting equipment required, **one** person can lift and move the instrument.

#### Step Action

1

Open the delivery box by cutting the adhesive tape at the top of the box.



#### Step Action

2

3

Take out the document placed at the top of the package and read the *Un- packing Instructions*.

#### Note:

Save the documents for future reference.



Take out the box placed at the top of the package. The box contains the accessories delivered with the instrument.



#### Step Action

Hold the red strap, and then lift the instrument out of the delivery box.



5

Open the strap lock and remove the strap.



Step	Action
6	Remove the foam cushion from the top of the instrument.



Remove the foam cushion from the bottom of the instrument by carefully lifting the instrument.



#### Step Action

Remove the plastic bag by gently tilting the system back and forth while pulling out the plastic bag.



#### **Unpack Frac30**

Follow the instructions below to unpack the Fraction collector.



Take care not to damage the Dispenser arm when lifting Frac30 or when removing the plastic bag.



#### NOTICE

Never lift the Frac30 by the Dispenser arm. This may damage the Fraction collector.

**Note:** Frac30 with packaging weighs about 6 kg. No lifting equipment required, one person can lift and move the Fraction collector.

#### Step Action

1 Open the Frac30 delivery box by cutting the adhesive tape at the top of the box.



- 2 Take out the document placed at the top of the package and read the *Unpacking Instructions*.
- 3 Holding the red strap, lift the fraction collector out of the delivery box. Place the Fraction collector on the laboratory bench.



# 4 Installation4.3 Unpack ÄKTA start and Frac30



5 Remove the foam cushion from the top of the Fraction collector.



#### Step Action

6 Remove the foam cushion from the bottom of the fraction collector by carefully lifting the Fraction collector.



Remove the plastic bag.

7



Step	Action
8	Remove the Bowl assembly from the Base unit:
	• Gently move the Dispenser arm counterclockwise to the end position.
	<ul> <li>Push the Drive assembly and hold it at the retracted position. At the same time, lift the Bowl assembly.</li> </ul>



Remove the foam cushion located on the Base unit.


Step	Action		
10	Re-assemble the Bowl assembly on to the Base unit:		
	• Orient the Bowl to match the aligning groove and the aligning features		

located on the bowl holder.

• Slightly push the Drive assembly laterally and lower the Bowl assembly onto the Base unit.





#### NOTICE

Never use the Dispenser arm assembly to lift or hold Frac30. To lift the module, use the handle provided on the bottom plate.

**Note:** Do not damage or break the warranty seal label during unpacking of Frac30.



## 4.4 Accessories package

## Illustration of the accessories package

The illustration below shows the accessories box and the user documentation included with  $\ddot{\mathsf{A}}\mathsf{K}\mathsf{T}\mathsf{A}$  start at delivery.

## ÄKTA start



Part	Description
1	Unpacking Instructions
2	System certificate
3	Product documentation
4	Maintenance Cue Card
5	System Cue Card
6	CD containing user documentation files and an unpacking video. The CD includes <i>Operating Instructions</i> and <i>Maintenance Manual</i> in English and translated versions.
7	Accessories box

## 4.5 Install ÄKTA start

#### Introduction

This section describes how to install ÄKTA start. The following actions must be performed:

- Install pump tubing.
- Connect power supply to ÄKTA start.
- Connect Frac30 to ÄKTA start.
- Connect ÄKTA start to the UNICORN start computer.



#### WARNING

Only use grounded power cords delivered or approved by GE.



#### WARNING

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



#### CAUTION

**Protective ground.** ÄKTA start must always be connected to a grounded power outlet.

## Install pump tubing

2

Follow the instructions below to install the pump tubing.

Step	Action
1	Open the top cover until it is fully open.



Place the tubing between the rollers and the track, press against the pump head inner wall.



#### Note:

Make sure that the pump tubing is not twisted or stretched against the rollers.

Step	Action			
3	Lower the top cover until it clicks into its fully closed position.			
	The track closes automatically and the tubing is stretched correctly as the track closes.			



## Connect power to ÄKTA start

Follow the instructions below to connect power to ÄKTA start.

Step	Action
1	Select the correct power cord to be used. ÄKTA start is delivered with 2 alter- native power cords:
	• Power cord with US-plug, 2 m.

• Power cord with EU-plug, 2 m.

#### Note:

Discard the unused power cord.

2

Connect the power cord to the Power input connector on the left side of the instrument and to a grounded wall outlet 100-240 VAC, 50/60 Hz.





#### CAUTION

ÄKTA start is filled with denaturated alcohol (50% C2H5OH (ethanol)) at delivery. **The denatured alcohol mixture can be hazardous to humans if consumed.** Flush out the denaturated alcohol before assembling, testing or integrating ÄKTA start into the intended process context.

#### Note:

For instructions on how to clean the flow path, see Section 8.3 Cleaning the system flow path, on page 222.

## Connect Frac30 to ÄKTA start



#### NOTICE

Frac30 should not be connected or disconnected from ÄKTA start when the instrument is powered ON.

Follow the instructions below to connect Frac30 to ÄKTA start.

# Step Action 1 Connect the Frac30 cable between the intended ports on the back of the Fraction collector and the instrument.

#### Note:

The supply voltage for Frac30 is distributed from ÄKTA start.

When the Frac30 cable is connected, the screws attached to the connector should be tightened.



- 2 Switch on ÄKTA start.
- 3 Enable the connection of Frac30 from the Instrument Display:
  - In the ÄKTA start home screen, tap Settings and service. Result: The Settings and service Screen 1 opens.



• In the Settings and service screen, tap Fraction collector.

Result: The Fraction collector screen opens.

Fi	action collector		?
	Enable Frac		
	Back		

• In the *Fraction collector* screen, tap *Enable Frac* to enable the connection of the Fraction collector.

Result: The following screen will be displayed.



#### Note:

The power on status of the Fraction collector is indicated by the white LED on the front of Frac30.

## Connect a computer to ÄKTA start

**Note:** Before connecting the computer to ÄKTA start, install the UNICORN start software on the computer. Refer to the UNICORN start 1.0 User Manual.

Follow the instructions below to connect a UNICORN start computer to ÄKTA start.

2

1 Connect power to the computer and monitor, and then switch on the computer and ÄKTA start.

*Result*: The instrument displays the **ÄKTA start** home screen.



Connect the PC Connection Cable between the connector marked as **PC Connection** on the back of ÄKTA start and a USB port on the computer.

6		
	-	
	•	
. ~		<u>.</u>

## Step Action 3 Launch UNICORN start and connect to ÄKTA start. For detailed instructions.

refer to UNICORN start 1.0 User Manual.

*Result*: The instrument displays the **ÄKTA start** home screen in connected state.

ÄKTA start	?
Method run	Manual run
Create method	Settings and service

#### Note:

Make sure that the system connection is established before starting the run. Always make sure to be in the **ÄKTA start** home screen (connected state) when trying to connect from the **System Control** module.



#### CAUTION

ÄKTA start is filled with denaturated alcohol (50% C2H5OH (ethanol)) at delivery. **The denatured alcohol mixture can be hazardous to humans if consumed.** Flush out the denaturated alcohol before assembling, testing or integrating ÄKTA start into the intended process context.

## 5 Prepare the system for a run

## About this chapter

This chapter describes how to start the instrument and prepare the system for a run.

## In this chapter

This chapter contains the following sections:

Section	See page
5.1 Flow path overview	84
5.2 Start the instrument	87
5.3 Calibrations	88
5.4 System performance	106
5.5 Connect a column	121
5.6 Run Prepare system methods	125
5.7 Sample application	135
5.8 Prepare the Fraction collector	147
5.9 Cold room operations	151
5.10 Starting a run	153

## 5.1 Flow path overview

## Illustration of the flow path

The illustration below shows the flow path for ÄKTA start. The flow path contains **Pump**, **Mixer**, **Valves**, and **UV**, **Conductivity** and **Pressure** monitors. The individual instrument modules are presented in the table below. For a detailed description of the modules, see the *ÄKTA start Maintenance Manual*.



Part	Description	Part	Description
1	Buffer valve	7	Injection valve (manual)
2	Mixer	8	Column
3	Sample valve	9	UV Monitor
4	Pump	10	Conductivity Monitor
5	Pressure sensor	11	Outlet valve
6	Wash valve	12	Fraction collector

## Inlet and outlet tubing

ÄKTA start is delivered with the entire flow path assembled and prefilled with storage solution (50% Ethanol). Details on the tubing types used along the flow path are presented in *Chapter 10 Reference information, on page 258*.

The table below lists the tubing connected to the instrument. Prepare the system for a run by connecting inlet and outlet tubing to the valve ports marked with orange arrows.

Module	Tube connection	Scope of use
Buffer valve	Port I (Buffer A)	Inlet tubing for buffer A
	Port II	Outlet tubing to the <b>Mixer</b> .
	Port III (Buffer B)	Inlet tubing for buffer B
Sample valve	Port I (Sample)	Inlet tubing used when the sample is applied via the <b>Pump</b> .
	Port II	Pump tubing.
	Port III	Inlet tubing from the <b>Mixer</b> .
Wash valve	Port I (Waste)	Outlet tubing used when cleaning the flow path or changing the buffer by running the <i>Pump Wash A/B</i> template.
	Port II	Inlet tubing from the <b>Pressure sensor</b> .
<b>→₩</b> ,	Port III	Outlet tubing to the <b>Injection valve</b>
Injection	Port <b>1</b>	Outlet; the tubing is connected to the column.
	Ports <b>2</b> and <b>5</b>	Inlet/outlet for connecting the sample loop.
	Port <b>3</b>	Inlet for injecting the sample into the loop.
	Port <b>4</b> (Waste)	Outlet tubing to waste, helps in washing or draining excess sample from the loop.
	Port <b>6</b>	Inlet; the tubing is connected to <b>Wash valve</b> .
Outlet valve	Port I (Waste)	Outlet tubing to the waste container.
	Port II	Inlet tubing from the <b>Conductivity</b> Monitor.
	Port III (Collection)	Outlet tubing to the Fraction collector.

## **Placement of buffer bottles**

Buffer bottles are placed in the Buffer tray on top of the instrument, as illustrated below. Sample bottle or tube may be placed on the bench on the left side of the instrument. A waste bottle may be placed on the bench on the right side of the instrument.





#### CAUTION

**Max. weight on Buffer tray.** Do not place bottles with a volume of more than 1 liter each on the Buffer tray. The total allowed weight on the Buffer tray is 5 kg.



#### CAUTION

Avoid spillage and overflow. Make sure that the waste tubing is inserted in an appropriate waste container and secured in place.

## 5.2 Start the instrument

## Switch on the instrument

Follow the instruction below to start the instrument.

Step	Action

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



*Result*: The instrument starts and initializes the display, showing the **ÄKTA start** home screen.



2

You can start using the instrument immediately. All modules are pre-calibrated from the factory.

## 5 Prepare the system for a run 5.3 Calibrations

5.3 Calibrations

## Introduction

This section describes how to calibrate the Instrument Display, **Pump**, and **Monitors**.

### In this section

This section contains the following subsections:

Section	See page
5.3.1 Calibration guide	89
5.3.2 Instrument Display calibration	90
5.3.3 Pressure sensor zero offset	92
5.3.4 Pump calibration	94
5.3.5 UV Monitor calibration	96
5.3.6 Conductivity Monitor calibration	100

## 5.3.1 Calibration guide

## When to calibrate

**Note:** The instrument is pre-calibrated at delivery, therefore no calibration is required when the instrument is installed. However, if the **System performance test** fails it is recommended to calibrate the modules.

The table below provides recommendations for when modules should be calibrated.

Module	When to calibrate
Instrument Display	• If there are any issues with the response of the touch screen.
Pressure sensor	• If the pressure is outside the range of $\pm$ 0.03 MPa, perform <b>Zero offset</b> .
Pump	• When chromatography run conditions are changed, e.g., viscosity of sample or buffer, temperature, back-pressure.
	• <b>Pump</b> and pump tubing requires calibration regularly. Recommended: once a week.
	• After the pump tubing has been replaced with new tubing.
	Note:
	Do not leave the pump tubing inside the <b>Pump</b> when the <b>Pump</b> is not running.
UV Monitor	• When the signal is unstable, or readings appear to be incorrect.
	• After cleaning, or after replacing the <b>UV flow cell</b> .
	• When error/warning is seen on power ON.
	• When baselining is ignored when the <b>UV flow cell</b> is clean.
	• Before and after performing runs in the cold room.
Conductivity Monitor	• When the signal is unstable, or readings appear to be incorrect.
	• After replacing the <b>Conductivity flow cell</b> .

2

## 5.3.2 Instrument Display calibration

## Instruction

Follow the instructions below to calibrate the Instrument Display.

#### Step Action

1 In the **ÄKTA start** home screen, tap **Settings and service** to access the instrument modules. Tap **Next:Next** to access the 3rd screen.

Result: The Settings and service screen will be displayed.



In the **Settings and service** screen, tap **Display** to access the touch screen options.

Result: The **Display** screen opens.



3 In the *Display* screen, tap *Touch screen calibration* to start the calibration. *Result*: A *Message* screen opens.

Touch Screen wi Do you want to c	ll be Calibrated now ontinue?	<i>.</i>
Yes	No	

Tap Yes to confirm and proceed with the calibration

or

Tap No to cancel the action and return to the Display screen.

4 In the four subsequent **Calibration** screens, tap precisely on the marked circles, as requested. The first **Calibration** screen is presented as an example.

Calibration in progress	
press the marked circle shown	

5

Tap *Exit* when the calibration is completed.

Calibration done press exit	Exit

#### Note:

Repeat the procedure if calibration fails.

## 5.3.3 Pressure sensor zero offset

## Zero offset

Follow the instructions below to set the pressure as zero when the **Pressure sensor** is exposed to atmospheric pressure only (**Zero offset**).

**Note:** Pressure Calibration is performed by a GE Service Engineer only, after the **Pressure sensor** is replaced.

#### Step Action

1 Disconnect the inlet tubing from the **Pressure sensor** in order to expose the sensor to atmospheric pressure only.

#### Note:

Make sure that the **Pump** is OFF before disconnecting the tubing.

2 In the **ÄKTA start** home screen, tap **Settings and service** to access the instrument modules.

Result: The Service and settings screen opens.

ettings and service	?
Fraction collector	Pressure sensor
Pump	System
	1/3
6	Next

3

In the **Settings and service** screen, tap **Pressure sensor** to access the **Pressure sensor** options.

Result: The Pressure sensor screen opens.

User opera	itions	
P set	0.00 MPa	Zero offset
GEHC serv	ice only	
		Calibrato

Step	Action
4	In the <b>Pressure sensor</b> screen, tap <b>Zero offset</b> . <i>Result</i> : A <b>Message</b> screen opens.
	Message
	Make sure "No Pressure" in the system
	Ok Cancel
5	Tap <b>OK</b> if there is no back pressure in the system (i.e. the inlet tubing from the <b>Pump</b> was disconnected from the <b>Pressure sensor</b> ). <i>Result</i> : The atmospheric pressure is set as zero.
6	Re-connect the inlet tubing from <b>Pump</b> to the <b>Pressure sensor</b> .

## 5.3.4 Pump calibration

4

#### Instruction

Follow the instructions below to calibrate the **Pump**.

Step	Action
1	Immerse the buffer inlet tubing A in demineralized (DM) water.
2	Place the outlet tubing from the <b>Wash valve</b> in a pre-weighed collection tube.

Note:

Before starting the calibration, prime the flow path with water and make sure that the outlet tubing where the pumped water is collected is filled with DM water. This ensures that the volume of the collected water corresponds to the pumped volume.

3 In the **ÄKTA start** home screen, tap **Settings and service** to access the instrument modules.

Result: The Settings and service - Screen 1 opens.



In the **Settings and service** screen, tap **Pump** to access the **Pump** options. *Result*: The **Pump** screen opens.



5

In the **Pump** screen, tap **Calibration**.

Result: The Calibration screen opens.

Calibration					[
Flow Rate	~	0.5	^	ml/min [	Start flow
Collected Volume	~	1.2	^	ml [	Calibrate
Back	]	[			

- 6 In the *Calibration* screen, set the intended flow rate in the range 0.5 to 5 ml/min. Use the up/down arrows to set the value or use numpad to type in the value.
- 7 In the **Calibration** screen, tap **Start flow** to start the **Pump**.

*Result*: The **Pump** starts and the pumped DM water will be collected in the pre-weighed collection tube.

Collect water for at least one minute in the pre-weighed collection tube.

- 8 Estimate accurately the volume of the water collected in the pre-weighed collection tube, then set the **Collected volume** value equal to this volume.
- 9 In the *Calibration* screen, tap *Calibrate*, then confirm the action (*OK*) to carry out the calibration.
- 10 Verify that the **Pump** was correctly calibrated. Repeat the steps 6 and 7 to confirm that the volume of the collected water corresponds to the intended flow rate.

#### Note:

If the collected water does not correspond to the required volume (i.e., the **Pump** does not deliver the liquid with the intended flow rate), inspect the condition of the pump tubing. Replace the pump tubing if necessary, and then perform re-calibration.

## 5.3.5 UV Monitor calibration

## Set the Cell path length

If the UV flow cell was replaced, a new Cell path length value has to be set.

**Note:** Make sure that the **UV flow cell** is assembled tightly, and the inlet and outlet tubing is fitted tightly to the **UV** Monitor. No stray light should enter the **UV** Monitor. Make sure that there are no air bubbles in the **UV flow cell**.

#### Prerequisites

- Buffer A: DM water
- Buffer B: freshly prepared 1% acetone solution, which is expected to give an Abs value of 340 mAU.

Follow the instructions below to set the *Cell path length*.

#### Step Action

1 In the **ÄKTA start** home screen, tap **Settings and service** to access the instrument modules, then tap **Next:Next**.

Result: The Settings and service Screen 3 opens.



2

In the Settings and service screen, tap UV to access the UV Monitor options.

*Result*: The **UV** screen opens.



3 In the **UV** screen, tap **Flow cell path length** to access the flow cell options. For a description of the **UV** Monitor options, refer to the ÄKTA start Maintenance Manual.

Result: The Flow Cell Path Length screen opens.

Abs	0.0	mAU	Set baseline
Cell		mm	
~	0.00	~	Save path length

In the Flow Cell Path Length screen:

set the *Cell path length* in the *Cell* field, corresponding to the new UV flow cell

Note:

The Cell path length is provided on the packing of the UV flow cell.

- tap *Save Path Length* to save the value.
- 4 If the *Cell path length* is not available, follow the instructions below to calculate the *Cell path length*.

Immerse the inlet buffer A tubing in DM water and the inlet buffer B tubing in 1% acetone solution.

- 5 In the *Flow Cell path length* screen, set the *Cell* value to 2.00 mm by using the up/down arrows or typing in the value.
- 6 Flush thoroughly and leave the **UV flow cell** filled with buffer A (DM water).

Tap **Set Baseline** to capture a new reference for the test.

Result: Abs should show a value close to 0 mAU.

- 7 Flush the **UV flow cell** with Buffer B (1% acetone) and leave the flow cell filled with 1% acetone. Note down the *new Abs* value.
- 8 Calculate the actual **Cell path length**:

*Cell* (mm) = 2.00 \* (*new Abs* value / 340)

Step	Action
9	Update the calculated <b>Cell</b> length value by using up/down buttons or typing in the value. Tap <b>Save Path Length</b> to save the value to permanent memory.
	Note:
	Observe that the <b>Abs</b> value now should be about $340 \pm 5\%$ mAU, confirming that normalization has been done.

## Calibrate the UV LED

Follow the instructions below to calibrate the **UV LED**.

**Note:** Make sure that the **UV flow cell** is assembled tightly, and the inlet and outlet tubes are fitted tightly to the **UV** Monitor. No stray light should enter the **UV** flow cell.

Step	Action
1	Immerse the buffer inlet tubing in DM water.
2	Flush the <b>UV flow cell</b> with DM water using the <b>Pump</b> . Make sure that there are no air bubbles in the <b>UV flow cell</b> .

#### Note:

Flush thoroughly with DM water to make sure that the flow cell is clean.

3 In the **ÄKTA start** home screen, tap **Settings and service** to access the instrument modules, then tap **Next:Next**.



Result: The Settings and service Screen 3 opens.

## In the Settings and service screen, tap UV to access the UV Monitor options. Result: The UV screen opens.

I.	?
UV LED calibration	Flow cell path length
Diagnostics	Configuration
Back	2

In the **UV** screen, tap **UV LED calibration**. For a description of the **UV** calibration parameters, refer to the ÄKTA start Maintenance Manual.

Result: The UV LED Calibration screen opens.

UV LED calif	oration				?
Signal	0.0	mV	Light	Str 0	ength
T amb	0.00	]•c	Calibrate	Sa	ve
Back		<b>L</b>			

6

5

- Set the *Light strength* value to 500 using up/down arrows or type in the value.
  - Tap Calibrate. A confirmation screen opens. Select OK to automatically search the Light strength value to get a minimum Signal response of 2500 mV.
  - If the *Signal* value is above 2500 mV, tap *Save*.

If the *Signal* value is below 2500 mV use the arrows to increase the *Light strength* until the *Signal* is above 2500 mV.

7 Tap **Save** to store the calibrated **Light strength** value.

## 5.3.6 Conductivity Monitor calibration

## Set the Cell constant value

2

3

If the flow cell was replaced, a new Cell constant value has to be set. Follow the instructions below to set the Cell constant value.

#### Step Action

1 In the **ÄKTA start** home screen, tap **Settings and service** to access the instrument modules, then tap **Next:Next**.

Result: The Settings and service screen 3 opens.



In the **Settings and service** screen, tap **Conductivity** to access the **Conductivity** Monitor options.

Result: The Conductivity screen opens.



In the **Conductivity** screen, tap **Configuration** to access the configuration options.

Result: The Configuration screen opens.

Set Cell Const	~	43.0	^	1/cm	
Set Ref Temp	~	20.0	^	°C	
	ſ	Save	1		

## Step Action 4 In the Configuration screen, set the Cell Constant value in the Set Cell Const field, for the new Conductivity flow cell by using the up/down arrows. Tap Save to save the new value. Note: Note:

The Cell Constant value is provided on the packaging of the **Conductivity** *flow cell*.

If a Cell constant value is not available, calibrate the **Conductivity flow cell** as explained in Calibrate the **Conductivity flow cell**, on page 103.

### Set the Reference Temperature

 
 Note:
 For the system to recalculate the measured conductivity provide the reference temperature in the temperature field Set Ref Temp.

 Make sure that the checkbox Enable Temperature Compensation in the Conductivity Calibration screen is checked.

Follow the instructions below to set the reference temperature.

#### Step Action

 In the Conductivity:Configuration screen, set the Reference Temperature (Set Ref Temp) value in the range 4°C to 35°C.



Tap **Save** to save the new reference temperature value.

2

5.3.6 Conductivity Monitor calibration

## Calibrate the temperature sensor

Follow the instructions below to calibrate the temperature sensor.

Step	Action
1	Place a precision thermometer in the path of the <b>UV flow cell</b> directly after the <b>Conductivity flow cell</b> and pump DM water through system with a flow rate of 0.5 ml/min.

2 In the Settings and service screen 3, tap Conductivity to access the Conductivity Monitor options.

Result: The Conductivity screen opens.

Conductivity	?
Calibration	Configuration
Advanced calibration	
Back	

3

In the Conductivity screen, tap Advanced calibration.

Result: The Advanced calibration screen opens.

Advanced calibration ?
Set actual temp 🗸 25.0 ^ °C Calibrate
GEHC service only
Cell resistance 0.0 Ohms
Calibrate sine gen
Back

4

Note the temperature and type it into the **Set Actual Temp** field, then tap **Calibrate** to carry out the temperature calibration.

#### Note:

Make sure that the temperature of the **Conductivity flow cell** has stabilized and measure the temperature of the calibration solution with a precision thermometer.

#### Note:

**Calibrate Sine Gen** is performed by a GE Service Engineer only, when the Main board is replaced.

## Calibrate the Conductivity flow cell

1

#### **Prequisites**

Calibration solution: 1.00 M NaCl or 100 mS/cm conductivity standard solution. Follow the instructions below to calibrate the **Conductivity flow cell**.

## Step Action

In the Conductivity screen, tap Calibration.

Conductivity	?
Calibration	Configuration
Advanced calibration	
Back	<u>۵</u>

Result: The Calibration screen opens.

Calibration	?
Set Conductivity 86.0 MS/cm Calibre	ate
Cell Temperature 25.0 °C	
Enable Temperature Compensation	
Back	

- 2 Note the current temperature of the calibration solution in the **Conductivity flow cell** as displayed in the **Cell Temperature** field.
- 3 In the *Calibration* screen, set the theoretical conductivity value at the *current* temperature in the *Set Conductivity* field and then tap *Calibrate* to carry out the conductivity calibration.

#### Note:

- If a certified conductivity standard solution is used, use the supplied theoretical conductivity value corresponding to the temperature in question.
- If a manually prepared 1.00 M NaCl calibration solution is used, get the conductivity value at the current temperature from the graph for conductivity of the 1.00 M NaCl as a function of temperature presented below.

5 Prepare the system for a run5.3 Calibrations5.3.6 Conductivity Monitor calibration

## Graph for Conductivity value

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



### Conductivity of 1.00 M NaCl at 20–30°C

Axis	Description
×	Temperature (°C)
У	Conductivity (mS/cm)

## 5.4 System performance

## Introduction

This section describes the **System performance method** and how to perform and evaluate the **System performance method** (system performance).

## In this section

This section contains the following subsections:

Section	See page
5.4.1 System performance method	107
5.4.2 System performance method from ÄKTA start	109
5.4.3 System performance method from UNICORN start	114
5.4.4 Switch valve timing	117

## 5.4.1 System performance method

### Introduction

The **System performance method** is performed to make sure that the system is performing within acceptable limits. It is recommended to run the test at the time of installation of the instrument or after replacement of modules such as **Pump**, **UV**, **Conductivity** or **Valves**. **System performance method** can also be used at any time to check the condition of the system, for example, after a prolonged storage of the system. The **System performance method** can be performed from both the Instrument Display and UNICORN start.

Note:

- Calibrate all the modules before starting the test.
  - Make sure that there is no column connected.
  - It is recommended not to edit any run parameters during a test in order to avoid the failure of the test.

#### **Requirements**

Required solutions are:

- Buffer A DM water
- Buffer B 1.0% acetone, 1.0 M NaCl
- Sample 1.0% acetone, 1.0 M NaCl (Buffer B)

**Note:** Make sure to prepare the buffer solutions accurately to avoid the test failure.

### Checklist

Before starting a *System performance method*, make sure that the following tasks are completed or met:

- Calibration of all the modules: Pressure sensor, Pump, UV and Conductivity.
- No column should be present in the flow path.
- Set the conductivity reference temperature to 20°C, save and enable the function.
- Immerse Buffer port A inlet in Buffer A (DM water).
- Immerse Buffer port B inlet in Buffer B (1.0% acetone, 1.0 M NaCl).
- Sample valve inlet immersed in sample (1.0% acetone, 1.0 M NaCl).
- Make sure that 1 ml Sample loop is filled with sample (1.0% acetone, 1.0 M NaCl).

- Make sure that 2 m of 0.5 mm ID tubing is connected to the **Outlet valve** at Waste position.
- When performing *System performance method* without Fraction collector, make sure the **Outlet valve** fractionation tubing is inserted into a pre-weighed beaker.
- When performing *System performance method* with Fraction collector, make sure the **Outlet valve** fractionation tubing is connected to Fraction collector with at least 5 pre-weighed tubes.
- Make sure that the system is prefilled with DM water.
- Make sure to take note of all the required observations by recording parameters while the *System performance method* is in progress. Enter the observed values in the System report template presented in *Section 11.1 System Performance Report, on page 276*.
# 5.4.2 System performance method from ÄKTA start

### Instruction

Follow the instructions below to initiate the *System performance method* from the Instrument Display.

**Note:** Insert a USB memory stick to save the results.

### Step Action

2

3

1 From the **ÄKTA start** home screen, tap **Method Run**.



#### In the Method run screen, tap Prepare system.



Select System performance method. To initiate the method, tap Select.

Select sy	stem method	?
	System cleaning	
<	System performance method	
		2/2
Back		Select

Step	Action
4	Tick the check box in order to save the results on a USB memory stick, and
	then tap <b>Run</b> to start the System Performance test.

#### Note:

Provide a unique file name.

Run parameters		?
System performa Save Result to USB	nce method Perf01	
	<u> </u>	1/1
	<b>a</b>	Run

Result: The Run view screen will be displayed.

UV absorbance	1.0	mAU	
Conductivity	25.0	mS/cm	
Flow rate	3.0	ml/min	Graph
Pressure	0.30	MPa	
Tube no.	6		

### Note:

- It is recommended not to edit any run parameters during a run to avoid the failure of the test.
- If required, the **System performance method** run can be ended before it is completed, tap **End** to abort the test.

```
5
```

Tap *Exit* to close the screen when the *System performance method* is completed.

UV absorbance	1.0	mAU	
Conductivity	25.0	mS/cm	
Flow rate	0.0	ml/min	Graph
Pressure	0.30	MPa	
Tube no.	6		

### Note:

Make sure not to unplug the USB memory stick until the system generates the report (BMP file).

6 Review the report whether the test passed or failed, based on the Acceptance criteria presented below.

# Acceptance criteria

Time (min)	Activity	Check	Approved interval
0	Pump wash	<b>Wash valve</b> posi- tion	Mobile phase out through <b>Waste</b>
1	1 ml/min, 0% B, flow through <b>Outlet valve</b> , <b>Waste</b> position	Back pressure	≤ 0.05 MPa
2	Repeat <b>UV Auto zero</b>		
3	5 ml/min	Back pressure	0.06 to 0.2 MPa
		UV level	± 10 mAU
		Conductivity level	±1mS/cm
4	1 ml/min, <b>Sample valve</b> , <b>Sam-</b>	Max. UV level	300 to 380 mAU
	pie position	Max. Conductivity level	65 to 95 mS/cm
7	1 ml/min, <b>Sample valve</b> , <b>Buffer</b> position		

### 5 Prepare the system for a run

5.4 System performance

5.4.2 System performance method from ÄKTA start

Time (min)	Activity	Check	Approved interval
10	Request switch Injection valve	Max. UV level	300 to 380 mAU
	to <b>Inject</b> position.	Max. Conductivity level	65 to 95 mS/cm
13	Request switch <b>Injection valve</b> back to <i>Load</i> position.		
15	Start gradient, 0 to 100% B in 10 minutes, start fractiona- tion/collection.		
19	End fractionation <sup>1</sup>	Weigh fraction no. 2, 3 and 4.	0.8 to 1.2 g
		Max. diff. between fractions	0.1 g
20	End collection <sup>2</sup>	Weigh beaker	4.2 to 5.8 g
25	End gradient, stay at 100% B	Gradient	Straight, no nega- tive dips.
28	50% B	Gradient level <sup>3</sup>	45 to 55% B
36	0 %B (Re equilibration)		
41	End	Check all connec- tions for leakage	No leakages.

- 1 With Fraction collector
- 2 Without Fraction collector
- 3 UV 50% B / UV 100% B

**Note:** If the **System performance method** fails, analyze the cause for the failure based on the acceptance criteria. Perform the following actions:

- *Re-calibrate the failed module*
- Use buffer with proper composition.
- Clean the failed module or entire system. Refer Chapter 8 Maintenance, on page 217 for more details on cleaning.
- Carefully follow the test instructions.
- Repeat the System performance method until it passes.

- If wavy or fluctuations in gradient is observed, then perform **Switch valve** *timing* optimization.
- If the test fails after following the above actions, replace the failed module.

# 5.4.3 System performance method from UNICORN start

# Instructions

Follow the instructions below to initiate the **System performance method** from UNICORN start.

Step	Action
1	Start the test from UNICORN start <b>System control:System:Performance</b> Test and Report.
2	Select method based on the Fraction collector configuration:
	• <b>Performance method with Frac</b> : when the Fraction collector is <i>enabled</i> .
	• <b>Performance method without Frac</b> : when the Fraction collector is <i>disabled</i> .
3	Read the method notes before starting the run.
4	Make a note of the result file location.
5	Run the <b>System performance method</b> .
6	The test report states whether the <b>System performance method</b> passed or failed.
	Manually verify Pressure limits, Fractionation/collection volumes, gradient levels and all connections for leakage during the test, using the Acceptance criteria presented below.

# Acceptance criteria

Time (min)	Activity	Check	Approved interval
1	1 ml/min, 0% B, flow through <b>Outlet valve</b> waste position	Back pressure	≤ 0.05 MPa
2	Repeat <b>UV Auto zero</b>	-	
3	5 ml/min	Back pressure	0.06 to 0.2 MPa
15	Start gradient, 0 to 100% B in 10 minutes, start fractiona- tion/collection.		

# 5 Prepare the system for a run

5.4 System performance

5.4.3 System performance method from UNICORN start

Time (min)	Activity	Check	Approved interval
19	End fractionation <sup>1</sup>	Weigh fraction no. 2, 3 and 4.	0.8 to 1.2 g
		Max. difference between fractions	0.1 g
20	End collection <sup>2</sup>	Weigh beaker	4.2 to 5.8 g
25	End gradient, stay at 100% B	Gradient <sup>3</sup>	Straight, no nega- tive dips.
41	End	Check all connec- tions for leakage	No leakage.

- 1 With Fraction collector
- 2 Without Fraction collector
- 3 UV 50%B / UV 100%B

**Note:** • Make sure to update the **Performance result** text file with manually observed recordings and then print the report.

• The other parameters are automatically checked and pass/fail reports are generated in the report. For detailed list of acceptance criteria, refer to Section 5.4.2 System performance method from ÄKTA start, on page 109.

# Illustration of the System performance test

The illustration below represents a typical *System performance method* result file generated from UNICORN start.



# **Note:** It is recommended to optimize the timing of switch valve when wavy gradients or fluctuations are observed. For detailed description, see Section 5.4.4 Switch valve timing, on page 117.

- **Note:** If the **System performance method** fails, analyze the cause for the failure based on the acceptance criteria. Perform the following actions:
  - *Re-calibrate the failed module*
  - Use buffer with proper composition.
  - Clean the failed module or entire system. Refer Chapter 8 Maintenance, on page 217 for more details on cleaning.
  - Carefully follow the test instructions.
  - Repeat the System performance method until it passes.
  - If wavy or fluctuations in gradient is observed, then perform **Switch valve** *timing* optimization.
  - If the test fails after following the above actions, replace the failed module.

# 5.4.4 Switch valve timing

### Introduction

*Switch valve timing* is used to optimize the switch valve (**Buffer valve**) timing of ÄKTA start. It is recommended to optimize the timing of switch valve when wavy gradients are obtained or when fluctuations in the step gradient are observed during either *System performance method* or chromatography runs.

- Note: The default Switch valve timing A is 4 sec.
  - Switch valve timing B is 5 sec.
  - Switch valve time can be set between 3.0 and 5.0 sec with 0.1 sec increments using Advanced timing.
  - The gradient fluctuations or wavy gradients are highly dependent on the flow rates. It is recommended to modify the switch valve timing if waviness/fluctuations are seen for specific flow rates.
  - After changing the Switch valve time, perform a System performance method to evaluate the gradient fluctuations/wavy gradients. Alternatively a manual run of 50% B for 10 min can also be performed to evaluate the gradient fluctuations/wavy gradients.

# Illustration of a typical operation

The illustration below shows the result of a **System performance method** where fluctuations were observed in the gradient (arrow).



The *Switch valve timing* was changed to 5 sec, the test was repeated and the result showed an acceptable gradient performance (arrow).





# Set Switch valve timing

Follow the instructions below to change the *Switch valve timing*. The *Switch valve timing* can be changed from the Instrument Display.

Step	Action
1	From the ÄKTA start home screen, tap Settings and service.
	Result: The Settings and service screen 1 opens.



2

### Tap **System**.

*Result*: The *System* screen opens.



3 Tap Switch valve timing.

Result: The Switch valve timing screen opens.

4 Select Switch valve timing B to change the Switch valve timing to 5 sec.

$\bigcirc$	Switch valve timing A (Default)	
	Switch valve timing B	
$\bigcirc$	Advanced timing	

- 5 Tap *Save* to save the changed timing.
- 6 If wavy gradients persist, or if fluctuations on step gradient levels are large, select *Advanced timing* to set switch valve timing other than *Switch valve timing A* and *Switch valve timing B*.

Switch valve timing	?
Switch valve timing A (Default)	
Switch valve timing B	
Advanced timing	
Switch valve time 🗸 4.5 🔨 Sec	
Back 🔂	Save

### Note:

It is recommended to use the **Advanced timing** if the options for **Switch valve timing A** or **Switch valve timing B** have not yielded satisfying results.

7

Set *Switch valve time* in the range of 3.0 to 5.0 sec.

Step	Action
8	Tap <i>Save</i> to save the optimized timing.
9	Perform the <b>System performance method</b> or 50% gradient runs until satis- factory results are obtained.

# 5.5 Connect a column

# Introduction

This section describes how to connect a column to ÄKTA start. Different types of column holders are available for the columns that are to be used with the instrument. See *Chapter 10 Reference information, on page 258* for selecting an appropriate column holder.

The column is connected in the flow path between the **Injection valve** and **UV** Monitor, as shown in the illustration of the flow path in Section 5.1 Flow path overview, on page 84.

# **Column placement**

Depending on column dimension, choose the appropriate location on the instrument to place the column. Column holder rails are located on the front and on the right side of the instrument, as shown in the image below.

- Front side of the instrument, for small columns (e.g., HiTrap™ columns)
- Right side of the instrument, for large columns (e.g., column length 60 cm)



5.5 Connect a column

# Connect a column

Follow the instructions below to connect a column to the instrument.

Step	Action		

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



2 Remove the column stoppers, mount the column on the union connector if the column type requires a union.





4

Connect the 0.75 mm i.d. PEEK tubing from the **Injection valve** port **1** to the column head.



5

### Step Action

Connect the 0.75 mm i.d. PEEK tubing from the bottom of the **UV** Monitor to the bottom of the column.

### Note:

The 0.75 mm i.d. PEEK tubing should not be disconnected from the **UV** Monitor inlet when the column is removed. See Section 8.3.1 Disconnect the column, on page 223.



**Note:** Do not overtighten when connecting columns. Overtightening might break the connectors or squeeze the tubing and thereby obstructing the flow.

# 5.6 Run Prepare system methods

# Introduction

This section describes how to prepare the flow path and the column before starting a chromatography run.

# In this section

This section contains the following subsections:

Section	See page
5.6.1 Pump wash A	126
5.6.2 Pump wash B	128
5.6.3 Washout fractionation tubing	130
5.6.4 Column preparation	133

# 5.6.1 Pump wash A

# Introduction

The *Pump wash A* method is used before the start of a new run or in case of changing the buffers. During *Pump wash A* the flow is directed through **Wash valve** to **Waste**.

- **Note: Pump wash A** is performed at 10 ml/min for 1 min through Buffer A port.
  - **Pump wash A** is important for preventing carryover and cross-contamination among Buffers/Samples.
  - It is recommended to first wash with DM water, and then wash with the buffer of choice.
  - The **Pump wash A** method cannot be edited.

## Requirements

Required solutions are:

- DM water
- Buffer solution

### Instruction

Follow the instructions below to perform a **Pump wash A** run. The **Pump wash A** procedure is initiated from the Instrument Display.

Step	Action
1	Immerse the buffer A inlet tubing in DM water or buffer.
2	In the ÄKTA start home screen tap <b>Method run</b>



### 3 In the *Method run* screen, tap *Prepare system*.



4

Select **Pump wash A** and then tap **Select** to initiate the method.

Select system	method	?
۲	Pump wash A	
0	Pump wash B	
$\bigcirc$	Washout fractionation tubing	
0	Column preparation	1/2
Back		Select

Result: The following screen opens.

UV absorbance	1.0	mAU	
Conductivity	25.0	mS/cm	
Flow rate	3.0	ml/min	Graph
Pressure	0.30	MPa	
Tube no.	6		

### Note:

If required, the **Pump wash A** run can be ended before it is completed by tapping **End** to stop the run.

- 5
- When the run is completed, tap *Exit* to close the *Pump wash A* screen.

UV absorbance	1.0	mAU	
Conductivity	25.0	mS/cm	
Flow rate	0.0	ml/min	Graph
Pressure	0.30	MPa	
Tube no.	6		

# 5.6.2 Pump wash B

# Introduction

The *Pump wash B* method is used before the start of a new run or in case of changing the buffers. During *Pump wash B* the flow is directed through **Wash valve** to **Waste**.

- **Note: Pump wash B** is performed at 10 ml/min for 1 min through Buffer B port.
  - **Pump wash B** is important for preventing carryover and cross-contamination among buffers and samples.
  - It is recommended to first wash with DM water, and then wash with the buffer of choice.
  - The Pump wash B method cannot be edited.

## Requirements

Required solutions are:

- DM water
- Buffer solution

### Instruction

Follow the instructions below to perform a **Pump wash B** run. The **Pump wash B** procedure is initiated from the Instrument Display.

Step	Action
1	Immerse the buffer B inlet tubing in DM water or buffer.
2	In the ÄKTA start home screen, tap <b>Method run</b> .



### 3 In the *Method run* screen, tap *Prepare system*.



4

Select **Pump wash B** and then tap **Select** to initiate the method.

Select system	method	?
0	Pump wash A	
۲	Pump wash B	
0	Washout fractionation tubing	
$\bigcirc$	Column preparation	1/2
Back		Select

Result: The following screen opens.

Pump wash in pro	ogress		?
UV absorbance	1.0	mAU	
Conductivity	25.0	mS/cm	
Flow rate	3.0	ml/min	Graph
Pressure	0.30	MPa	
Tube no.	6		
Edit run	Hole	d Pause	End

If required, the *Pump wash B* run can be ended before it is completed by tapping *End* to stop the wash in advance.

When the run is completed, tap *Exit* to close the *Pump wash B* screen.



5

# 5.6.3 Washout fractionation tubing

# Introduction

The *Washout fractionation tubing* method is used to clean the fractionation tubing. It is recommended while collecting the fractions without Fraction collector using **Outlet valve**, and in between different runs using the Fraction collector.

**Note:** Flow is diverted from fractionation tubing to collection tube, through **Outlet** valve.

# Requirements

Required cleaning solutions are:

- DM water
- Buffer solution

## Instruction

Follow the instructions below to perform a *Washout fractionation tubing* run. The *Washout fractionation tubing* procedure is initiated from the Instrument Display.

Step	Action
1	Immerse the buffer inlet tubing in DM water or buffer.
2	Remove the column from the flow path and re-connect the flow path. For detailed instructions, see Section 8.3.1 Disconnect the column, on page 223.

- 3 Place the fractionation tubing in the waste container.
- 4 In the **ÄKTA start** home screen, tap **Method run**.



6

7

### 5 In the *Method run* screen, tap *Prepare system*.



Select **Washout fractionation tubing** and then tap **Select** to initiate the method.

Select syste	em method	?
	Pump wash A	
	Pump wash B	
	Washout fractionation tubing	
	Column preparation	1/2
Back		Select

Set the required run parameters:

- Flow rate, flow rate (ml/min)
- Pressure limit, pressure limit (MPa)
- Run volume, run volume (ml)

Run parameters				?
Column volume		1.0	~	ml
Flow rate	~	1.0	^	ml/min
Pressure limit	~	0.30	^	MPa
Run volume	~	5.0	^	ml
				Run

Tap **Run** to initiate the method.

5 Prepare the system for a run 5.6 Run Prepare system methods

5.6.3 Washout fractionation tubing

### Step Action

Result: The following screen opens.

UV absorbance	1.0	mAU	
Conductivity	25.0	mS/cm	
Flow rate	3.0	ml/min	Graph
Pressure	0.30	MPa	
Tube no.	6		

### Note:

If required, the **Washout fractionation tubing** run can be ended before it is completed by tapping **End** to stop the washout in advance.

8

When the run is completed, tap *Exit* to close the *Washout fractionation tubing* screen.

UV absorbance	1.0	mAU	
Conductivity	25.0	mS/cm	
Flow rate	0.0	ml/min Grap	h
Pressure	0.30	MPa	11
Tube no.	6		

# 5.6.4 Column preparation

# Introduction

The *Column preparation* method is used to prepare a new column or to equilibrate the column. It is recommended to equilibrate columns before starting a new run.

### **Requirements**

Required solution is:

• Buffer solution

### Instructions

Follow the instructions below to prepare the column for a run. The **Column preparation** procedure is initiated from the Instrument Display.

Step	Action
1	Immerse the buffer inlet tubing in the intended buffer.
2	Connect the column into the flow path. For detailed instructions, see Section 5.5 Connect a column, on page 121.

3 In the **ÄKTA start** home screen, tap **Method run**.



4

In the *Method run* screen, tap *Prepare system*.

Method run	?
Quick start	Templates
User defined	Prepare system

5 Prepare the system for a run 5.6 Run Prepare system methods 5.6.4 Column preparation

#### Step Action

5

Select *Column preparation* and then tap *Select* to initiate the method. For detailed instructions, see *Section 6.4.5 Prepare system methods, on page 189.* 

Select sys	stem method	?
	O Pump wash A	
	O Pump wash B	
	O Washout fractionation tubing	/
	Column preparation	1/2
Back		Select

- 6
- Set the required run parameters and then tap *Run* to initiate the method.



Result: The following screen opens.

olumn preparatic	on in pro	ogress	?
UV absorbance	1.0	mAU	
Conductivity	25.0	mS/cm	-
Flow rate	3.0	ml/min	Graph
Pressure	0.30	MPa	
Tube no.	6		
Edit run	Hole	d Paus	e End

#### Note:

If required, the **Column preparation** run can be ended before it is completed by tapping **End** to stop the run.

7 When the run is completed, tap *Exit* to close the *Column preparation* screen.

# 5.7 Sample application

# Sample application

The table below shows the different modes of sample application available for ÄKTA start. The sample application technique can be selected from the Instrument display in the *Run parameters* screen or from UNICORN start. For details, see *Chapter 6 Operations* from the Instrument Display, on page 155 and UNICORN start 1.0 User Manual.

Sample volume	Sample application	Loop type
25 µl to 5 ml	via <b>Loop</b>	Sample loop
10 ml to 150 ml	via <b>Loop</b>	Superloop™, 10 ml Superloop, 50 ml Superloop, 150 ml
> 5 ml	via <b>Pump</b> , from the <b>Sample</b> <b>valve</b> port I (Sample)	-

**Note:** Make sure to load only the recommended volume of sample in the column to obtain good results. For details, see the Column instructions.

# Injection valve description

The **Injection valve** enables the application of a sample onto the column from a sample loop connected to the valve. The illustration below shows the different positions of the **Injection valve**. The **Injection valve** position can be changed manually by turning the lever to the left (**Load Sample** position) or the the right (**Inject to column** position).



### Valve position: Load Sample

Port connection	Function
6 - 1	Default route for the system flow path
3 - 2	Directs the liquid manually injected through port <b>3</b> to the sample loop. <b>Note:</b> A sample loop or a Superloop is connected to the ports <b>2</b> and <b>5</b> of the <b>Injection valve</b> .
5 - 4	Directs the liquid from the sample loop, through port <b>4</b> , to the waste container. <b>Note:</b> The path indicated by light orange arrows in the illustration above is used during the manual filling of the loop (Sample or Superloop) through port <b>3</b> .

### Valve position: Inject to column

Port connection	Function
6 - 5	Diverts the system flow path to the sample loop.
2 - 1	Directs the liquid from the sample loop to the column so that the sample loaded into the loop is transferred to the column.

# Connect a sample loop

Follow the instructions below to connect a sample loop to the **Injection valve**.

Step	Action
1	Connect the sample loop between ports <b>2</b> and <b>5</b> of the <b>Injection valve</b> .
2	Make sure that waste tubing is connected to port <b>4</b> of the <b>Injection valve</b> .

# **Connect a Superloop**

Follow the instructions below to connect a Superloop to the Injection valve.

Step	Action
1	Attach an appropriate column holder to the column holder rail on the right side edge of the instrument.
2	Make sure that the Superloop is filled with liquid according to the Superloop instructions.
3	Attach the Superloop to the column holder.
4	Connect the tubing from the bottom of the Superloop to port <b>2</b> of the <b>Injec-</b> tion valve.



# Prime the sample tubing using the Pump

Follow the instructions below to prime the sample tubing using DM water/Buffer, before loading the sample using the **Pump**.

Step	Action
1	Connect a 1 mm i.d. ETFE tubing to port I (Sample) of the <b>Sample valve</b> .
2	Immerse the other end of the sample inlet tubing in the DM water/Buffer container.
3	From the Instrument Display, select <i>Manual Run</i> . For more details on manual run, see <i>Section 6.3 Perform a manual run, on</i> page 162.
4	Tap the forward arrow to access the run parameters on screen 2/2.

5

Toggle to set **Sample valve** position to **Sample** so that the flow is delivered from the sample inlet.



- 6 Tap *Run* to start the run.
- 7 End the run manually once the priming with required volume of DM water/buffer has completed.

### Load the sample using the Pump

The sample can be applied directly using the **Pump** through the **Sample valve**. The direct sample application technique allows the application of sample volumes larger than 5 ml.

Follow the instructions below to apply the sample directly using the Pump.

Step Action	Ste	р	A	ct	ion
-------------	-----	---	---	----	-----

- 1 Connect a 1 mm i.d. ETFE tubing to port I (Sample) of the **Sample valve**.
- 2 Immerse the other end of the sample inlet tubing in the sample container.
- 3 From the Instrument Display, select **Method Run**.



Choose *Quick start* or *Templates*. For details, see *Section 6.4 Perform a method run, on page 170*.

4

5.7 Sample application

### Step Action

In the *Run parameters* screen, select the sample application via the *Pump*.

Run	parameters					?
	Sample from	0	Pump 🤇	L	оор	
	Sample volume	~	0.1	^	ml	
	Equilibration volume	~	5.0	^	CV	-
	Wash unbound volume	~	15.0	^	CV	2/3

#### Note:

- When the sample is applied via the **Pump**, the **Injection valve** has to be manually set to position **Load** (default).
- Make sure to wash the sample inlet tubing with buffer A before immersing the tubing into the sample tube. Make sure to keep sufficient volume of sample to avoid air entering the tubing.
- Make sure that there are no trapped air bubbles in the tubing.
- Prefill the sample tubing with sample before the start of the run to ensure that the tubing is filled with sample.
- 5 In the *Run parameters* screen, set the sample volume and the other required parameters. For details, see *Operation Overview, on page 157*.
- 6 Tap *Run* to start the run.

# Prime the sample loop before injecting sample

A sample loop allows the injection of small sample volumes onto the column. The sample application via the loop is performed in two steps:

- 1 Loading the sample loop with sample.
- 2 Injecting the sample from the sample loop onto the column.

Follow the instructions below to prime the sample loop using DM water/Buffer, before injecting the sample using the manual **Injection valve**.

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

1 Fill a syringe with DM water/buffer.

#### Note:

Make sure that the **Injection valve** is set to position **Load Sample**.



4 Repeat steps 1 to 3 using at least 5 times the loop volume, before loading the sample.

# Load the sample into the sample loop

Follow the instructions below to load the sample into the sample loop.

**Note:** Make sure to flush the loop with DM water and buffer using at least 5 times the loop volume before injecting the sample.

# Step Action

1 Fill a syringe with sample.

5.7 Sample application



### Note:

Make sure that the **Injection valve** is set to position **Load Sample**, which allows to fill the sample loop from the fill port **3**.

3 Carefully load the 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 overfill the loop to make sure that the loop is completely filled. Excess of sample will leave the valve through port **4**.

4 From the Instrument Display select:

*Method run*, then choose *Templates*. For details, see *Section 6.4 Perform a method run, on page 170* 

5 In the *Run parameters* screen, select the sample application via the **Loop** and set all the required run parameters. For details, see *Chapter 6 Operations from the Instrument Display, on page 155.* 

Switch the **Injection valve** position to the **Inject** position, when the following screen is shown on the Instrument Display.



#### Note:

Make sure that the Injection valve is in Load position before starting the run.

6 After manually switching position, acknowledge the message by tapping *Continue.* 

The sample will be injected onto the column when the **Injection valve** is switched manually to position **Inject to column** during the run.

7 Switch the **Injection valve** position to the **Load** position, when the following screen is shown on the Instrument Display.



- 8 After manually switching the position of the **Injection valve**, acknowledge the message by tapping **Continue**.
- **Note:** For Binding techniques (AC/IEX) it is advisable to empty the loop with at least 3 times the loop volume to achieve good/high sample recovery. This is not advised for non binding techniques (DS/GF) as there are sample volume limitations due to the size of the column used.

### 5 Prepare the system for a run 5.7 Sample application

# Load the sample from a Superloop

Superloop allows the injection of larger volumes of sample (10 to 150 ml) onto the column. Follow the instructions below to apply the sample using a Superloop.

Step	Action
1	Fill a large volume syringe with sample.
2	Connect the syringe to the <b>Injection valve</b> port <b>3</b> and carefully inject the sample into the Superloop.

#### Note:

Make sure that the **Injection valve** is set to position **Load sample**, which allows to fill the capillary loop from the fill port **3**.



3 From the Instrument Display select:

*Method run*, then choose *Quick start* methods or *Templates*. For details, see *Section 6.4 Perform a method run, on page 170*.

4 In the *Run parameters* screen, select the sample application via the **Loop** and set all the required run parameters. For details, see *Operation Overview*, on page 157.
#### Step Action

5

Switch the **Injection valve** position to the **Inject** position, when the following screen is shown on the Instrument Display.



#### Note:

Make sure that the **Injection valve** is in **Load** position before starting the run.

- 6 After manually switching position, acknowledge the message by tapping *Continue*.
- 7 Switch the **Injection valve** position to the **Load** position, when the following screen is shown on the Instrument Display.



The sample will be injected onto the column when the **Injection valve** is switched manually to the position **Inject to column** during the run.



# 5 Prepare the system for a run 5.7 Sample application

Step	Action
8	After manually switching the position of the <b>Injection valve</b> , acknowledge the message by tapping <b>Continue</b> .

## 5.8 Prepare the Fraction collector

## **Prepare the Fraction collector**

Fractions are collected in tubes using the Fraction collector. Follow the instructions below to prepare the Fraction collector if the Fraction collector is going to be used during a run.

The following types of tubes can be placed in the tube holder of the Bowl assembly:

- Eppendorf<sup>™</sup> tubes (1.5 ml or 2 ml)
- 5 ml tubes (12 × 75 mm)
- Centrifuge tubes (10 to 12 ml)
- Falcon™ tubes (15 ml)



#### NOTICE

The Fraction collector should be connected or disconnected from the instrument only when ÄKTA start is switched off.

**Note:** Make sure that the Fraction collector is properly installed. See Connect Frac30 to ÄKTA start, on page 78.

#### Step Action

1 Insert a sufficient number of collection tubes in to the Bowl assembly.

#### Note:

All the tubes must be of the same length and diameter and there should be no empty spaces in the sequence.

2 Connect a 0.75 mm i.d. PEEK tubing to the **Outlet valve** port **III** (Collection).

#### Note:

The tubing must be about 50 cm long to ensure proper placement of the Fraction collector and free movement of the dispenser arm.

3 Loosen the nut of the tubing holder and insert the outlet tubing into the tubing holder and tighten the nut.

#### Note:

The PEEK tubing should extend slightly (2 to 3 mm) out of the tubing holder. Make sure that the extended length of the tubing is short enough to avoid any collision with the test tubes during fractionation.

## 5 Prepare the system for a run

5.8 Prepare the Fraction collector



4

Fit the tubing holder into the corresponding port on the Dispenser arm (i.e., the outer or inner port is chosen according to the type of collection tubes inserted in the Bowl assembly).



Gently move the arm to the dispensing position. 5



## Set the delay volume

The delay volume represents the volume between the **UV** and the Fraction collector or the outlet that is used. The delay volume settings are done to make sure that the fractions collected during fractionation correspond to the fractions indicated in the chromatogram.

When the Fraction collector is enabled, the delay volume is collected in the first tube (T1) and the elution volume is collected in the subsequent tubes. Without the Fraction collector enabled, the delay volume is collected in the collection beaker (the total collected volume in the collection beaker will be *delay volume* + *elution volume*).

As the delay volume is affected by the length and diameter of the tubing, it should be set according to the tubing used. Follow the instructions below to set the delay volume.

#### Step Action

1 In the **ÄKTA start** home screen, tap **Settings and service** to access the instrument modules.



Result: The Settings and service Screen 1 opens.

2 In the **Settings and service** screen, tap **System** to access the system options.





#### 5 Prepare the system for a run 5.8 Prepare the Fraction collector

#### Step Action

3

In the **System** screen, tap **Delay volume setting** to access the settings. *Result*: The **Delay volume settings** screen opens.

Delay volume setting	?	
Outlet valve to Fraction collector		
Tube ID     mm     Tube length     mm       v     0.75     ^     500     ^		
Total Delay volume (UV to Fraction collector) 0.49 ml		
Back		

4

Enter the internal diameter (ID) in the *Tube ID* field and length of the tubing from the **Outlet valve** to Fraction collector in the *Tube length* fields, and then tap *Save*.

*Result*: The total delay volume from **UV** to Fraction collector is displayed in the *Total delay volume* field.

#### Note:

The delay volume from **UV** to **Outlet valve** is constant (0.27 ml) in all ÄKTA start instruments if the recommended tubing lengths and i.d. are used.

#### Note:

Make sure to use recommended length and ID of the PEEK tubing from **UV** to **Outlet valve** to avoid incorrect calculation of the delay volume.

## 5.9 Cold room operations

## Introduction

When purifying biomolecules that are temperature sensitive, chromatography runs are performed in a cold room.

## Preparation

Follow the instructions below to prepare the instrument for a run in the cold room.

Step	Action
1	Place ÄKTA start in the cold room.
2	If a UNICORN start computer is connected to the instrument, leave the computer outside the cold room.
3	Allow the instrument to stabilize at the temperature of the cold room.
4	Tighten all connections and pump DM water through the system to check for leaks.
5	Tighten any leaking connector.

## Starting a run

Before starting a run, make sure that the temperature of the buffers has reached the temperature set in the cold room.

**Note:** The measured temperature of the system is the temperature in the **Conductivity flow cell**, which can differ from the ambient temperature.

## Removal from the cold room

Follow the instructions below to remove the instrument from the cold room.

Step	Action
1	Switch off the instrument and disconnect the power cable before moving the instrument out of the cold room.
2	Loosen all connections to prevent them sticking when the system returns to room temperature.

Step	Action
3	Allow the instrument to stabilize at room temperature for at least a few hours.
4	Tighten all connections and pump DM water through the system to check for leaks.
5	Tighten any leaking connector.

## 5.10 Starting a run

## **Final checks**

Before starting a run, make the checks recommended below to prevent that problems are not encountered once the run has been started.

#### Buffer

- Check that the buffer inlet tubings A and B are immersed in the correct bottles containing the buffers of interest.
- Check that there is sufficient buffer available.

#### Waste outlet

- Check that the outlet tubings leading to waste from the **Wash valve**, **Injection valve**, and the **Outlet valve** are placed in the waste container.
- Check that the waste container is not full and that there is provision for the volume diverted to it during the run.

#### **Fraction collector**

• If the fraction collector, Frac30 is going to be used during the run, check that the fraction collector is prepared and filled with collection tubes and the **Outlet valve** collection PEEK tubing is connected to the Fraction collector and also that the Fraction collector is enabled.

#### Column

• Check that the correct column has been connected and equilibrated (if equilibration is not included in the method).

#### Sample

• Make sure that the sample is ready to be loaded via **Pump**, Loop, or Superloop.

#### Pump

• Make sure to place the pump tubing properly over the pump head. Make sure to close the pump hood before starting the run.

#### **Result storage**

• If the result of the run has to be saved when using without UNICORN start, make sure that a USB memory stick is connected to the instrument.

#### **UNICORN start**

- Check that ÄKTA start is connected to a PC with UNICORN start installed.
- Make sure that the system connection is established before starting the run. For details, refer to UNICORN start 1.0 User Manual.

## 5 Prepare the system for a run 5.10 Starting a run

## Start a run

A chromatography run is performed on ÄKTA start is by either using a *Quick start* method or *Template*, or by running the system manually. A run can be started from the Instrument Display or from UNICORN start by selecting one of the run options available with the instrument.

Detailed instructions for starting a run are presented in *Chapter 6 Operations from the Instrument Display, on page 155.* For starting a run from UNICORN start, refer to *UNICORN* start 1.0 User Manual.

# 6 Operations from the Instrument Display

## About this chapter

This chapter describes how to operate the instrument, perform a run and the procedures after a run.

## In this chapter

This chapter contains the following sections:

Section	See page
6.1 Introduction	156
6.2 Fractionation	159
6.3 Perform a manual run	162
6.4 Perform a method run	170
6.5 Procedures after a run	192
6.6 Manage methods and files	195

## 6.1 Introduction

## Workflow

A typical workflow for ÄKTA start is presented below. All the operations in the workflow can be controlled from the instrument display. A chromatographic run can be performed by either using a *Quick start* method, *Template*, or by operating the system manually. The options for starting a run from the instrument display are:

- Method run
- Manual run

Detailed instructions are presented in *Section 6.3 Perform a manual run, on page 162* and *Section 6.4 Perform a method run, on page 170.* 



Procedures required after a run, such as the cleaning of the column, the system flow path, can also be performed manually or using methods available in the *Prepare system* menu.

Calibration of the modules and service can be performed from the *Settings and service* screen. Detailed instructions for calibration are presented in *Section 5.3 Calibrations, on page 88*.

Method management operations such as create, edit, and import a method can be performed from the *Create method* screen. For detailed instructions, see *Section 6.6 Manage methods and files, on page 195.* 

## **Operation Overview**

The **ÄKTA start** home screen displays four different options for the user to select and perform operations from. Instructions for each operation are presented in separate sections in this chapter. For a description of the options available in the main screen, see *Chapter 3 System description, on page 33*.

ÄKTA start	?
Method run	Manual run
Create method	Settings and service

## Checklist

Make sure that the system is correctly prepared. Check that:

- The system is prepared according to *Chapter 5 Prepare the system for a run, on* page 83 and the modules are calibrated according to *Section 5.3 Calibrations, on* page 88.
- A suitable column has been selected for the application. Consider target protein, pressure range, and optimal flow rate.
- A suitable sample application technique will be used. See Section 5.7 Sample application, on page 135.
- The buffer inlet tubing is immersed into correct buffer bottles. Consider the volume required for the intended application.
- The waste tubing is inserted into an appropriate waste container. Consider container size and its material.
- No tubing is twisted or blocked and the flow path is free from leakage.

#### 6 Operations from the Instrument Display 6.1 Introduction

- The Fraction collector configuration needs to be either enabled or disabled as required.
- If the Fraction collector is used, make sure to use tubes with the same tube size.
- The delay volume is set.

## Specifications of run parameters

Parameter	Range	Increment
Flow rate	0.5 to 5.0 ml/min	0.1
Column volume (CV)	1 to 1000 ml, 0 to 150 ml	1 ml
Pressure limit	0.1 to 5 bar	0.1 bar
Sample volume	<b>Pump</b> : 0.1 to 1000.0 ml, 0.1 to 1000 ml	0.1 ml
	Sample loop: 0.1 to 1000 ml	0.1 ml
	Superloop: 0.1 to 1000 ml	0.1 ml
Wash volume	0.0 to 50.0 CV	0.1 CV
Equilibration volume	0.0 to 50.0 CV	0.1 CV
Elution volume	0.0 to 100 CV	0.1 CV
Target B concentration (%)	0 to 100%	1%
Gradient length	0.0 to 100.0	0.1
Fractionation volume	0.5 to 15 ml	0.1 ml

## 6.2 Fractionation

## **Fractionation options**

For many purification schemes it is important to collect fractions of the eluent. ÄKTA start offers the fractionation options presented in the table below.

Instrument configuration	Fractionation options
ÄKTA start + UNICORN start + Frac30	<ul> <li>Fixed volume fractionation</li> <li>Peak fractionation <ul> <li>Level based</li> </ul> </li> </ul>
	- Slope based
ÄKTA start + UNICORN start	<ul> <li>Single Peak collection</li> <li>Level based</li> </ul>
ÄKTA start + Frac30	Fixed volume fractionation
ÄKTA start	Collection of elution volume

## Handling the delay volume

For setting the delay volume refer to Section 5.8 Prepare the Fraction collector, on page 147.

#### **Fractionation with Frac30**

The delay volume is collected in the first tube (T1), followed by the rest of the fractions in the subsequent tubes.

#### **Fractionation without Frac30**

The delay volume is collected in the collection container along with the fraction (i.e., Total collected volume = delay volume + fraction volume).

## Fractionation using ÄKTA start

For detailed instructions on fractionation operations using UNICORN start, refer to UNICORN start 1.0 User Manual.

## ÄKTA start with Frac30

A representative chromatogram depicting fractionation using fixed volume fraction collection in ÄKTA start with Frac30 is shown below.

**Note:** Make sure to set fraction volumes that suit the column being used and use an adequate number of collection tubes.



## ÄKTA start using the Outlet valve (without Frac30)

A representative chromatogram depicting collection using the **Outlet valve** in ÄKTA start (without Frac30) is shown below.



## 6.3 Perform a manual run

## Introduction

This section describes how to start a manual run by configuring the run parameters from the instrument display and how to control an ongoing run.

## In this section

This section contains the following subsections:

Section	See page
6.3.1 Manual run	163
6.3.2 Monitor and control the run	165

## 6.3.1 Manual run

## Start a run

Follow the instruction below to start a manual run.

**Note:** Make sure that a USB memory stick is connected to the instrument. If the instrument does not detect a USB memory stick, the result will not be saved.

#### Step Action

1 In the **ÄKTA start** home screen, tap **Manual run** to access the run parameters for a manual run.



Tap the forward arrows to access additional run parameters.



- Set run parameters:
  - Flow rate, flow rate (ml/min)
  - **Pressure limit**, pressure limit (MPa)
  - Conc B, buffer B concentration (%)

Use up/down arrows to set the values, or use the numpad to type in the values.

- Tick the checkbox *Save Result to USB* if you want to save the result. The result file name can be edited by setting the digits in the range 00 to 99.
- Tap *Run* if no other parameters needs to be set.

2

## 6 Operations from the Instrument Display

6.3 Perform a manual run

6.3.1 Manual run

#### Step Action

#### Note:

Make sure that the values for the Flow rate and Pressure limit are appropriate for the chosen column. Refer to the column manual for details.

If the pressure reaches above the set limit, the instrument will enter the **Pause** state.

3



- Toggle to set which valve positions are open:
  - **Sample valve**: set as **Buffer** or **Sample** so that the flow is delivered from either the buffer inlets or the sample inlet.
  - Wash valve, set as Column or Waste to direct the flow to either the column or to waste.
  - **Outlet valve**, set as **Collection** or **Waste** to direct the flow to either the Fraction collector or to waste.
- Set the *Fractionation volume*, the volume of the fraction to be collected when the Fraction collector is enabled.

Use up/down arrows to set the value, or use numpad to type in the value.

#### Note:

To collect fractions, make sure that the Fraction collector is enabled. If the Fraction collector is enabled, place the required number of tubes of adequate volume in the Bowl assembly.

If enabled, the Fraction collector will home to position 1 at the beginning of every run.

• Tap *Run* to start the run.

Result: The Run view screen will be displayed.

## 6.3.2 Monitor and control the run

## Overview

From the *Run view* screen, the user can monitor and control the ongoing run.

UV absorbance	1.0	mAU		
Conductivity	25.0	mS/cm		
Flow rate	3.0	ml/min	Graph	A
Pressure	0.30	MPa		
Tube no.	6			

The following options are available:

Option	Description
Graph	Displays the run-time UV absorbance curve.
Edit run	Allows the user to edit the run parameters of the ongoing run.
Pause	Temporarily pauses the run by stopping the <b>Pump</b> , hence no flow of liquid in the flow path.
End	Terminates the current run.

## View the chromatogram

Follow the instruction below to view the chromatogram of the ongoing run.

Run view				?
UV absorbance	1.0	mAU		
Conductivity	25.0	mS/cm		
Flow rate	3.0	ml/min	Graph	A
Pressure	0.30	MPa		<u>.</u>
Tube no.	6			

In the *Run view* screen, tap the graph icon to view the chromatogram.

6 Operations from the Instrument Display

6.3 Perform a manual run

6.3.2 Monitor and control the run



The graph displays the UV curve. The Y axis displays the UV absorbance (mAU) and X axis the time (min).

Tap the return arrow to return to the *Run view* screen.

## Edit the run

Follow the instruction below to edit the run parameters of an ongoing run.

-	Run view ?				
	UV absorbance	1.0	mAU		
	Conductivity	25.0	mS/cm		
	Flow rate	3.0	ml/min	Graph	
	Pressure	0.30	MΡα		
	Tube no.	6			

In the *Run view* screen, tap *Edit run* to access the run parameters of the ongoing run.

Step	Action	
2	Edit run	?
	Conc B	✓ 50.0 ∧ %
	Flow rate	✓ 3.0 ∧ ml/min
	Fractionation	Stop
	Fractionation volume	✓ 0.2 ∧ ml 1/2
		Cancel Execute

• Edit the run parameters:

Conc B, buffer B concentration (%)

Flow rate, flow rate (ml/min)

Fractionation volume, fractionation volume (ml)

Toggle *Fractionation* between *Start* and *Stop* to start or stop the fractionation.

Use up/down arrows to adjust the values, or use the numpad to type in the values.

If no other parameters need to be set, tap *Execute* to implement the changes. To ignore the changes, tap *Cancel*.

• Tap the forward arrow to access additional run parameters to edit.

#### Note:

Before starting the run, set the status of the Fraction collector to enabled or disabled as required.

3



- Toggle as needed to set which valve positions are open:
  - **Sample valve**: set as **Buffer** or **Sample** so that the flow is delivered from either the buffer inlets or the sample inlet
  - Wash valve: set as Column or Waste to direct the flow to either the column or to waste
  - **Outlet valve**: set as **Collection** or **Waste** to direct the flow to either the Fraction collector or to waste

6 Operations from the Instrument Display

6.3 Perform a manual run

6.3.2 Monitor and control the run

Step	Action	
	• Tick the <i>Autozero UV</i> checkbox if a UV baseline to zero is required.	
	<ul> <li>Tick the Set event mark checkbox if you need to set an event mark in the chromatogram.</li> </ul>	n
	<ul> <li>After you have set the run parameters, tap <i>Execute</i> to implement the changes.</li> </ul>	е

## Pause the run

Follow the instruction below to pause an ongoing run.

#### Step Action

1 In the *Run view* screen, tap *Pause* to temporarily pause the run by stopping the **Pump**.

Run view			?
UV absorbance	1.0	mAU	
Conductivity	25.0	mS/cm	
Flow rate	3.0	ml/min	Graph
Pressure	0.30	MPa	
Tube no.	6		
Edit run		Pause	End

2

#### To continue the run, tap **Continue**.

			?
UV absorbance	1.0	mAU	
Conductivity	25.0	mS/cm	
Flow rate	3.0	ml/min	Graph
Pressure	0.30	MPa	
Tube no.	6		
Edit run		Contin	ue End

#### Note:

The run is paused and there is no flow of liquid in the flow path as the **Pump** is stopped.

## End the run

Follow the instruction below to end an ongoing run.

Step	Action					
1	In the <b>Run view</b>	screer	n, tap <b>E</b>	<b>nd</b> to termin	ate the ru	n.
	Run view			?		
	UV absorbance	1.0	mAU			
	Conductivity	25.0	mS/cm	-		
	Flow rate	3.0	ml/min	Graph		
	Pressure	0.30	MPa	:		
	Tube no.	6				
	Edit run		Ραι	se End		

*Result*: A Message screen that requires to confirm the action opens.

Message			
	Are you sure you want to end the run?		
	Yes No		

Tap **Yes** to confirm that you want to terminate the run *or* tap **No** to cancel the action and return to the **Run view** screen.

#### Note:

When ending a run before it is completed, the partial result is stored on the USB memory stick.

The USB memory stick stores the result files which can be viewed using UNICORN start. Also a BMP file is generated which can be viewed from any PC. For details, refer to Section 6.6.3 BMP result file, on page 208.

Do not remove the USB memory stick until the system generates the report (BMP file).



2



Tap *Exit* to close the *Run ended* screen.

## 6.4 Perform a method run

## Introduction

This section describes the types of methods that can be selected for a run.

## In this section

This section contains the following subsections:

Section	See page
6.4.1 Select a method type	171
6.4.2 Quick start	173
6.4.3 Templates	179
6.4.4 User defined methods	187
6.4.5 Prepare system methods	189

## 6.4.1 Select a method type

## **Method types**

Four different method types can be selected to perform a method run. The different method types are specified below.

*Quick start*: Allows the user to run methods like affinity, Ion exchange, Gel filtration and Desalting with method parameters predefined.

*Templates*: Allows the user to edit and run the predefined methods Affinity, Ion exchange, Gel filtration and Desalting.

User defined: Allows the user to run user created methods or USB imported methods.

*Prepare system*: Allows the user to perform system operations, such as Pump wash, Column preparation, Cleaning and System performance test.

The **Quick start** methods and **Templates** available with ÄKTA start are briefly described in Section 6.4.2 Quick start, on page 173 and Section 6.4.3 Templates, on page 179.

For a description of the *Prepare system* methods, see Section 6.4.5 Prepare system methods, on page 189, and Section 8.3 Cleaning the system flow path, on page 222.

Method type	Option
Quick start	<ul> <li>AC step 1 ml HiTrap</li> <li>AC step 5 ml HiTrap</li> <li>DS 5 ml HiTrap</li> </ul>
	<ul> <li>DS 53 ml HiPrep™</li> <li>IEX step 1 ml HiTrap</li> </ul>
	<ul> <li>IEX step 5 ml HiTrap</li> <li>IEX aradient 1 ml HiTrap</li> </ul>
	<ul> <li>IEX gradient 5 ml HiTrap</li> <li>GF 16/60 HiPrep</li> </ul>
Templates	<ul> <li>Affinity (AC)</li> <li>Desalting/buffer exchange (DS)</li> <li>Ion exchange (IEX)</li> <li>Gel filtration (GF)</li> </ul>
User defined	Methods created by the user, based on the predefined templates.

6 Operations from the Instrument Display6.4 Perform a method run6.4.1 Select a method type

Method type	Option
Prepare system	• Pump wash A
	• Pump Wash B
	Washout fractionation tubing
	Column preparation
	System cleaning
	System performance method

## Select a method

2

Follow the instruction below to select a method.



In the **ÄKTA start** home screen, tap **Method run** to access the method types available with the instrument.

Method run ?
Quick start Templates
User defined Prepare system

Select one of the following methods:

- Quick start
- Templates
- User defined
- Prepare system

## 6.4.2 Quick start

## Introduction

Quick start contains "ready to run" methods to purify most common proteins based on Affinity, Ion exchange, Gel filtration and Desalting techniques. Run parameters like column volume, flow rate, equilibration and elution mode, and volume are predefined in the method. The user needs to enter only the sample volume. For detailed description of each *Quick start* method, refer to *ÄKTA start System Cue Card*.

**Note:** If required, the run parameters can be changed using the **Edit run** option during an ongoing run.

## **Quick start techniques**

The table below describes the various kinds of quick start techniques that a user can choose, based on application requirements.

Method	Chromatography Technique	Details
AC step 1 ml/5ml HiTrap	Affinity Chromatography	Bound proteins are eluted in a single step, using a single elution buffer.
		proteins, for example Histidine-tagged proteins.
DS 5 ml/53ml HiTrap™	Desalting	Proteins are eluted in a single step, using a single elution buffer.
IEX step 1 ml/5ml HiTrap	lon Exchange Chromatography	Bound proteins are eluted in a single, step using a single elution buffer.
IEX gradient 1 ml/5ml HiTrap	lon Exchange Chromatography	Bound proteins are eluted using two buffers with linear increase in the concen- tration of buffer B, over a specified time followed by a step of 100% B.
GF 16/60 HiPrep	Gel Filtration	Proteins are eluted in a single step, using a single elution buffer.

**Note:** It is recommended to use the appropriate column as indicated in the template names. For example, use HiTrap 1 ml column when selecting AC/IEX step1 ml HiTrap, or 5 ml column when selecting AC/IEX step 5ml HiTrap.

# 6 Operations from the Instrument Display6.4 Perform a method run6.4.2 Quick start

2

## Start a run

Follow the instruction below to start a run based on a *Quick start* method.

**Note:** Make sure that a USB memory stick is connected to the instrument. If the instrument does not detect a USB memory stick, the result will not be saved.

Step	Action	
1	Method run	?
	Quick start	Templates
	User defined	Prepare system
	1	2

In the *Method run* screen, tap *Quick start* to access the templates.

Quick start		?
	AC step 1 ml HiTrap	
	🔵 AC step 5 ml HiTrap	
	🔵 DS 5 ml HiTrap	2
	O DS 53 ml HiPrep	1/3
Back		Select
Quick start		?
Quick start	IEX step 1 ml HiTrap	?
Quick start	<ul> <li>IEX step 1 ml HiTrap</li> <li>IEX step 5 ml HiTrap</li> </ul>	?
Quick start	IEX step 1 ml HiTrap     IEX step 5 ml HiTrap     IEX gradient 1 ml HiTrap	?
Quick start	IEX step 1 ml HiTrap     IEX step 5 ml HiTrap     IEX gradient 1 ml HiTrap     IEX gradient 5 ml HiTrap	?

Step Act
----------



• To select a Quick start method, tap a radio button

or

Tap the forward arrow to access additional **Quick start** methods.

• To continue with the selected method, tap Select.

#### Note:

3

Make sure to load the recommended sample volume for the selected column. If large Gel filtration columns are used, it is recommended to pre-equilibrate the column before starting the run.

 
 Run parameters
 ?

 Sample from
 Pump

 Sample volume
 5.0 ^ ml

 Save Result to USB
 ACO1

 1/1
 Immodel Run

• The mode of sample application is **Pump** (default).

#### Note:

Sample application using **Pump** is used for all **Quick start** methods to automate sample loading or to have an unattended chromatographic run.

#### Note:

Sample application via **Loop** is not applicable.

6 Operations from the Instrument Display6.4 Perform a method run6.4.2 Quick start

#### Step Action

• Enter the sample volume in the Sample volume field .

Use up/down arrows to set the values, or use numpad to type in the values.

- Tick the checkbox *Save Result to USB* if you want to save the result. The result file name can be edited by setting the digits in the range 00 to 99.
- Tap *Run* to start the run.

Result: The **Run view** will be displayed.

#### Note:

Other run parameters can be edited using the **Edit run** option available in the **Run view** screen.



UV absorbance	1.0	mAU
Conductivity	25.0	mS/cm
Flow rate	3.0	ml/min Grap
Pressure	0.30	MPa
Tube no.	6	

In the *Run view* screen, the following options are available to monitor and control the ongoing run (for details, see *Section 6.3.2 Monitor and control the run, on page 165*):

#### Step Action

*Graph*, to view the chromatogram.

Edit run, to change any run parameters in the current run.

*Hold*, to temporarily hold the run, with current set flow rate, valve positions and B concentration.

Pause, to pause the current run.

End, to end the run before it is completed.

#### Note:

The run begins with a default pump wash. Pump wash is performed at 10 ml/min for 1 min with 30 sec of buffer B wash followed by 30 sec of buffer A wash.

*Edit run* is disabled when the pump wash is in progress. During pump wash the flow is directed through *Wash valve* to *Waste*.

#### Note:

The USB memory stick stores the result files which can be viewed using UNICORN start. Also a BMP file is generated which can be viewed from any PC. For details, refer to Section 6.6.3 BMP result file, on page 208.

Do not remove the USB memory stick until the system generates the report (BMP file).

## Hold the run

Follow the instruction below to hold an ongoing run.

1	Run view			?
	UV absorbance	1.0	mAU	
	Conductivity	25.0	mS/cm	
	Flow rate	3.0	ml/min	Graph
	Pressure	0.30	MPa	
	Tube no.	6		

In the *Run view*, tap *Hold* to temporarily hold the run.

#### Note:

Not applicable for a manual run. Hold option is active only in a method run.

6 Operations from the Instrument Display 6.4 Perform a method run 6.4.2 Quick start



To resume the run, tap *Resume*.

#### Note:

During hold the run is temporarily interrupted, with current flow rate, gradient and valve positions sustained.

## 6.4.3 Templates

## Introduction

ÄKTA start provides four method templates based on the most commonly used purification techniques. The templates are provided with default run parameters. The parameters can also be changed to suit the run conditions. New methods can be created and saved from these predefined templates in the **Create method** option.

This section describes how to start a run using *Templates*.

## **Predefined method templates**

The user can create customized purification methods based on the templates available on the instrument. The predefined templates available with ÄKTA start are described below.

Method	Description
Affinity (AC)	Affinity Chromatography separates molecules based on the reversible interaction between the target protein and the specific ligand attached to a chromatography matrix.
lon exchange (IEX)	Ion Exchange Chromatography is based on the reversible interaction between a charged protein and an oppositely charged chromatography medium.
Gel filtration (GF)	Gel filtration, also known as size-exclusion chro- matography, is a chromatography technique that separates molecules based on differences in the molecular size.
Desalting/buffer exchange (DS)	Desalting is a gel filtration technique that allows rapid group separation of high molecular weight substances from low molecular weight sub- stances. Small molecules like salt, free labels and other impurities are efficiently separated from the high molecular weight substances of interest.

6 Operations from the Instrument Display6.4 Perform a method run6.4.3 Templates

## Affinity (AC) or Ion exchange (IE)

Follow the instruction below to start a run based on Affinity (AC) or Ion exchange (IEX).

- **Note:** Make sure that a USB memory stick is connected to the instrument. If the instrument does not detect a USB memory stick, the result will not be saved.
- **Note:** Before starting the run, set the status of the Fraction collector to enabled or disabled as required.

## Step Action

1

2

Quick start	Templates
User defined	Prepare syste

In the *Method run* screen, tap *Templates* to access the different templates.



- Tap a radio button to select a template that suits your application.
- To continue with the selected technique, tap *Select*.

Column volume	~	1.0	^	ml
Flow rate	~	1.0	^	ml/min
Pressure limit	~	0.30	^	MPa
Save Result		AC01	-Y	1/3


#### Step Action

- Set the run parameters:
  - Column volume, column volume (ml)
  - Flow rate, flow rate (ml/min)
  - Pressure limit, pessure limit (MPa)
- Tick the checkbox Save Result to USB if you want to save the result. The
  result file name can be edited by setting the digits in the range 00 to 99.
- Tap the forward arrow to access additional run parameters.

#### Note:

Make sure that the values for the **Column volume**, **Flow rate** and **Pressure limit** are appropriate for the chosen column. Refer to the column manual for details.

If the pressure reaches above the set limit, the instrument will enter the **Pause** state.

#### Note:

The USB memory stick stores the result files which can be viewed using UNICORN start. Also a BMP file is generated which can be viewed from any PC. For details, refer to Section 6.6.3 BMP result file, on page 208.

Do not remove the USB memory stick until the system generates the report (BMP file).

4



• Select the mode of sample injection in the *Sample from* field: to be applied via *Pump* or via *Loop*. For detailed instructions on sample application, refer to *Section 5.7 Sample application, on page 135*.

6 Operations from the Instrument Display6.4 Perform a method run6.4.3 Templates

#### Step Action

- Set the run parameters:
  - Sample volume, the volume of sample to be loaded onto the column
  - **Equilibration volume**, the volume of buffer A required for for equilibrating the column.
  - **Wash unbound volume**, volume of buffer needed after the sample application to wash off the unbound molecules

#### Note:

5

For AC/IEX methods when loading sample through loop it is advisable to empty the loop with 3 times the loop volume to achieve good sample recovery.

Run J	parameters			?
	Elution option	Isocratic (	Gradient	
	Conc B	<ul><li>✓ 100</li></ul>	^ %	
	Elution volume	<ul> <li>✓ 5.0</li> </ul>	∧ CV	
	Fractionation volume	× 1.0	∧ ml	3/3
				Run

- Configure the run parameters for *Elution Option* set as *Isocratic*:
  - Conc B, concentration of buffer B to elute the bound protein
  - *Elution volume*, volume needed to elute the bound protein from the column
  - **Fractionation volume**, volume of the fraction to be collected when the Fraction collector is enabled

or

<	Elution option	) Iso	cratic 🤇	Gra	dient	
	Target conc B	~	100	^	%	
	Gradient volume	~	5.0	^	CV	
	Fractionation volume	~	1.0	^	ml	3/3

#### Step Action

- Configure the run parameters for *Elution Option* set as *Gradient* (Bound proteins are eluted with continuous change of buffer B composition to increase eluent strength over specified time):
  - **Target conc B**, maximum buffer B concentration level to be set in the gradient
  - **Gradient volume**, volume needed to elute the bound protein from the column
  - *Fractionation volume*, volume of the fraction to be collected when the Fraction collector is enabled.
- Tap back arrow to view or edit run parameters.
- Tap *Run* to start the run.

Result: The Run view screen will be displayed.

UV absorbance	1.0	mAU	
Conductivity	25.0	mS/cm	
Flow rate	3.0	ml/min	Graph 🚶
Pressure	0.30	MPa	
Tube no.	6		

#### Note:

The run begins with a default pump wash. Pump wash is performed at 10 ml/min for 1 min with 30 sec of buffer B wash followed by 30 sec of buffer A wash.

*Edit run* is disabled when the pump wash is in progress. During pump wash the flow is directed through *Wash valve* to *Waste*.

#### Gel filtration, Desalting/Buffer Exchange

Follow the instruction below to start a run based on Gel Filtration or Desalting template.

- **Note:** Make sure that a USB memory stick is connected to the instrument. If the instrument does not detect a USB memory stick, the result will not be saved.
- **Note:** Before starting the run, set the status of the Fraction collector to enabled or disabled as required.

6 Operations from the Instrument Display6.4 Perform a method run6.4.3 Templates

2

3

Step	Action	
1	Method run	?
	Quick start	Templates
	User defined	Prepare system

In the *Method run* screen, tap *Templates* to access the predefined method templates.

Templates		?
0	Affinity (AC)	
$\bigcirc$	Desalting/buffer exchange (DS)	
$\bigcirc$	on exchange (IEX)	
	Gel filtration (GF)	
Back		Select

- Tap a radio button to select a template that suits your application (e.g., *Gel filtration*).
- To continue with the selected technique, tap *Select*.



#### Step Action

- Set the run parameters:
  - Column volume, column volume (ml)
  - Flow rate, flow rate (ml/min)
  - Pressure limit, pressure limit (MPa)

Use up/down arrows to set the values, or use numpad to type in the values.

- Tick the checkbox Save Result to USB if you want to save the result. The
  result file name can be edited by setting the digits in the range 00 to 99.
- Tap the forward arrow to access additional run parameters.

#### Note:

Make sure that the values for the **Flow rate** and **Pressure limit** are appropriate for the chosen column. Refer to the column manual for details.

If the pressure reaches above the set limit, the instrument will enter the **Pause** state.

#### Note:

The USB memory stick stores the result files which can be viewed using UNICORN start. Also a BMP file is generated which can be viewed from any PC. For details, refer to Section 6.6.3 BMP result file, on page 208.

Do not remove the USB memory stick until the system generates the report (BMP file).





- Select the mode of sample injection in the *Sample from* field: to be applied via *Pump* or via *Loop*. For detailed instructions on sample application, refer to *Section 5.7 Sample application, on page 135*.
- Set the run parameters:
  - Sample volume, the volume of sample to be loaded in to the column
  - **Equilibration volume**, the volume of buffer A required for for equilibrating the column.

6 Operations from the Instrument Display6.4 Perform a method run6.4.3 Templates

#### Step Action

#### Note:

Washout unbound is not applicable for GF/DS methods.

#### Note:

Make sure to load the recommended sample volume for selected column. If large GF columns are used, it is recommended to pre-equilibrate the column before starting the run.

Run	Surumeters					Ŀ
<	Conc B	~	0	~	%	
	Elution volume	~	1.0	^	CV	
	Fractionation volume	~	4.0	^	ml	3/3
						Run

- Set the run parameters:
  - *Elution volume*, volume of buffer needed to elute the protein from the column
  - **Fractionation volume**, volume of fractions to be collected when the Fraction collector is enabled.
- Tap *Run* to start the run.

Result: The Run view screen will be displayed.

#### Note:

**Conc B** is not applicable for GF/DS as elution takes place only with a single buffer (buffer A).

#### Note:

The run begins with a default pump wash. Pump wash is performed with buffer A at 10 ml/min for 30 sec.

*Edit run* is disabled when the pump wash is in progress. During pump wash the flow is directed through *Wash valve* to *Waste*.

<sup>5</sup> 

## 6.4.4 User defined methods

#### Start a run

Follow the instruction below to start a run based on a user created or USB imported method.

**Note:** Make sure that a USB memory stick is connected to the instrument. If the instrument does not detect a USB memory stick, the result will not be saved.

# Step Action 1 Method run Quick start Templates User defined Prepare system Image: Compare system Image: Compare system

In the *Method run* screen, tap *User defined* to access the user created methods.

AC	201	
O D:	\$06	

- Tap a radio button to select a user method to run.
- To continue with the selected user method, tap Select.

Sample from	Pump Loop	
Sample volume	✓ 5.0 ∧ ml	
Save Result to USB	Result01	
		1

In the <i>Method run</i> screen, tap <b>Us</b> methods.
Select method
AC01

2

3

6 Operations from the Instrument Display6.4 Perform a method run6.4.4 User defined methods

4

#### Step Action

Set the run parameters:

- Select the mode of sample injection in the *Sample from* field: to be applied via **Pump** or via **Loop**. For detailed instructions on sample application, refer to *Section 5.7 Sample application, on page 135*.
- Sample volume, the volume of sample to be loaded in to the column
- Tick the checkbox Save Result to USB if you want to save the result. The
  result file name can be edited by setting the digits in the range 00 to 99.
- Tap *Run* to start the selected method.

Result: The Run view screen will be displayed.

Run view			?
UV absorbance	1.0	mAU	
Conductivity	25.0	mS/cm	
Flow rate	3.0	ml/min	Graph
Pressure	0.30	MPa	
Tube no.	6		
Edit run		Pause	End

In the *Run view* screen, the following options are available to monitor and control the ongoing run (for details, see *Section 6.3.2 Monitor and control the run, on page 165*):

Graph, to view the chromatogram.

Edit run, to change any run parameters in the current run.

Hold, to hold the current run.

Pause, to pause the current run.

End, to end the run before it is completed.

#### Note:

The USB memory stick stores the result files which can be viewed using UNICORN start. Also a bmp file is generated which can be viewed from any PC. For details, refer to Section 6.6.3 BMP result file, on page 208.

Do not remove the USB memory stick until the system generates the report (.bmp file).

#### Note:

USB imported methods which were created in UNICORN start cannot be edited in the instrument. Use UNICORN start to edit those methods.

## 6.4.5 Prepare system methods

#### Introduction

Predefined methods for the preparation and the cleaning of the system are available with ÄKTA start. Use the *Prepare system* methods to clean the entire system flow path when needed, and to fill the system with storage solution when the instrument is not going to be used for a period of time. For detailed instructions, see *Section 8.3 Cleaning the system flow path, on page 222.* 

The system methods available with ÄKTA start are listed below:

- Pump wash A
- Pump wash B
- Washout fractionation tubing
- Column preparation
- System cleaning
- System performance method

The **Pump wash A/B**, **Washout fractionation tubing**, and **Column preparation** methods required for the system preparation are presented in detail in Section 5.6 Run Prepare system methods, on page 125. The **System performance method** is presented in Section 5.4 System performance, on page 106.

#### System cleaning

Follow the instruction below to perform a system cleaning run. For detailed instructions on cleaning the system using the **System cleaning** template, see Section 8.3.2 System cleaning, on page 224.

Step	Action	
1	Method run	?
	Quick start	Templates
	User defined	Prepare system
		2

In the *Method run* screen, tap *Prepare system* to access the system methods.

6 Operations from the Instrument Display 6.4 Perform a method run

6.4.5 Prepare system methods

3



In the Select system method screen,

- Select System cleaning.
- To continue with the System cleaning method, tap Select.

	System cleaning
1	Please follow instructions 1. Disconnect the column 2. Connect injection valve to UV with suitable tubing and connectors 3. Place the buffer tubings (A & B) in cleaning solution When completed press Continue
	Continue

- Perform the operations described on the display:
  - 1 Disconnect the column.
  - 2 Connect Injection value to UV with suitable tubing and connectors.
  - 3 Place the buffer tubings (A & B) in cleaning solution.

For detailed instructions, see Section 8.3.2 System cleaning, on page 224.

• Tap *Continue* to start the *System cleaning* run.

ustem cleaning i	n progra			2
stem cleaning i	n progre			•
UV absorbance	1.0	mAU		
Conductivity	25.0	mS/cm		
Flow rate	3.0	ml/min	Graph	
Pressure	0.30	MPa		
Tube no.	6			

While the *System cleaning* run is in progress, wait for the run to complete.

#### Note:

If required, the **System cleaning** run can be ended before it completes by tapping **End**.

UV absorbance	1.0	mAU	
Conductivity	25.0	mS/cm	
Flow rate	0.0	ml/min	Graph
Pressure	0.30	MPa	
Tube no.	6		

Tap *Exit* to close the *System cleaning* screen when the *System cleaning* run is completed.

5

#### 6 Operations from the Instrument Display 6.5 Procedures after a run

## 6.5 Procedures after a run

#### Introduction

This section briefly describes:

- How to evaluate a recorded result
- How to clean the instrument after a run.
- How to prepare the system for storage if the instrument is not going to be used for a period of time.

The instrument and the columns should be cleaned between the runs. This will prevent, for example, carryover and cross-contamination among samples, protein precipitation, and clogging of the column or flow path. For further details on cleaning and maintenance procedures, see *Chapter 8 Maintenance, on page 217*.

#### Evaluate a run

After a chromatographic run, the result stored on a USB memory stick can be transferred to UNICORN start where it can be viewed, and evaluated. The result holds a complete record of the run, including method, system settings, chromatogram, and run log. The result can be also viewed by using the BMP result file that is generated and stored on the USB memory stick. For details, refer to Section 6.6.3 BMP result file, on page 208.

Detailed instructions for the transfer of the result are presented in *Section 6.6 Manage methods and files, on page 195.* 

Detailed instructions for the evaluation of a result are presented in *Chapter 7 Operations from UNICORN start, on page 210*, and in the *UNICORN start 1.0 User Manual.* 

#### Clean the system

After a run is completed, perform the following:

- Remove the column from the flow path and re-connect the flow path. For detailed instructions, see Section 8.3.1 Disconnect the column, on page 223.
- Rinse the flow path with cleaning solution and/or DM water using either **System** *cleaning* or the **Pump wash** methods, as required. For detailed instructions, see *Section 8.3 Cleaning the system flow path, on page 222.*
- If required, remove the tubes from the Fraction collector. If there is any spillage, clean the Bowl assembly with DM water.
- Clean all spills on the instrument and on the bench using a damp cloth.

• Empty the waste container.

#### Clean and store the column

After a run is completed, perform the following:

- Disconnect the column from the flow path.
- Clean the column off-line according to the column instructions.

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

- Fill the column with the storage solution recommended in the column data sheet.
- Detach the column from the instrument and store it according to the column recommendation.

#### System storage

If the instrument is not going to be used for a period of time, fill the system and the inlets with storage solution (DM water, or 20% ethanol). For detailed instructions, see *Section 8.7 Storage of the instrument, on page 236.* 

### Power off the instrument

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



**Note:** When the instrument is switched off or not in use, make sure to open the pump hood and release the pump tubing from the pump head.

# 6.6 Manage methods and files

#### Introduction

This section describes how to create, edit, import and delete methods on ÄKTA start. For information about how to create a method using the UNICORN start, see *Chapter 7 Operations from UNICORN start, on page 210* or the *UNICORN start 1.0 User Manual.* 

#### In this section

This section contains the following subsections:

Section	See page
6.6.1 Create method	196
6.6.2 Handling the USB memory stick	205
6.6.3 BMP result file	208

6 Operations from the Instrument Display6.6 Manage methods and files6.6.1 Create method

## 6.6.1 Create method

2

#### Create method menu

The *Create method* menu allows the user to create a new method, edit, import, and delete a method from the ÄKTA start Instrument Display.

Follow the instructions below to access the Create method options.

Step	Action	
1	ÄKTA start	?
	Method run	Manual run
	Create method	Settings and service

In the ÄKTA start home screen, tap Create method.

Create	Edit
USB import	Delete

In the *Create method* screen, the following options are available:

Create, to create a new method using a predefined template

*Edit*, to edit a method or change run parameters for user created methods stored on instrument

**USB Import**, to import a method developed on UNICORN start to the instrument using a USB memory stick

Delete, to delete a method that is stored on the instrument

#### Create a method

Follow the instruction below to create a method using a predefined template.

Step	Action	
1	Create method	?
	Create	Edit
	USB import	Delete
		1

In the *Create method* screen, tap *Create*.

Result: The **Select templates** screen opens.

۲	Affinity (AC)
C	Desalting/buffer exchange (DS)
C	Ion exchange (IEX)
C	Gel filtration (GF)

- Tap a radio button to select a template.
- Tap Create to create a method based on the selected technique.

For details on the templates available in ÄKTA start, refer to *Section 6.4.3 Templates, on page 179.* 

Column volume	<ul> <li>✓ 1.0</li> </ul>	∧ ml
Flow rate	<ul><li>✓ 1.0</li></ul>	ml/min
Pressure limit	<ul><li>✓ 0.30</li></ul>	∧ MPa
Save method as	AC01	1/2

- Set the run parameters:
  - Column volume, column volume (ml)
  - Flow rate, flow rate (ml/min)
  - Pressure limit, pressure limit (MPa)

3

2

6 Operations from the Instrument Display 6.6 Manage methods and files 6.6.1 Create method

4

#### Action Step

Select Save Method as field if you want to set a method name. The file • name can be edited by setting the digits in the range 00 to 99.

#### Note:

Provide a unique method name. The created method will be saved under the **User defined** methods menu.

#### Note:

Make sure that the values for the Column volume, Flow rate and Pressure *limit* are appropriate for the chosen column. Refer to the column manual for details.

If the pressure reaches above the set limit, the instrument will enter the **Pause** state.



- Select the mode of sample injection in the Sample from field: to be ap-• plied via **Pump** or via **Loop**. For detailed instructions on sample application, refer to Section 5.7 Sample application, on page 135.
- Set the run parameters: ٠
  - Sample volume, the volume of sample to be loaded onto the column -
  - Equilibration volume, the volume of buffer A required for for equili-brating the column
  - Wash unbound volume, volume of buffer needed after the sample application to wash off the unbound molecules

#### Note:

The Washout unbound volume is applicable only for AC/IEX techniques.

Step	Actio	n				
5	Set p	arameters				?
	-	Elution option	Isoc	aratic 🔘 G	radient	
		Conc B	~	100 /	%	
		Elution volume	~	5.0	cv	
		Fractionation	~	1.0 /	ml	3/3
						Save

- Configure the run parameters for *Elution Option* set as *Isocratic*:
  - Conc B, concentration of buffer B to elute the bound protein
  - *Elution volume*, volume needed to elute the bound protein from the column
  - **Fractionation volume**, volume of the fraction to be collected when the Fraction collector is enabled

or

Elution option	O Iso	cratic 🤇	Gro	dient	
Target conc B	~	100	^	%	
Gradient volume	~	5.0	^	CV	
Fractionation	~	1.0	^	ml	3/3

- Configure the run parameters for *Elution Option* set as *Gradient* (Bound proteins are eluted with continuous change of buffer B composition to increase eluent strength over specified time):
  - **Target conc B**, maximum buffer B concentration level to be set in the gradient
  - **Gradient volume**, volume needed to elute the bound protein from the column
  - **Fractionation volume**, volume of the fraction to be collected when the Fraction collector is enabled.
- Tap *Save* to save the new method.

*Result*: A *Message* screen that requires to confirm the action will be displayed.

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Step	Action	
6	Message	
	Are you sure you want to save the method?	
	Yes No	

Tap **Yes** to confirm the saving of the method *or* tap **No** to cancel the action and return to set the run parameters.

#### Note:

The created method will be saved under the **User defined** methods menu.

The system can store up to 10 methods only. Delete existing methods if new methods need to be stored.

#### Edit a method



Follow the instructions below to edit a user defined method.

In the *Create method* screen, tap *Edit* to access the methods.

Result: The Select method to edit screen opens.

Step	Action	
2	Select method to edit	?
	Method 1	
	Method 2	
	O Method 3	
	Method 4	
	Back 🔂	Edit

- In the Select method to edit screen, tap a radio button to select a user method.
- Tap *Edit* to start editing the run parameters for the selected method.

Set p	arameters		?
	Column volume	~ 2.0 ^	ml
	Flow rate	~ 3.0 ^	ml/min
	Pressure limit	v 0.40 ^	MPa
			1/3
			Save
Set p	arameters		?
	Sample from	Pump L	.oop
	Sample volume	~ 5.0 <b>^</b>	ml
<	Equilibration	~ 0.0 ^	cv
	Wash unbound	✓ 15.0 ^	CV 2/3
			Save
Set p	arameters		?
<	Elution option	Isocratic Gro	Idient
	Conc B	~ 20 ^	%
	Elution volume	~ 5.0 <b>^</b>	CV
	Fractionation	✓ 2.0 ∧	ml 3/3
			Save

3

6 Operations from the Instrument Display6.6 Manage methods and files6.6.1 Create method

Step	Action
	<ul> <li>Select Save Method as field if you want to set a method name. The file name can be edited by setting the digits in the range 00 to 99.</li> </ul>
	Note:
	Provide a unique method name, for example AC02, DS05. The created method will be saved under the <b>User defined</b> methods menu.
	USB imported methods which were created using UNICORN start canno be edited from the instrument. Use UNICORN start to edit those methods
4	Message
	Are you sure you want to save the method?
	Yes No
	Tap <b>Yes</b> to confirm the saving of the method

or

Tap No to cancel the action and return to set the run parameters.

#### Import a method

Follow the instruction below to import a method stored on a USB memory stick.

**Note:** Make sure that the USB memory stick containing the user defined methods is connected to the instrument. For details about exporting a method, see Section 6.6.2 Handling the USB memory stick, on page 205.



In the Create method screen, tap USB Import to access the methods.

Step	Action	
2	Select method to import from USB	?
	Method 1	
	Method 2	
	Back	Import

- Tap a radio button to select a method.
- Tap *Import* to import the method.

*Result*: A *Message* screen that requires to confirm the action will be displayed.

-			

Are you sure you want to <b>import</b> the method?

Tap Yes to confirm the import of the selected file

or

Tap **No** to cancel the action and return to the file list.

#### Note:

The imported methods will be saved under the **User defined** methods menu. Only one method can be imported at a time. If multiple methods are to be imported, repeat the steps described above.

#### Note:

If the system memory is full, delete existing methods before importing a new method.

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#### Delete a method

 Step
 Action

 1
 Create method

 Create
 Edit

 USB import
 Delete

 Image: Create
 Image: Create

Follow the instruction below to delete a user method.

In the Create method screen, tap Delete to access the methods.

Select method	to delete	?
(	Method	
6	Method	
0	Method	
(	Method	
Cancel		Delete

- Tap a checkbox to select a method.
- Tap *Delete* to delete the method.

*Result*: A *Message* screen that requires to confirm the action opens. or

To cancel the action and return to the *Create method* screen, tap *Cancel*.

#### Note:

Multiple files can be deleted at the same time.

Message
Are you sure you want to delete the selected
method(s)?
Yes
No

Tap **Yes** to confirm the deletion of the selected files *or* tap **No** to cancel the action and return to the file list.

3

2

## 6.6.2 Handling the USB memory stick

#### Introduction

ÄKTA start provides the user with the option to store result data on a USB memory stick. The USB memory stick is used to save a result, BMP file and also for transferring methods between the instrument and UNICORN start. The USB memory stick is also used to generate a System error report.

# **Note:** • The result files will be saved in the GE folder which is automatically created by the instrument once the USB memory stick is plugged in.

• At any given point of time only 10 results can be stored in the GE folder. To save further results, transfer the result files to another folder, PC or rename the GE folder.

# Store result on a USB memory stick

Follow the instructions below to store results generated in ÄKTA start, on a USB memory stick.

Step	Action
1	Connect a USB memory stick to the instrument, via the USB port.
2	Start a <b>Manual run</b> or <b>Method run</b> .
3	In the <b>Run parameters</b> screen, tick the <b>Save results to USB</b> check box in order to save the generated results on the USB memory stick.
	When the run is complete, the results are saved in a GE folder.
4	A BMP result file is also generated. Hence, the chromatogram can be viewed without the use of UNICORN start.
5	Export the results to $UNICORN$ start to view the chromatogram and evaluate.
Note:	Make sure that the USB memory stick is not removed during the run. For more details, see Do's and Dont's while handling the USB memory stick, on page 205.

# Do's and Dont's while handling the USB memory stick

• Make sure that the USB memory stick is inserted completely into the instrument.

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- Max supported USB memory stick is 32 GB. Minimum 1 GB free space is required to execute read/write operations.
- Only FAT32 file system is supported and this needs to be taken into account while formatting the memory stick.
- Only unplug the USB memory stick when the instrument display is in the Home screen.
- It is preferred to keep a minimum number of files on the memory stick. Once you take a backup of the files, delete them from the memory stick and then save onto a computer. Avoid keeping unnecessary files in the memory stick.
- Avoid using folders named GE on the USB memory stick. However, you may use folders named for example GE\_ or GExyz.
- Always take backup of complete GE folder from USB memory stick and do not backup individual files. It is recommended to take backups whenever you have completed important runs.

# Result file import from USB stick to UNICORN start.

Follow the instructions below to export a result file generated in ÄKTA start, and import it into UNICORN start.

Step	Action
1	Open the <i>Evaluation</i> module in UNICORN start.
2	Select <i>File:Import:Import ÄKTA start results from USB</i> , and then import the result files to a desired location on the computer.
3	View, analyze, report or print the result file.

# Method Export from UNICORN start to USB memory stick

Follow the instructions below to export a method created in UNICORN start, to a USB memory stick.

Step	Action
1	Create a method using the <i>Method Editor</i> module in UNICORN start.
2	Connect a USB memory stick to the computer.

Step	Action
3	Select <i>File:Export:Export Method</i> in order to export the created method to a USB memory stick, connected to the computer.
	Note:
	Make sure that the method is stored in a GE folder.

## Method Import to ÄKTA start – USB import

Follow the instructions below to import a method from UNICORN start, to ÄKTA start.

Step	Action
1	Create a method using the <i>Method Editor</i> module in UNICORN start.
2	Select <i>File:Export:Export Method for ÄKTA start to USB</i> in order to export the created method to a USB memory stick.
3	Connect the USB memory stick to ÄKTA start.
	<b>Note:</b> Make sure that the method is stored in a GE folder.
4	From the <b>ÄKTA start</b> Home screen, tap <b>Create method</b> : <b>USB import</b> .
5	Select the method to import.

## 6.6.3 BMP result file

#### Introduction

In order to provide the user with the option to view the result image using another type of software outside of ÄKTA start and UNICORN start, the instrument provides the feature to export the result in BMP format. This format facilitates viewing of the generated result without UNICORN start.

#### Feature of exported result

- The result file in BMP format can be opened and viewed using Operating Systems (OS) such as Microsoft® Windows® operating system and Mac OS™.
- The BMP result is saved and exported, if user selects the option to *Save result to USB* before the start of a run.
- The result file offers the user UV curve data with fractionation marks.
- The result file contains 4 hours of run data.
- The BMP file displays the necessary legends like Product name, run details, UV, Frac marks.
- In case of shutdown or power failure, the partial result will not be available. However, forcible ending of the run by the User results in the export of result as BMP.

#### Generate a BMP file

Step	Action
1	Connect a USB memory stick to the instrument.
2	Start a <b>Method run</b> or <b>Manual run</b> .

#### Step Action

3 Tick the *Save results to USB* check box in order to save the results to the USB memory stick.

#### Note:

A BMP file is only generated when the result is saved.



When the run is completed, the result will be saved and a BMP file will be generated.

#### Note:

4

5

Do not unplug the USB memory stick when the BMP file generation is in progress.

Transfer and connect the USB memory stick to a computer or else the image file will not be generated. Open the generated BMP file, view or print the result.



# 7 Operations from UNICORN start

#### About this chapter

This chapter gives a brief insight into the four modules of UNICORN start: **System Control** *Method Editor*, *Evaluation* and *Administration*.

#### In this chapter

This chapter contains the following sections:

Section	See page
7.1 System Control	211
7.2 Method Editor	213
7.3 Evaluation	215
7.4 Administration	216

#### Introduction

UNICORN start brings the following capabilities:

- Simple and flexible method creation.
- Easy system control using process picture and real time monitoring of manual and method runs.
- Ability to evaluate and compare results.
- Create and print PDF reports.
- Ability to manage (store, archive\retrieve, backup\restore) results generated from ÄKTA start.

For detailed instructions on operations from UNICORN start, refer to the UNICORN start 1.0 User Manual.

# 7.1 System Control

#### Introduction

The **System Control** module is used to start, view, and control a run.

# Illustration of the System Control user interface



#### Main features

The main features of the System Control module are listed below:

- A flow scheme representing the real time flow path with indications of the different modules in wet side of the instrument. Current run status of the system is displayed.
- The ability to control the instrument via simple clicks on the flow path, for example, to turn the valves, set flow rates, change B concentrations and start/stop fractionation.

- A real time chromatogram depicting the complete run with curves including UV, conductivity, system flow, gradient concentrations, fraction marks, run logs and pressure.
- The ability to perform manual and method runs.
- Ability to run predefined methods like *Quick start*, *Templates* and *Prepare system methods*.
- Ability to perform System performance method.
- Ability to generate a System error report.
- **Note:** When the Fraction collector is enabled, the process picture will display a Fraction collector image. If the Fraction collector is disabled, a collection beaker image is displayed.

# 7.2 Method Editor

#### Introduction

The *Method Editor* module provides flexibility to create or edit the chromatography methods.

# Illustration of the *Method Editor* module

The user interface of *Method Editor* is illustrated below.

Method Settings	· hands date					
Prime and Equilibration	T					
*		Detaipe 12 concertion		- 10	310-1000	
Eample Application		Set elabor volume	- 16.0	or .	pt-100.0	
*						
Wesh and unknowed	O Dealert eluter					
•	Shert terget 128 core	ettical [11] [51	(J) 2 - 102 (T)			
Elder and Practisnation						
*	JI A	Integet 1.8 converter	( 10.0)	$^{32}$	21.703	
Prime and Equilibration	:X	Set product vitation	(	ev.	84-101	
		107				
	Practiceation	Partnesse allega				
		Practice alize type	Pasthatientie			Approx.
	-	Fund fractionalism volume	dure #23-15.0			Past Fra
	1000	Test factoration volume		11. HD1-110		

#### **Main features**

The main features of the *Method Editor* module are listed below:

- Ability to create methods from predefined templates like Affinity, Ion Exchange, Desalting and Gel Filtration.
- Flexibility to create customized methods by dragging and dropping chromatography phases such as *Prime and Equilibration*, *Sample Application*, *Wash Out Unbound*, *Elution and Fractionation*.

#### 7 Operations from UNICORN start 7.2 Method Editor

• The methods created from *Method Editor* can either be run directly from *System Control* or exported to a USB memory stick to run them from ÄKTA start directly.

# 7.3 Evaluation

#### Introduction

The *Evaluation* module is used to manage and evaluate the results from chromatography runs.

# Illustration of the *Evaluation* module

The user interface of *Evaluation* is illustrated below.



#### Main features

The main features of the *Evaluation* module are listed below:

- Open and view existing chromatogram results.
- Compare two curves or chromatograms.
- Perform peak integration analysis.
- Create and print PDF reports.
- Import results from ÄKTA start via a USB memory stick.

# 7.4 Administration

## Introduction

The *Administration* module is used to manage the UNICORN start database and to review UNICORN start and system logs.

# Illustration of the Administration module



#### **Main features**

The main features of the *Administration* module are listed below:

- Ability to back up and restore operations, and archive and retrieve operations.
- Review the UNICORN start and system logs.
# 8 Maintenance

# About this chapter

This chapter describes the maintenance program for ÄKTA start and provides instructions for routine maintenance.

## In this chapter

This chapter contains the following sections:

Section	See page
8.1 Regular maintenance	218
8.2 Cleaning before planned maintenance/service	221
8.3 Cleaning the system flow path	222
8.4 Cleaning the UV flow cell	227
8.5 Cleaning the Conductivity flow cell	229
8.6 Other cleaning procedures	230
8.7 Storage of the instrument	236
8.8 Replacement of tubing and filters	238

# 8.1 Regular maintenance

# Introduction

Regular maintenance is important for safe and trouble-free operation of ÄKTA start. The user should perform preventive maintenance on a daily, weekly and monthly basis.

## Safety precautions

To avoid damage to person when performing maintenance on the instrument, follow the instructions below.



#### WARNING

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



#### WARNING

**Disconnect power**. Always switch off power to the instrument before replacing any component on the instrument, unless stated otherwise in the user documentation.



#### WARNING

**Spillage Hazard**. Avoid spillage of fluids on the surfaces of the instrument which have cables, plugs and other wirings. Be careful if there is spillage of fluids on the tray while trying to remove the tray from ÄKTA start.

 $\triangle$ 

#### CAUTION

Always use appropriate personal protective equipment during operation and maintenance of ÄKTA start.

# Periodic maintenance schedule

The periodic maintenance schedule provides a guide to the user to ensure proper routine maintenance of ÄKTA start.

Interval	Maintenance action	See section
Daily	Visually inspect the instrument for leakages in the flow path. Check the <b>Pump</b> for leakage. If there are signs of liquid leaking from the <b>Pump</b> , check the in- tegrity of the pump tubing and the tubing con- nections. <b>Note:</b> Make sure that the pump tubing is not left inside	-
	the <b>Pump</b> when it is not in use.	
	Clean the column and the system flow path af- ter use and leave the system filled with DM water.	Section 6.5 Procedures after a run, on page 192
	<b>Note:</b> If the instrument is not going to be used for a few days, prepare the system for storage.	Section 8.3 Cleaning the system flow path, on page 222
Weekly, or when required	Calibrate the <b>Pump</b> .	Section 5.3 Calibra- tions, on page 88
	Visually inspect the inlet filters and clean them if necessary.	Section 8.6.1 Cleaning the inlet filters, on page 231
Monthly, or when required	Clean the system flow path with 1 M NaOH and rinse with DM water. <b>Note:</b> Cleaning may be necessary more or less frequent- ly, depending on the system usage and the nature of the samples.	Section 8.3.2 System cleaning, on page 224
Yearly, or when re- quried	Visually inspect the drive sleeve on the Fraction collector. Replace if worn out.	Maintenance Manual, Chapter 7 - Disassem- bly

# Other required maintenance

The following maintenance should be performed by the user of ÄKTA start when required.

Maintenance action	See section
Clean the instrument externally	Section 8.6.2 Cleaning the instrument externally, on page 232
Clean the Fraction collector	Section 8.6.3 Cleaning the Fraction collector, on page 233
Perform System cleaning	Section 8.3.2 System cleaning, on page 224
Clean the <b>UV flow cell</b>	Section 8.4 Cleaning the UV flow cell, on page 227
Clean the <b>Conductivity cell</b>	Section 8.5 Cleaning the Conductivity flow cell, on page 229
Calibrate the touch screen	Section 5.3.2 Instrument Display calibration, on page 90
Calibrate the UV flow cell	Section 5.3.5 UV Monitor calibration, on page 96
Calibrate the <b>Conductivity cell</b>	Section 5.3.6 Conductivity Monitor calibration, on page 100
Pressure sensor zero offset	Section 5.3.3 Pressure sensor zero offset, on page 92
Replace the inlet filters	Section 8.8.1 Replace the inlet filters, on page 239
Replace the tubing and connec- tors	Section 8.8.2 Replace the tubing and connectors, on page 240

# 8.2 Cleaning before planned maintenance/service

# Cleaning before planned maintenance/service

To ensure the protection and safety of service personnel, all equipment and work areas must be clean and free of any hazardous contaminants before a Service Engineer starts maintenance work.

Please complete the checklist in the *On Site Service Health & Safety Declaration Form*, or the *Health & Safety Declaration Form for Product Return or Servicing*, depending on whether the instrument is going to be serviced on site or returned for service, respectively. Copy the form you need from *Section 10.4 Health and Safety Declaration Form, on page 269* or print it from the PDF file available on the User Documentation CD.

# 8.3 Cleaning the system flow path

# Introduction

Cleaning of the system flow path is performed to prevent carryover between runs, contamination in the flow path and as a routine maintenance protocol.

Cleaning the system flow path is usually performed by using *System cleaning* or *Pump wash* methods.

Note:

Before cleaning the system flow path, remove the column from the flow path. For detailed instructions, see Section 8.3.1 Disconnect the column, on page 223.



#### WARNING

Hazardous biological agents during run. When using hazardous biological agents, run the *System cleaning* method to flush the entire system tubing with 1M NaOH and subsequently with distilled water, before service and maintenance. NaOH can cause health injurious, avoid spillage.



#### CAUTION

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

Tip:

If hazardous chemicals are used for system or column cleaning, wash the system or columns with a neutral solution in the last phase or step.

## In this section

This section contains the following subsections:

Section	See page
8.3.1 Disconnect the column	223
8.3.2 System cleaning	224

# 8.3.1 Disconnect the column

## Introduction

The column should be removed from the flow path before cleaning the system flow path. The flow path has to be re-connected between the manual **Injection valve** port 1 and the **UV** inlet.

For column cleaning procedures and storage instructions, refer to the column catalogue.

#### Instruction

Follow the instructions below to remove the column and re-connect the flow path.

Step	Action
1	Disconnect the tubing from the column, as indicated by the arrows in the illustration below (1).

2 Re-connect the flow path between the **Injection valve** and the **UV** Monitor, as indicated by the arrows in the illustration below (2). Join the tubing by using the union mounted on the tubing connected to the **UV flow cell**.



# 8.3.2 System cleaning

# Introduction

The **System cleaning** method is used to clean the instrument flow path. **System cleaning** is recommended to be performed to prevent carryover between runs, contamination in the flow path, as a routine maintenance protocol and to prepare system for storage.

- *Note:* Cleaning is important for preventing cross-contamination and bacterial growth in the instrument.
  - Prepare cleaning solutions of recommended concentration to ensure proper cleaning.
  - It is recommended not to end the run before completion.
  - It is recommended to clean the inlets and outlets (sample tubing, fractionation tubing) from the **Edit run** screen.

# **Required solutions**

The following cleaning solutions are required:

- 1 M NaOH
- DM water

# Instruction

Follow the instructions below to clean the system flow path. The **System cleaning** procedure is initiated from the Instrument Display.

Step Action

- 1 Remove the column from the flow path and re-connect the tubing.
- 2 Immerse both the buffer inlets in 1M NaOH.
- 3 In the **ÄKTA start** home screen, tap **Method run**.



Step Action	Ste	р	Α	cti	on
-------------	-----	---	---	-----	----

4

5

6

In the *Method run* screen, tap *Prepare system*.



Select **System cleaning** and then tap **Select** to initiate the method. For detailed instructions, see Section 6.4.5 Prepare system methods, on page 189.

Select sy	stem method	?
	System cleaning	
<	O System performance method	
		2/2
Back		Select

- Perform the operations presented on the display:
  - 1 Disconnect the column.
  - 2 Connect Injection valve to UV with suitable tubing and connectors.
  - 3 Place the buffer tubings (A & B) in cleaning solution.
  - Tap Continue to start System cleaning.

System cleaning
Please follow instructions 1. Disconnect the column 2. Connect injection valve to UV with suitable tubing and connectors 3. Place the buffer tubings (A & B) in cleaning solution When completed press Continue
Continue

#### 8 Maintenance

8.3 Cleaning the system flow path

8.3.2 System cleaning

Step	Action
7	When the run is completed, tap <i>Exit</i> to close the <i>System cleaning</i> screen.
	Note:

- Do not leave NaOH in the instrument in order to avoid damage of the system. Wash the complete flow path with water.
- Check the pH after a wash with water to make sure a complete removal of NaOH.

UV absorbance	1.0	mAU	
Conductivity	25.0	mS/cm	
Flow rate	3.0	ml/min	Graph
Pressure	0.30	MPa	
Tube no.	6		

# 8.4 Cleaning the UV flow cell

## **Maintenance interval**

Clean the **UV flow cell** every six months, or when required. A clean flow cell is essential for the acceptable performance of the **UV** Monitor.



#### NOTICE

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

#### **Required solutions**

The following solutions are required:

- Cleaning solution: 10% surfactant detergent solution, such as Decon<sup>™</sup> 90, Deconex<sup>™</sup> 11, RBS 25, 1M HCl or 1M NaOH.
- DM water
- **Note:** It is recommended to use 10% surfactant detergent solution for cleaning the **UV flow cell**.
  - Heat the 10% surfactant detergent solution to 40°C to increase the cleaning efficiency.
  - If NaOH is used, perform cleaning at 1ml/min and reduce the hold time to 5 min in step 3 of the method mentioned below.
  - NaOH should not be left in the flow cell for more than 20 minutes and proper care should be taken to remove the NaOH completely from the flow cell.

# Cleaning the UV flow cell in-place

Follow the instructions below to clean the UV flow cell.

Note:	Before cleaning the UV flow cell, remove the column from the flow path and
	re-connect the flow path. See Section 8.3.1 Disconnect the column, on page 223.

# Step Action 1 Immerse the inlet tubing in cleaning solution.

Step	Action
2	Start a manual run and pump cleaning solution at 5 ml/min through the <b>UV flow cell</b> for 10 minutes and pause the run.
3	Leave the <b>UV flow cell</b> filled with cleaning solution for 15 minutes.
4	Immerse the inlet tubing in DM water.
5	Resume the run and rinse the flow cell thoroughly with DM water.

# 8.5 Cleaning the Conductivity flow cell

#### Maintenance interval

Clean the **Conductivity flow cell** when the **Conductivity** Monitor shows a slow response or when the conductivity measurements are not comparable to any of the previous results.

### **Required solutions**

The following solutions are required:

- 1 M NaOH
- DM water

# Cleaning the Conductivity flow cell in-place

Follow the instructions below to clean the Conductivity flow cell.

**Note:** Before cleaning the **Conductivity flow cell**, remove the column from the flow path and re-connect the flow path. See Section 8.3.1 Disconnect the column, on page 223.

#### Step Action

- 1 Immerse the inlet tubing (either A or B) in 1M NaOH.
- 2 Start a manual run and pump 1M NaOH at 1 ml/min through the flow cell for 10 minutes.
- 3 Pause the run. Leave the **Conductivity flow cell** filled with 1M NaOH for 15 minutes.
- 4 Immerse the inlet tubing in DM water.
- 5 Resume the run and rinse the flow cell thoroughly.

#### Note:

- Do not leave NaOH for a very long time in flow cell to avoid damage. Rinse the flow path with water thoroughly.
- Make sure that the NaOH is completely removed. The conductivity reading in the **Run view** screen should be < 1 mS/cm.

# 8.6 Other cleaning procedures

# Introduction

This section provides instructions for additional cleaning procedures to be performed by the user of ÄKTA start.

# In this section

This section contains the following subsections:

Section	See page
8.6.1 Cleaning the inlet filters	231
8.6.2 Cleaning the instrument externally	232
8.6.3 Cleaning the Fraction collector	233

# 8.6.1 Cleaning the inlet filters

## Maintenance interval

Clean the inlet filters when required, for example when the visual inspection shows that the filters are clogged.

# **Required solutions**

The following solutions are required:

- 1M NaOH
- DM water

#### Instruction

Follow the instructions below to clean the inlet filters.

Step	Action
1	Pull off the support net and the inlet filter from the inlet filter holder. See Section 8.8.1 Replace the inlet filters, on page 239.
2	Immerse and leave the inlet filter and the support net in 1 M NaOH for about 2 hours.
3	Remove the inlet filter and the support net from the NaOH solution and rinse thoroughly with DM water.
4	Fit the inlet filter into the support net, and press it into position on the inlet filter holder.

# 8.6.2 Cleaning the instrument externally

# **Maintenance interval**

Clean the instrument externally when required. Do not allow spilled liquid to dry on the instrument.

# **Required material**

The following materials are required:

- Cleaning cloth
- Mild cleaning agent or 20% ethanol

## Instruction

Follow the instructions below to clean the instrument externally.

Step	Action	
1	Check that no run is in progress.	
2	Switch off the instrument.	
3	Wipe the surface with a damp cloth. Wipe off stains using a mild cleaning agent or 20% aqueous ethanol.	
4	Let the instrument dry completely before using it.	

# 8.6.3 Cleaning the Fraction collector

#### Maintenance interval

Clean the fraction collector when required, for example in case of liquid spill in the Bowl assembly.

### **Required material**

The following materials are required for cleaning the Bowl assembly:

- Water
- 20% ethanol
- Cleaning cloth

#### Instruction

Follow the instructions below to disassemble and clean the Bowl assembly.

Step	Action
1	Check that no run is in progress.
2	Switch off ÄKTA start and disconnect the Frac30 Cable.
3	Remove the collection tubes and disassemble the Bowl assembly from the Base unit:

- Gently move the Dispenser arm counterclockwise to the end position (1)
- Push the drive assembly and hold it at the retracted position (2)
- Lift and remove the Bowl assembly (3)

#### 8 Maintenance

8.6 Other cleaning procedures

8.6.3 Cleaning the Fraction collector

#### Step Action



- 4 Wash the Bowl under a water tap. Use a mild detergent if required, and rinse thoroughly with water.
- 5 Wipe the surface with a damp cloth. Wipe off stains using water.
- 6 Let the Bowl assembly dry completely before re-assembling.
- 7 Re-assemble the Bowl assembly on to the Base unit:
  - Orient the Bowl to match the aligning groove and the aligning features on the Bowl holder (1)
  - Lower the bowl assembly on to the Base unit (2) and push the drive assembly to allow the Bowl assembly to get in position (3)



#### Removing the tube holder

**Note:** The tube holder is not intended to be open by the user. If required, the tube holder may need to be removed from the Bowl assembly for cleaning.

Follow the instructions below to remove and then place back the tube holder in the Bowl assembly.

#### Step Action

1 To remove the test tube holder, unsnap the snap locks one by one and sequentially for ease of removal.



2

To fit the test tube holder on the Bowl base:

• Align the holder with the help of the aligning groove.



• Snap fit the part by operating one snap at a time in a continuous sequence by pressing at the edge of the part.

# 8.7 Storage of the instrument

# **Required material**

The following materials are required:

- DM water
- 20% ethanol
- 0.75 mm i.d. PEEK tubing
- Waste container

## Short term storage

If the system is not going to be used for a few days, follow the instructions below to prepare the system for short term storage.

**Note:** Before cleaning the flow path, remove the column and re-connect the flow path. See Section 8.3.1 Disconnect the column, on page 223.

Step	Action
1	Immerse the buffer and the sample inlet tubing in DM water.
2	In the <b>ÄKTA start</b> home screen, tap <b>Manual run</b> . For detailed instructions, see Section 6.3 Perform a manual run, on page 162.

Set the run parameters according to the table below.

Parameter	Setting
Flow rate	1 ml/min
Pressure limit	0.5 MPa
Select Buffer/Sample	Buffer
Wash valve	Column
Outlet valve	Waste

3 Tap *Run* to start the manual run.

Pump 20 ml of DM water through the system.

#### Step Action

4 Tap *Edit run* and set the run parameters as indicated below.

*Result*: The sample inlet tubing and the outlet tubing for fraction collection will be flushed with DM water.

Parameter	Setting
Select Buffer/Sample	Sample
Outlet valve	Collection

- 5 Pump 20 ml of DM water through the system.
- 6 End the run and leave the system filled with DM water during the storage period.

#### Long term storage

If the system is not going to be used for more than 4 days, follow the instructions below to prepare the system for long term storage.

**Note:** Before cleaning the flow path, remove the column and re-connect the flow path. See Section 8.3.1 Disconnect the column, on page 223.

## Step Action

- 1 Immerse the buffer and the sample inlet tubing in 20% ethanol.
- Start a manual run and pump 20 ml of 20% ethanol through the system.
   Use the same run parameters recommended for the short term storage procedure.
- 3 Edit the run and set the run parameters to clean the sample inlet tubing and the outlet tubing for fraction collection.
- 4 Pump 20 ml of 20% ethanol through the system.
- 5 End the run and leave the system filled with 20% ethanol during the storage period.

# 8.8 Replacement of tubing and filters

# Introduction

This section describes how to replace tubing and connectors, and how to replace the inlet filters.



# In this section

This section contains the following subsections:

Section	See page
8.8.1 Replace the inlet filters	239
8.8.2 Replace the tubing and connectors	240

# 8.8.1 Replace the inlet filters

#### Maintenance interval

Replace the inlet filters when required, for example when the visual inspection shows that the filters are clogged or damaged.

### **Required items**

The following items are required:

- inlet filters
- support nets
- **Note:** An Inlet Filter Set containing inlet filters and support nets is included in the accessories kit.

### Instruction

Follow the instruction below to replace an inlet filter and a support net.

**Note:** The inlet filters are mounted on the inlet tubing at the end that will be immersed in the buffer solution.

#### Step Action

1 Pull off the inlet filter and the support net from the inlet filter holder.





2 Fit the new support net and inlet filter, and press the filter in position into the inlet filter holder.

# 8.8.2 Replace the tubing and connectors

# **Maintenance interval**

Replace tubing and connectors when required, for example when the tubing is clogged or bent and the flow is obstructed.

**Note:** Before starting to replace tubing and connectors, clean the system flow path with DM water, then remove the inlet tubing from water.

# **Required items**

The following item are required:

- Tubing and connectors
- Tubing cutter

# Instruction

Follow the instructions below to replace tubing and connectors.

- **Note:** To replace the pump tubing (Marprene™ tubing, Part No. 29-0240-12), follow the instruction presented in the ÄKTA start Maintenance Manual.
- **Note:** To replace the tubing that connects the **UV** monitor to the **Conductivity** monitor use the pre-bent tubing supplied with the system.

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

#### Step Action

3

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





#### CAUTION

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

#### Note:

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

- 5 Put the labels back on the new tubing.
- 6 Mount the connectors on the tubing.

For fingertight connectors:

• Slide the connector onto the tubing.

For tubing connectors 1/8":

- Slide the connector onto the tubing.
- Slide the ferrule onto the tubing with the thick end towards the end of the tubing.
- 7 Insert the tubing with connector into the port. Make sure to insert the tubing all the way into the bottom of the port.
- 8 Tighten the connector fully.

# 9 Troubleshooting

# About this chapter

This chapter describes troubleshooting and corrective actions for ÄKTA start.

# In this chapter

This chapter contains the following sections:

Section	See page
9.1 Introduction to troubleshooting	243
9.2 Basic troubleshooting	245
9.3 System error report	256

# 9.1 Introduction to troubleshooting

### Introduction

The sections in this chapter describe the basic troubleshooting for ÄKTA start and include a general checklist that should be completed prior to the troubleshooting work. How to generate a System error report for service purposes is also presented. For replacement of modules and other module specific problems and corrective actions, refer to the ÄKTA start Maintenance Manual.

Refer to the UNICORN start 1.0 User Manual for problems related to the software.

**Note:** For detailed description of Warning messages, refer to the ÄKTA start Maintenance Manual.

#### **Troubleshooting procedure**

To troubleshoot ÄKTA start, follow these steps:

Step	Action	
1	Follow the checklists below, for system, flow path, and purification.	
2	If problems remain, search for solutions in <i>Section 9.2 Basic troubleshooting</i> , <i>on page 245</i> .	
3	If problems remain after corrective actions, generate a System error report (see Section 3.3.4 Description of Settings and service, on page 51) and contact your local GE Service Engineer.	

## System checks

• Does the instrument display show the ÄKTA start home screen?



• Does the ventilation of the instrument function? Contact a GE Service Engineer if the fans at the bottom of the instrument stop to run.

# Flow path checks

- Is all tubing connected correctly, as shown in *Section 5.1 Flow path overview*, on page 84?
- Is there leakage at any of the connections? Tighten the connections if required.
- Is any tubing bent or twisted? Adjust the tubing position to make sure that the flow of liquid is smooth, or replace the tubing if required.
- Is the buffer inlet tubing immersed in correct buffer solutions? See Section 5.10 Starting a run, on page 153.
- Are the inlet filters clean? Clean the inlet filters, or replace the filters if required. See Section 8.6.1 Cleaning the inlet filters, on page 231, and Section 8.8.1 Replace the inlet filters, on page 239.
- Is the column connected correctly? See Section 5.5 Connect a column, on page 121.

# **Purification checks**

- Has the column been cleaned and prepared according to the recommendations in the column instruction manual?
- Has the sample been adjusted to binding buffer conditions?
- Has the sample been clarified by centrifugation and/or filtration prior to the sample loading?
- Are the correct buffers used for the chosen columns and proteins?
- Check buffers for precipitations and contamination. Adjust the required temperature wherever needed.
- Are the chosen columns suitable for the chosen target proteins?
- Have the stable baseline for UV and Conductivity been established?

# 9.2 Basic troubleshooting

# Introduction

This section describes problems that can occur with the Display, UV and Conductivity measurements, problems with the wet side modules, possible causes, and the recommended corrective actions. If error codes are displayed on the Touch screen, refer to *ÄKTA start Maintenance Manual*.

# Display

Description	Possible cause	Action
The Instrument display does not show anything	No power.	Check that the Power cord is connected and that the Power Switch is switched on.
	The Instrument display is dam- aged.	Contact a GE Service Engineer.
	Communication problem, no sig- nals to the display.	Contact a GE Service Engineer.
Issues with the touch response	Calibration may have gone bad.	Re-calibrate the Touch screen. For details, refer to the ÄKTA start Maintenance Manual.
The Display color is not proper	Display malfunc- tion. Display inter- face is cable loose.	Perform a <b>Display:Color test</b> , refer to ÄKTA start Maintenance Manual. If there is a pattern mismatch, contact a GE Ser- vice Engineer.
The Display Back- light is not working	Display malfunc- tion. Display inter- face is cable loose.	Perform a <b>Display:Diagnostic:Backlight</b> <b>test</b> , refer to ÄKTA start Maintenance Manual. Check whether the Backlight is changing or not. If it is not changing, contact a GE Service Engineer.

# UV curve

Description	Possible cause	Action
Noisy UV signal, signal drift or insta- bility	If UV noise level is above 10 mAU, air bubbles are trapped in the flow cell.	Remove the air bubbles trapped in the <b>UV flow cell</b> by flushing the cell with DM water or buffer. If persistent, clean the <b>UV flow cell</b> . See Section 8.4 Cleaning the UV flow cell, on page 227.
	Buffer is impure.	Check if the signal is still noisy with DM water.
	Air dissolved in the buffer or air bub- bles seen in the buffer inlet tub- ings.	Degas the buffer before use. Use a tech- nique available in the laboratory, such as vacuum degassing or sonication. Perform Pump wash to remove the air bubbles from the flow path.
	<b>UV flow cell</b> not clean.	Clean the <b>UV flow cell</b> . See Section 8.4 Cleaning the UV flow cell, on page 227.
	UV flow cell not mounted properly, locknut not tight- en.	Remove the protective cover and check the flow cell. Tighten the locknut.
Ghost peaks	Air dissolved in the buffer.	Degas the buffer before use. Use a tech- nique available in the laboratory, such as vacuum degassing or sonication.
	Flow path not clean.	Clean the system flow path. See Sec- tion 8.3 Cleaning the system flow path, on page 222.
	Column not clean.	Clean the column according to the col- umn instructions.
Low sensitivity	<b>UV flow cell</b> not clean	Clean the <b>UV flow cell</b> . See Section 8.4 Cleaning the UV flow cell, on page 227.
Waves on the gra- dient	Switch valve tim- ing not optimized.	Perform <b>Switch valve timing</b> to optimize the switch valve timing. See Section 5.4.4 Switch valve timing, on page 117.

Description	Possible cause	Action
Warning 111: UV intensity low	<ul> <li>UV Module is not calibrated properly.</li> <li>Flow cell is not clean.</li> <li>Flow cell is not mounted prop- erly.</li> <li>UV LED intensi- ty is below de- sired level.</li> </ul>	Mount flow cell securely and re-calibrate the <b>UV</b> module with the flow cell filled with DM water. If the problem persists, replace the <b>UV</b> module. For details, refer to the ÄKTA start Maintenance Manual.
Warning 112: UV intensity high	<ul> <li>Calibration was not per- formed on a clean flow cell.</li> <li>Flow cell is not mounted prop- erly.</li> </ul>	Mount the flow cell securely, clean and re-calibrate <b>UV</b> module with the flow cell filled with DM water. For details, refer to the <i>ÄKTA start Maintenance Manual</i> .
Warning 115: Flush flow cell and mount securely	Flow cell not mounted properly or is optically mis- aligned	Mount the flow cell properly. Re-calibrate the <b>UV</b> module with flow cell filled with DM water.
Warning 116: UV base lining ignored	Flow cell is not clean.	Clean the flow cell thoroughly with DM water. Re-calibrate the <b>UV</b> module with the flow cell filled with DM water.

# Conductivity curve

Description	Possible cause	Action
Incorrect or unsta- ble reading or Out of specification conductivity val- ues	<b>Conductivity</b> cell not clean.	Clean the <b>Conductivity</b> cell. See <i>Sec-</i> <i>tion</i> 8.5 <i>Cleaning the Conductivity flow</i> <i>cell, on page 229.</i>
	Cable improperly connected to the <b>Conductivity</b> Mon- itor.	Check that the <b>Conductivity</b> module cable is connected properly to the connector behind the flow cell.
	Pump malfunction.	Check that the <b>Pump</b> functions properly. See <b>Pump</b> troubleshooting.
	Temperature sen- sor not calibrated.	Calibrate the temperature sensor. See Section 5.3 Calibrations, on page 88.
	Non-equilibrated column.	Check that the column is equilibrated. If necessary, clean the column.
	<b>Buffer valve</b> mal- function.	Check the operation of the <b>Buffer valve</b> . Refer to the ÄKTA start Maintenance Manual.
	The 0.5 mm ID (195 mm) PEEK tubing is not connected between the <b>Con- ductivity</b> Monitor and the <b>Outlet</b> <b>valve</b> .	Connect 0.5 mm ID PEEK tubing. See Section 8.8.2 Replace the tubing and connectors, on page 240.
	Failure of compo- nent in Main board	Contact a GE Service Engineer.
Baseline drift or noisy signal	Air trapped in the conductivity flow cell.	Remove the air trapped in the flow cell by flushing the cell with DM water or buffer. If persistent, clean the <b>Conductiv-</b> <b>ity flow cell</b> . See Section 8.5 Cleaning the Conductivity flow cell, on page 229.
	Non-equilibrated column.	Check that the column is equilibrated. If necessary, clean the column.
	Pump malfunction.	Check that the pump functions properly. See <b>Pump</b> troubleshooting.

Description	Possible cause	Action
Conductivity mea- surement with the same buffer changes over time at constant tem- perature	Flow cell not clean.	Clean the <b>Conductivity flow cell</b> , see Section 8.5 Cleaning the Conductivity flow cell, on page 229.
Waves on the gra- dient	Pump or Buffer valve malfuntion.	Check that the <b>Pump</b> and the <b>Buffer</b> <b>valve</b> operate properly. Refer to ÄKTA start Maintenance Manual.
	Switch valve tim- ing not optimized.	Perform <i>Switch valve timing</i> to optimize the switch valve timing. See <i>Section 5.4.4</i> <i>Switch valve timing, on page 117.</i>
	Wrong absolute conductivity value.	<b>Conductivity flow cell</b> not calibrated Recalibrate the <b>Conductivity flow cell</b> . See Section 5.3 Calibrations, on page 88.
Temperature sen- sor not calibrated		Calibrate the temperature sensor. See Section 5.3 Calibrations, on page 88.
Calibration solu- tion (1.00 M NaCl) not correctly pre- pared		Prepare a new calibration solution and recalibrate the <b>Conductivity flow cell</b> .
Ghost peaks in the gradient profile	A charged sample was detected (e.g., a protein).	Clean the <b>Conductivity flow cell</b> , see Section 8.5 Cleaning the Conductivity flow cell, on page 229.
	Air bubbles pass through the flow cell.	Check for loose tubing connections.
Non-linear gradi- ents or slow re-	<b>Buffer valve</b> mal- function.	See <b>Buffer valve</b> troubleshooting.
sponse to %B changes	Irregular flow.	Check that the <b>Pump</b> functions properly. See <b>Pump</b> troubleshooting.
	Tubing not clean.	Make sure that the tubing is washed properly. Run <b>System cleaning</b> to clean the system flow path. See Section 8.3 Cleaning the system flow path, on page 222.

# Pump

Description	Possible cause	Action
Erratic flow	Air bubbles in the flow path.	Remove the air bubbles trapped in the flow path by flushing the flow path with DM water or buffer according to the pro- cedure below:
		• remove the column and re-connect the flow path (see Section 8.3.1 Disconnect the column, on page 223)
		<ul> <li>perform a manual run with a flow rate of 5 ml/min for around 10 min- utes</li> </ul>
		<ul> <li>observe the graph until the pulsations are no more visible and the curve is stable</li> </ul>
		Note:
		If there is air trapped in the flow path, the flow is not accurate and pulsations can occur, affecting the output signals.
	Worn out pump tubing.	Replace the pump tubing. See Sec- tion 8.8.2 Replace the tubing and connec- tors, on page 240.
Inaccurate flow rate	<b>Pump</b> not calibrat-ed.	Calibrate the <b>Pump</b> . See Section 5.3 Calibrations, on page 88.
	Pump tubing not properly posi- tioned.	Place the pump tubing in the pump hood maintaining equal distance on both the sides.
	Worn out pump tubing.	Replace the pump tubing. See Sec- tion 8.8.2 Replace the tubing and connec- tors, on page 240.
No flow	Pump tubing is not fixed inside the pump hood.	Fix the pump tubing inside the pump hood and start the run.
	Failure of compo- nent in Main board	Troubleshoot the <b>Pump</b> and if the prob- lem still persists, contact a GE Service Engineer.

Description	Possible cause	Action
Leakage	Tubing connection	Check the tubing connections. Re-tighten or replace if necessary.

# Mixer

Description	Possible cause	Action
Leakage	Tubing connection.	Check the tubing connections. Re-tighten or replace if necessary.

# **Fraction collector**

Description	Possible cause	Action
The fractions col- lected fall outside the collection	The Bowl Assembly improperly fitted on the Base unit.	Make sure that the Bowl is fitted properly on the base, not tilted.
lubes.	Dispenser arm not in correct position.	Check that the dispenser arm is in the dispensing position. Notice the alignment of the markings on the dispenser arm.
	Drive sleeve is worn out and slip- page occurs.	Replace the drive sleeve. Refer to the ÄKTA start Maintenance Manual
Fraction collector is not homing at the start of the run. Fraction the inst option abled.	Fraction collector is not connected to the instrument.	Connect the Fraction collector to ÄKTA start
	Fraction collector option is not en- abled.	Enable the Fraction collector option from the <b>Settings and service</b> screen.

# Buffer valve/Sample valve/Wash valve/Outlet valve

Description	Possible cause	Action
External leakage	Tubing connec- tions	Check the tubing connections. Tighten or replace if required.
Internal leakage	Internal parts may be worn	Replace valve. Refer to ÄKTA start Maintenance Manual.
High back-pres- sure	Flow path not clean	Clean the system flow path. See Sec- tion 8.3 Cleaning the system flow path, on page 222.
Valve not switch- ing position	Internal parts may be worn	Replace valve. Refer to ÄKTA start Maintenance Manual.
		If the valve does not function even after replacement, contact a GE Service Engineer.

# Injection valve

Description	Possible cause	Action
The valve switch- ing to wrong posi- tion	Valve parts incor- rectly assembled after replacement	Make sure that the marking etched on the valve aligns correctly with the same marking on the instrument. Refer to ÄKTA start Maintenance Manual.
External leakage	Tubing connec- tions	Check the tubing connections. Tighten or replace if required.
Internal leakage	Internal parts may be worn	Replace valve. Refer to ÄKTA start Maintenance Manual.
High back-pres- sure	Flow path not clean	Clean the system flow path. See Sec- tion 8.3 Cleaning the system flow path, on page 222.
Unable to load sample to the loop	Manual injection valve is in the <b>In-</b> <b>ject</b> position.	Turn the <b>Injection valve</b> to <b>Load</b> position while loading the sample to the loop.
	The Valve or the loop is blocked	Clean the system flow path. If problem persists, replace the valve/loop.
#### **Pressure sensor**

Description	Possible cause	Action
<i>Error 501:</i> Over pressure	<ul> <li>Blockage in tubing, valves or in the column.</li> <li>Improperly selected flow rate.</li> </ul>	<ul> <li>Check the tubing and valves by disconnecting one at a time. Clean or replace when a blockage is found.</li> <li>Clean the column/separation media with suitable solution (1 M NaOH) or replace the column with a new one.</li> <li>Check the specification of the columns for correct flow rate.</li> </ul>
<b>Pressure sensor</b> is not functioning. Pressure curve is not displayed on the screen.	<b>Pressure sensor</b> failure or failure of component in Main board.	Contact a GE Service Engineer.

#### **User Information Messages**

Description	Possible cause
Instruction ignored message: <b>Instruction</b> <b>ignored, not allowed to set the inlets</b> <b>during gradient</b> .	This message is shown when it is not possible to give the instruction during <i>Gradient</i> .
Occurrence message: The instruction Outlet valve is not possible to issue dur- ing an active fractionation.	This message appears after the delay volume has passed and no fractionation is active.
Occurrence message: Instruction ignored. Stop fractionation is only allowed during fractionation.	This message appears when the instruc- tion <i>Fractionation</i> has not been execut- ed, the <i>Stop fractionation</i> instruction shall be ignored and an occurrence shall be issued.
Warning message: Instruction ignored. Peak fractionation is not allowed during fractionation or Single peak collection.	This warning message appears when an instruction is not possible to execute during <i>Single peak collection</i> or during <i>Fractionation</i> .

Description	Possible cause
Warning message: Last tube has been reached; change tubes in the fraction collector and press Continue to continue the run with fractionation. Press Cancel fractionation to continue the run without fractionation flow is diverted to Flow through/Waste position.	This message appears when the last tube has been reached.
Occurrence message: Last tube has been reached and the run has continued without fractionation.	This message appears if <b>Continue</b> without fractionation is selected.
Warning message: Instruction ignored. Single peak collection is not allowed during 'Fractionation' or 'Peak fractiona- tion'.	This message appears when one tries to execute <b>Single peak collection</b> dur- ing fractionation.
Warning message: Turning Outlet valve is not allowed during Single peak fraction-ating.	This message is displayed to show that during <i>Single peak fractionation</i> the <b>Outlet valve</b> shall not be able to turn.
Occurrence message: Instruction ignored. Stop single peak collection is only al- lowed during single peak collection.	This message is displayed when the in- struction <i>Single peak collection</i> has not been executed.
USB removed abruptly.	This message appears when the user removes the USB memory stick while read or write operation is being execut- ed.

#### **USB** memory stick connection

To troubleshoot possible issues encountered when connecting a USB memory stick to ÄKTA start, follow the procedure presented in the flow chart below.



# 9.3 System error report

#### Introduction

A System error report can be generated during a troubleshooting case with information about the problem. The report can then be sent to GE Service Engineer for action.

#### Generate a System error report

Follow the instructions below to generate a System error report.

**Note:** Make sure that a USB memory stick is connected to the instrument. If the instrument cannot detect a USB memory stick, the report file cannot be saved.

#### Step Action

1 In the **ÄKTA start** home screen, tap **Settings and service** to access Screen 1 (see Section 3.3.4 Description of Settings and service, on page 51), and then select **System**.

#### Settings and service Screen 1



2

Insert a USB memory stick into the USB port located on ÄKTA start.

In the System screen, select Export system error report to USB.

Delay volume setting	Firmware update
Switch valve timing	Export system

*Result*: Two files with the names INSTLOG.TXT and ERRORLOG.TXT are exported to the USB memory stick.

#### Step Action

4

3 Remove the USB stick from the USB port and connect it to a computer. Check the content of the system report files INSTLOG.TXT and ERRORLOG.TXT. The content should look similar to the images below.



Use the system report in further contacts with GE Service Engineer.

# 10 Reference information

#### About this chapter

This chapter lists the technical specifications of ÄKTA start. The chapter also includes a list of wetted materials, a chemical resistance guide, Health and Declaration form for service, and ordering information.

#### In this chapter

This chapter contains the following sections:

Section	See page
10.1 Specifications	259
10.2 Chemical resistance	265
10.3 Literature	268
10.4 Health and Safety Declaration Form	269
10.5 Ordering information	271

# 10.1 Specifications

#### Introduction

This section lists the specification data of ÄKTA start. For component data see the ÄKTA start Maintenance Manual.

#### System specifications

Parameter	Data
System configuration	Benchtop system
Control system	Instrument Display and/or UNICORN start
Connection between PC and instrument	USB
Dimensions ( $W \times D \times H$ )	340 mm × 280 mm × 360 mm
Weight (excluding packaging)	8 kg
Power supply	100 to 240 V AC ±10%, 50/60 Hz
Power consumption	95 VA
Transient overvoltages	Overvoltage category II
Fuse	Fast blow glass tube type, F5AL250V
Enclosure protective class	IP21
Tubing and connectors:	·
Inlet	PTFE tubing, length 100 cm, i.d. 1.6 mm, 5/16-24 UNF connections
Buffer valve to Mixer	PEEK tubing, Length 15 cm, i.d. 0.75 mm, 10-32 UNF connections
Mixer to Sample valve	PEEK tubing, Length 23 cm, i.d. 0.75 mm, 10-32 UNF connections
Sample valve to Pressure sensor (via Pump)	Marprene tubing, Length 25 cm, i.d. 0.8 mm, 10-32 UNF connections
Pressure sensor to Wash valve	PEEK tubing, Length 13 cm, i.d. 0.75 mm, 10-32 UNF connections

Parameter	Data
Wash valve to Injection valve	PEEK tubing, Length 17 cm, i.d. 0.75 mm, 10-32 UNF connections
Injection valve to Column	PEEK tubing, Length 15 cm, i.d. 0.75 mm, 10-32 UNF connections
Column to <b>UV</b>	PEEK tubing, Length 15 cm, i.d. 0.75 mm, 10-32 UNF connections
UV to Conductivity	PEEK tubing, Length 20 cm, i.d. 0.75 mm, 10-32 UNF connections
Conductivity to Outlet valve	PEEK tubing, Length 19 cm, i.d. 0.50 mm, 10-32 UNF connections
Outlet valve to Frac30	PEEK tubing, Length 50 cm , i.d. 0.75 mm, 10-32 UNF connections
Waste tubing	ETFE tubing, Length 60 cm i.d. 1.0 mm, Fingertight connector, 1/16"
Sample tubing	ETFE tubing, Length 25 cm, i.d. 1.0 mm, Fingertight connector, 1/16"

### Equipment noise level

Parameter	Value
Noise emission	< 60 dB A

#### **Environmental ranges**

Parameter	Data
Operation site	Indoor use
Altitude	Maximum 2000 m
Storage and transport temperature range	-25°C to +60°C
Humidity	20% to 80%, non-condensing
Pollution degree	2

#### **Operating range**

Parameter	Data
Operating temperature range	+4°C to +35°C
Relative humidity	20% to 80%, non-condensing

#### Pump

Parameter	Data
Pump type	Peristaltic Pump. Single channel, four roller pump head with low pulsation
Flow rate	0.5 to 5 ml/min (operating range) 10 ml/min (Wash flow)
Flow rate specifications	<ul> <li>Accuracy:         <ul> <li>Flow rate ≤ 1 ml/min: ±15%</li> <li>Flow rate &gt; 1 ml/min: ±10%</li> </ul> </li> <li>Precision:         <ul> <li>Flow rate ≤ 1 ml/min: ±15%</li> <li>Flow rate &gt; 1 ml/min: ±10%</li> </ul> </li> <li>Condition: 0.8 to 2 cP and fresh Pump tubing.</li> </ul>
Pressure range	0 to 0.5 MPa (5 Bar)
Viscosity range	0.6 to 5 cP

#### Mixer

Parameter	Data
Mixing principle	Static mixer
Mixer volume	0.4 ml

# Valves: Buffer, Sample, Wash and Outlet

Parameter	Data
Туре	Solenoid type switch valve
No. of Ports	<ul> <li>3 ports:</li> <li>Buffer valve and Sample valve: 2 in - 1 out</li> <li>Wash valve and Outlet valve: 1 in - 2 out</li> </ul>

#### Injection valve

Parameter	Data
Туре	Rotary type manual valve
Function	Sample injection through <b>Loop</b> .
No. of Ports	6 ports

### **Gradient formation**

Parameter	Data
Gradient flow rate range	0.5 to 5 ml/min
Gradient composition accuracy	± 5% Conditions: 5% to 95% B, 1 to 5 ml/min, 0.8 to 2 cP and fresh pump tubing.

#### **Pressure sensor**

Parameter	Data
Placement of sensor	Pressure sensor is located after the Pump.
Range	0 to 0.5 MPa (5 Bar)
Accuracy	± 0.05 MPa

Parameter	Data
Wavelength range	280 nm $\pm$ 3 nm. Single wavelength
Absorbance range	-0.1 to +2 AU
Linearity	Within $\pm$ 5% up to 1.5 AU
Operating pressure	0 to 0.5 MPa
Flow cell	2 mm optical path length. Total cell volume is 30 $\mu l.$

### Conductivity

Parameter	Data
Conductivity range	0 mS/cm to 300 mS/cm
Resolution	1 mS/cm
Accuracy	$\pm$ 5% or $\pm$ 2 mS/cm (whichever is greater)
Operating pressure	0 to 0.5 MPa
Flow cell Volume	22 µl
Temperature monitor range	4°C to 35°C
Temperature monitor ac- curacy	± 10% or ± 5°C (whichever is greater)

#### Frac30

Parameter	Data	
Number of fractions	Up to 30	
Vessel type	Centrifuge tubes (10 to 12 ml)	
	• Falcon tubes (15 ml)	
	• Eppendorf tubes (1.5 ml or 2 ml)	
	• 5 ml tubes (12 × 75 mm)	

#### 10 Reference information

#### 10.1 Specifications

Parameter	Data
Fraction volumes	0.5 to 15 ml
Flammable liquids	No
Delay Volume ( <b>UV</b> to Dis- penser head)	0.49 ml (default)
Dimensions (W $\times$ D $\times$ H)	270 × 280 × 285 mm
Weight	5 Кд

# 10.2 Chemical resistance

#### Flow path

All chemicals and concentrations are for short- term (< 1 day) use only, ambient temperature < 25°C, if not other stated. Long-term use is defined as 1 month.

The flow path shall withstand the following suggested chemicals.

Chemical	Concentration	CAS no. /EEC no.	Usage
Aqueous buffers, pH 2 to 12	-	N/A	Separation
Acetone	10%	67-64-1/ 200-662-2	
Acetic acid	6% (1 M)	64-19-7/200-580-7	CIP
Ammonium sulphate	3 M	77-83-20-2/231-984-1	Purification of plasmids
Arginine	2 M	74-79-3/200-811-1	Wash, using pro- tein A gels, Refold- ing
Benzyl alcohol	4%	100-51-6/202-859-9	Cleaning and storage of columns
Decon 90	10%	1310-58-3/215-181-3	Cleaning
Dimethyl sulphoxide (DMSO)	5%	67-68-5/200-664-3	CIP, RPC, Cell sepa- ration
DTT	100 mM	3483-12-3/222-468-7	Reducing agent
DTE	100 mM	6892-68-8/229-998-8	Reducing agent
TCEP (Tris(2-Car- boxyethyl)Phosphine)	100 mM	51805-45-9/	Reducing agent
EDTA	100 mM	6381-92-6/205-358-3	Buffer additive
Ethanol	96%	64-17-5/200-578-6	Storage (Long term use)
Ethylene glycol	30%	112-60-7/203-989-9	Buffer additive
Glycerol	30%	56-81-5/200-289-5	Buffer additive
Glycine	0.5 M	56-40-6/200-272-2	Cleaning of MAb binding media

#### 10 Reference information

10.2 Chemical resistance

Chemical	Concentration	CAS no. /EEC no.	Usage
Guanidine hydrochloride	6 M	50-01-1/200-002-3	Denaturing of proteins
Hydrochloric acid	0.1 M	7647-01-0/231-595-7	CIP
Imidazole	1 M	288-32-4/206-019-2	Affinity
Isopropanol	70%	67-63-0/200-661-7	CIP
Methanol	100%	67-56-1/200-659-6	RPC, CIP
Mercaptoethanol	20 mM	60-24-2/200-464-6	Reducing agent
Potassium phosphate basic	1 M	16788-57-1/231-834-5	Buffer
SDS	1%	151-21-3/205-788-1	Detergent
Sodium chloride	4 M	7647-14-5/231-598-3	CIP
Sodium hydroxide, NaOH	1 M	1310-73-2/215-185-5	CIP
Sodium sulphate	1 M	7757-82-6/231-820-9	Buffer
Triton™-X 100	1%	9002-93-1/	Detergent
Tween™ 20	1%	9005-64-5/500-018-3	Detergent
Urea	8 M	57-13-6/200-315-5	Buffer additive, denaturing agent
Water	100%	N/A	(Long term use)

## Wet side and painting

Chemical	Concentration	CAS no. /EEC no.
Decon 90	10%	1310-58-3/215-181-3
Ethanol	20%	64-17-5/200-578-6
Hydrochloric acid	0.1 M	7647-01-0/231-595-7
Isopropanol	70%	67-63-0/200-661-7
Triton-X 100	1%	9002-93-1/
Tween 20	1%	9005-64-5/500-018-3

Chemical	Concentration	CAS no. /EEC no.
Spray with commercial house cleaning detergent.	5%	N/A

### Display

Chemical	Concentration	CAS no. /EEC no.
Aqueous buffers, pH 2 to pH 12	-	N/A
Decon90	10%	1310-58-3/215-181-3
Ethanol	20%	64-17-5/200-578-6
Hydrochloric acid	0.1 M	7647-01-0/231-595-7
Isopropanol	70%	67-63-0/200-661-7
Sodium chloride	1 M	7647-14-5/231-598-3
Sodium hydroxide	0.5 M	1310-73-2/215-185-5
Triton-X 100	1%	9002-93-1/
Tween 20	1%	9005-64-5/500-018-3
Spray with commercial house cleaning detergent.	5%	N/A

## 10.3 Literature

For further information related to ÄKTA start, refer to the following:

- ÄKTA start Maintenance Manual
- UNICORN start 1.0 User Manual
- ÄKTA start System Cue Card
- ÄKTA start Maintenance Cue Card
- Data file

## 10.4 Health and Safety Declaration Form

#### On site service



#### **On Site Service Health &** Safety Declaration Form

#### Service Ticket #:

To make the mutual protection and safety of GE service personnel and our customers, all equipment and work areas must be clean and free of any hazardous contaminants before a Service Engineer starts a repair. To avoid delays in the servicing of your equipment, please complete this checklist and present it to the Service Engineer upon arrival. Equipment and/or work areas not sufficiently cleaned, accessible and safe for an engineer may lead to delays in servicing the equipment and could be subject to additional charges.

Yes	No	Please review Provide expla	Please review the actions below and answer "Yes" or "No". Provide explanation for any "No" answers in box below.			
		Instrument ho Please rinse tu residue. Ensure wipe test or ot	Instrument has been cleaned of hazardous substances. Please rinse tubing or piping, wipe down scanner surfaces, or otherwise ensure removal of any dangerous residue. Ensure the area around the instrument is clean. If radioactivity has been used, please perform a wipe test or other suitable survey.			
		Adequate space installation. In prior to GE arri	ce and clearance is provided to all some cases this may require cust val.	ow safe access for instrum omer to move equipment f	ent service, repair or rom normal operating location	
		Consumables any area that	such as columns or gels, have b may impede access to the instru	een removed or isolated fi iment.	rom the instrument and from	
		All buffer / wo Excess contai	All buffer / waste vessels are labeled. Excess containers have been removed from the area to provide access.			
Provide explana for any answer:	Provide explanation for any "No" answers here:					
Equipm	nent typ	e / Product No:		Serial No:		
I hereby area ha	I hereby confirm that the equipment specified above has been cleaned to remove any hazardous substances and that the area has been made safe and accessible.					
Name:		Company or institution:				
Position job title	n or e:			Date (YYYY/MM/DD):		
Signed:	:			·	·	

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#### Product return or servicing



#### Health & Safety Declaration Form for Product Return or Servicing

Return authorization number:	and/or Service Ticket/Request:	
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To make sure the mutual protection and safety of GE personnel, our customers, transportation personnel and our environment, all equipment must be clean and free of any hazardous contaminants before shipping to GE. To avoid delays in the processing of your equipment, please complete this checklist and include it with your return.

- 1. Please note that items will NOT be accepted for servicing or return without this form
- 2. Equipment which is not sufficiently cleaned prior to return to GE may lead to delays in servicing the equipment and could be subject to additional charges
- 3. Visible contamination will be assumed hazardous and additional cleaning and decontamination charges will be applied

Yes	No	Please specify if	cify if the equipment has been in contact with any of the following:				
		Radioactivity (ple	ease specify)				
		Infectious or haz	ardous biological	substances (p	lease specify)		
		Other Hazardous	s Chemicals (pleas	e specify)			
Equipmo you for o	ent must additionc	be decontamina Il information cor	ted prior to servic ncerning the syste	e / return. Ple m / equipme	ase provide a telephone r nt.	number where GE can contact	
Telepho	one No:						
Liquid o	ınd/or ga	s in equipment is	5:	Water			
				Ethanol	Ethanol		
				None, empty			
				Argon, Hel	Argon, Helium, Nitrogen		
				Liquid Nitro	ogen		
			Other, please specify				
Equipm	ent type	/ Product No:			Serial No:		
I hereby area ha	/ confirm is been m	that the equipm ade safe and acc	ent specified abo cessible.	ve has been c	leaned to remove any haz	ardous substances and that the	
Name:					Company or institution:		
Position or job title:			Date (YYYY/MM/DD)				
Signed:							

To receive a return authorization number or service number. please call local technical support or customer service.

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# 10.5 Ordering information

For ordering information visit www.gelifesciences.com/AKTA.

#### **Accessories list**

Part	Accessory description	Code no.
Pump	Marprene Tubing	29-0240-12
	Peristaltic Pump	29-0239-92
Solenoid valve	Buffer valve	29-0238-95
	Sample valve	29-0238-96
	Wash valve	29-0238-97
	Outlet valve	29-0238-98
Manual Injection valve	Injection valve, Manual	29-0239-58
	Valve kit, Manual INV	29-0239-17
Mixer	Mixer, ÄKTA start	29-0239-60
UV	UV module, ÄKTA start	29-0240-18
	Flow Cell 2 mm UPC-900	29-0113-25
Conductivity	Conductivity Cell, ÄKTA start	29-0240-21
Sample loops	Sample Loop, PEEK, 10 µl	18-1120-39
	Sample Loop 100 µl, INV-907	18-1113-98
	Sample Loop 500 µl, INV-907	18-1113-99
	Sample Loop 1.0 ml, INV-907	18-1114-01
	Sample Loop 2.0 ml, INV-907	18-1114-02
	Sample Loop 5 ml, PEEK	18-1140-53
	Sample Loop	18-1161-24
Superloop™	Superloop 10 ml ÄKTA	18-1113-81
	Superloop 50 ml ÄKTA	18-1113-82
	Superloop 150 ml	18-1023-85

#### 10 Reference information 10.5 Ordering information

Part	Accessory description	Code no.
Fittings	Tubing Connector 1/8"	18-1121-17
	Ferrule for 1/8" tubing	18-1121-18
	Union Luer Female/HPLC Male	18-1112-51
	Fingertight Connector 1/16"	18-1112-55
	Stop plug 1/16", PKG/5	18-1112-52
	Stop plug, 5/16", PKG/5	18-1112-50
	Union, 1/16" female/1/16" female, for 1/16" o.d. tubing, titanium	18-3855-01
	Union Valco F/F	11-0003-39
	Fill port	18-1127-66
Tubing	Inlet tubing Kit, ÄKTA start	29-0240-32
	Complete tubing kit, ÄKTA start	29-0240-34
	PEEK tubing i.d. 0.75 mm (1/16")	18-1112-53
	PEEK tubing i.d. 1.0 mm (1/16")	18-1115-83
	PEEK tubing, 2 m/i.d. 0.5 mm/o.d. 1/16"	18-1113-68
Cables	Mains cable, 115 V	19-2447-01
	Mains cable, 220 V	19-2448-01
	Cable Assy OTH USB	29-0240-36

#### 10 Reference information 10.5 Ordering information

Part	Accessory description	Code no.
Miscellaneous	Inlet filter assembly	18-1113-15
	Inlet filter set, 10 Filters/Nets	18-1114-42
	Screw lid GL45 kit, ÄKTA	11-0004-10
	Tubing cutter	18-1112-46
	Column clamp o.d. 10 to 21 mm	28-9563-19
	Short column holder	18-1113-17
	T-Slot holders	29-0240-38
	Buffer tray ÄKTA start	29-0240-39
	Accessory Box	29-0240-37
	Operating Instructions, printed	29-0270-57
	Maintenance Manual, printed	29-0603-08
	Injection kit	18-1110-89
Software	UNICORN start DVD, license access code and manual package	29-0187-51
Frac30	Frac30 Assembly	29-0230-51
	Drive sleeve	19-6067-02
	Tubing holder	18-6464-01
	Bowl Assembly, Frac30	29-0240-45
	Cable Assembly, Frac30	29-0240-65

## ÄKTA start spare parts

Item	Code no.
Packaging Kit for ÄKTA start	29-0320-87
Packaging Kit for Frac30	29-0337-03

#### 10 Reference information 10.5 Ordering information

#### Service tools

Item	Code no.
Torx driver T10	29-0031-71
Torx driver T20	28-9513-03
Flat screwdriver	56-4656-00

# 11 Appendix

#### About this chapter

This appendix presents a template for a *Report of the* **System performance method**. The Report has to be filled in with observations collected during the **System performance method**, which is performed either from ÄKTA start or from UNICORN start.

#### In this chapter

This chapter contains the following sections:

Section	See page
11.1 System Performance Report	276

# 11.1 System Performance Report

## Test performed from ÄKTA start

Time (min)	Activity	Check	Approved interval	Observations
0	Pump wash	<b>Wash valve</b> posi- tion	Mobile phase out through <b>Waste</b>	
1	1 ml/min, 0% B, flow through <b>Outlet valve</b> , <b>Waste</b> position	Back pressure	≤ 0.05 MPa	
2	Repeat <b>UV Auto zero</b>			
3	5 ml/min	Back pressure	0.06 to 0.2 MPa	
		UV level	± 10 mAU	
		Conductivity lev- el	± 1 mS/cm	
4	1 ml/min, <b>Sample valve</b> ,	Max. UV level	300 to 380 mAU	
Sample po	Sample position	Max. Conductivi- ty level	65 to 95 mS/cm	
7	1 ml/min, <b>Sample valve</b> , <b>Buffer</b> position			
10	Request switch Injection	Max. UV level	300 to 380 mAU	
	valve to <i>inject</i> position.	Max. Conductivi- ty level	65 to 95 mS/cm	
13	Request switch <b>Injection</b> <b>valve</b> back to <i>Load</i> posi- tion.			
15	Start gradient, 0 to100% B in 10 minutes, start fraction- ation/collection.			
19	End fractionation <sup>1</sup>	Weigh fraction no. 2, 3 and 4.	0.8 to 1.2 g	
		Max. diff. be- tween fractions	0.1 g	

Time (min)	Activity	Check	Approved interval	Observations
20	End collection <sup>2</sup>	Weigh beaker	4.2 to 5.8 g	
25	End gradient, stay at 100% B	Gradient	Straight, no negative dips.	
28	50% B	Gradient level <sup>3</sup>	45 to 55% B	
36	0% B (Re equilibration)			
41	End	Check all connec- tions for leakage	No leakages.	

1 With Fraction collector

2 Without Fraction collector

3 UV 50% B / UV 100% B

# Test performed from UNICORN start

Time (min)	Activity	Check	Approved inter- val	Observations
1	1 ml/min, 0% B, flow through <b>Outlet valve</b> waste position	Back pressure	≤ 0.05 MPa	
2	Repeat <b>UV Auto zero</b>			
3	5 ml/min	Back pressure	0.06 to 0.2 MPa	
15	Start gradient, 0 to 100% B in 10 minutes, start fraction- ation/collection.			
19	End fractionation <sup>1</sup>	Weigh fraction no. 2, 3 and 4.	0.8 to 1.2 g	
		Max. difference between fractions	0.1 g	
20	End collection <sup>2</sup>	Weigh beaker	4.2 to 5.8 g	
25	End gradient, stay at 100% B	Gradient <sup>3</sup>	Straight, no nega- tive dips.	
41	End	Check all connec- tions for leakage	No leakage.	

1 With Fraction collector

2 Without Fraction collector

3 UV 50%B / UV 100%B

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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

