ÄKTA™ start

System Cue Card

Contents

System Overview

ÄKTA start is a simple and modern liquid chromatography system intended 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 should not be used in any clinical procedures, or for diagnostic purposes.

The System Cue Card describes how to prepare the system for a chromatography run, start and monitor a run, and evaluate the result.

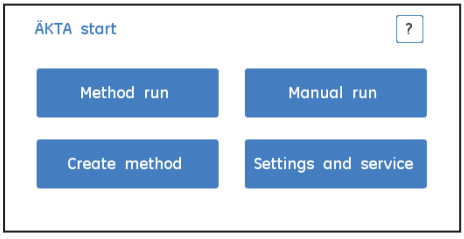
System overview

Part	Description
1	ÄKTA start
2	Frac30, Fraction collector
3	PC with UNICORN™ start software

Main features of ÄKTA start

- ÄKTA start is operated in two modes: 1) using Instrument Display (touch screen), and 2) using UNICORN start.
- ÄKTA start is offered with a dedicated Fraction collector, Frac30.
- Quick start methods available for easy purification of common tagged/untagged proteins.
- Method templates available for Affinity Chromatography (**AC**), Ion Exchange Chromatography (**IEX**), Gel Filtration (**GF**) and Desalting (**DS**).
- Predefined methods available for cleaning the system flow path, flow cells (UV and Conductivity) and testing the system performance.

Instrument Display - home screen



The ÄKTA start home screen displays four options for the user to select and perform operations from.

Method run: run predefined methods

Manual run: start and control the system in real time

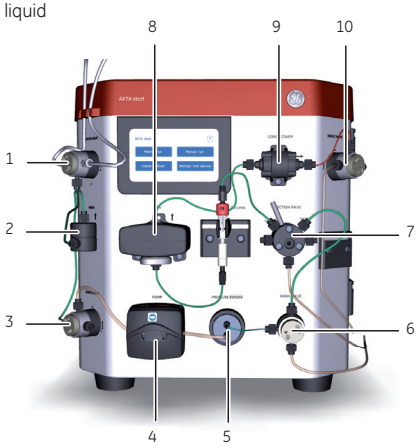
Create method: create, edit, import and delete methods

Settings and service: configure, calibrate, troubleshoot and diagnose modules

Instrument illustration

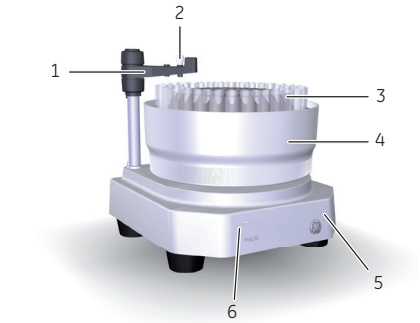
The illustration below shows the locations of the modules placed on the wet side of the instrument. The modules have the following functions:

- To deliver the liquid in the system flow path and divert the flow as required
- To monitor the UV absorbance and conductivity of the liquid



Part	Description	Part	Description
1	**Buffer valve**	6	**Wash valve**
2	**Mixer**	7	**Injection valve**
3	**Sample valve**	8	**UV** Monitor
4	**Pump**	9	**Conductivity** Monitor
5	**Pressure sensor**	10	**Outlet valve**

Frac30, Fraction collector

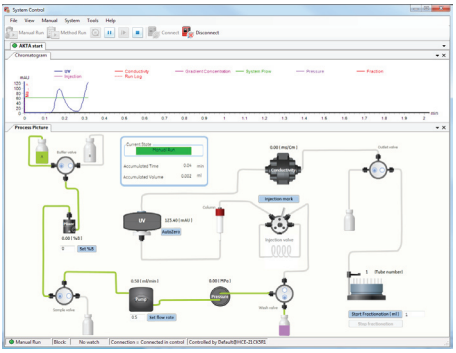


Part	Description	Part	Description
1	Dispenser arm	4	Bowl assembly
2	Tubing holder	5	Base unit
3	Collection tubes	6	Power indicator

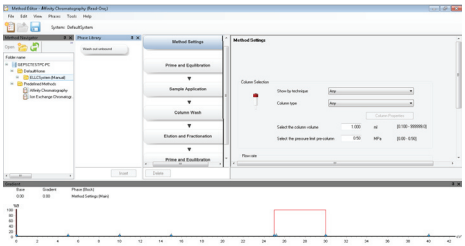
UNICORN start overview

UNICORN start is a software package for:

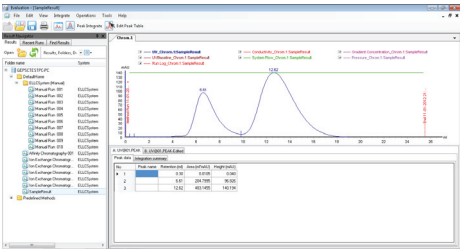
- Controlling the ÄKTA start instrument



- Creating and editing methods



- Evaluating and analyzing the results from ÄKTA start



Module	Features
System Control	Provides an intuitive and easy-to-use interface to control ÄKTA start. Performs and monitors manual chromatography runs. Performs and monitors automated predefined or user defined chromatography method runs. Performs system performance method runs.
Method Editor	Provides the flexibility to automate the chromatography runs. Allows creation of methods from predefined chromatography templates like Affinity, Gel Filtration, Ion Exchange and Desalting. Gives flexibility to create a customized method by dragging and dropping the chromatography phases such as Prime and Equilibration, Sample Application, Elution and Fractionation, etc. Allows exporting methods to a USB flash drive for importing them into ÄKTA start.
Evaluation	Allows viewing and presentation of results, including creation of PDF reports. Allows various evaluation operations on curves and chromatograms including comparison, peak integration etc. Allows importing results from ÄKTA start and exporting results to other formats.
Adminis-tration	Allows administration of the UNICORN start database for backup, restore, archive and retrieve operations. Allows reviewing of UNICORN start and system logs.

Safety

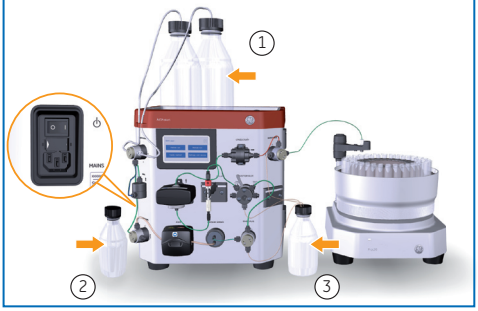
Read *ÄKTA start Operating Instructions* before using the instrument.

System preparation

Preparation workflow

Instruction

1. Place the buffer bottles (A & B) in the buffer tray and immerse the buffer inlet tubing (A & B) in the respective buffer bottles (1).
2. Place the sample inlet tubing in the sample container or prefill the sample loop with the sample (2).
3. Place all the waste tubing in the waste bottle (3).
4. Make sure the pump tubing is installed in the Pump.
5. Fit the collection tubing to the Fraction collector.
6. Switch on the instrument.
7. Calibrate the instrument modules.



Connect a PC to ÄKTA start

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

Instruction

1. Connect power to the computer and monitor and start the computer.
2. Connect the PC Connection Cable between the connector marked as **PC Connection** at the back of ÄKTA start and a USB port on the computer.
3. Launch UNICORN start and connect to ÄKTA start.

Connect Frac30 to ÄKTA start

Note: *Frac30 should not be connected or disconnected when ÄKTA start is switched ON.*

Instruction

1. Connect the Frac30 Cable between the intended ports at the back of Frac30 and ÄKTA start.
2. Switch on ÄKTA start.
3. Enable connection of Frac30 from Instrument Display.

Fractionation

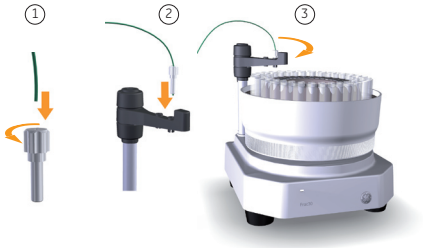
For many purification schemes it is important to collect fractions of the eluent. ÄKTA start offers the fractionation options presented in the table below.

Instrument configuration	Fractionation options
ÄKTA start + UNICORN start + Frac30	• Fixed volume fractionation • Peak fractionation ◊ Level based ◊ Slope based
ÄKTA start + UNICORN start	• Single Peak collection ◊ Level based
ÄKTA start + Frac30	• Fixed volume fractionation
ÄKTA start	• Collection of elution volume

Prepare the Fraction collector

Instruction

1. Place a sufficient number of collection tubes (1.5 ml/ 5 ml/15 ml) in the Bowl assembly.
2. Fit the outlet tubing into the tubing holder (1).
3. Fit the tubing holder into the corresponding port on the Dispenser arm (2).
4. Gently move Dispenser arm to dispensing position (3).
5. Enable Frac30 from Instrument Display (***Home*** > ***Settings and service***>***Fraction collector***>***Enable Frac***).
6. Set the delay volume (***Home***>***Settings and service***> ***System***>***Delay volume setting***).



Calibration

Calibrate the modules in the order described below to ensure optimal performance of the system. For instructions, refer to *ÄKTA start Operating Instructions.*

Instruction

Wash the entire flow path of the instrument with demineralized (DM) water, then calibrate the modules.

Display
Touch Screen calibration

Pressure Sensor
Zero Offset

Pump
Flow rate calibration

UV
UV LED calibration

Conductivity
Temp. sensor calibration
Cond. flow cell calibration

A calibration guide for when to calibrate the modules is available in *ÄKTA start Maintenance Cue Card.*

Note: *At delivery, the instruments are pre-calibrated. Based on the results of the system performance test the user can decide if re-calibrations are required.*

Prefill the flow path

Cleaning/prefilling the flow path including all inlets and outlets is performed using the system methods listed below. For instructions on how to prime the sample inlet tubing and sample loop, refer to *ÄKTA start Operating Instructions*, section *5.7 Sample application*. For instructions on how to perform system methods, refer to *ÄKTA start Maintenance Cue Card*.

- ***Pump wash A/Pump wash B*** (prime buffer A & B inlets)
- ***Washout fractionation tubing*** (clean fractionation tubing)
- ***Column preparation*** (prepare/equilibrate a column)
- ***System cleaning*** (clean the flow path using 1 M NaOH and DM water)

The flow path can also be prefilled by using a manual run.

Connect a column

For detailed instructions on how to connect a column, refer to *ÄKTA start Operating Instructions*.

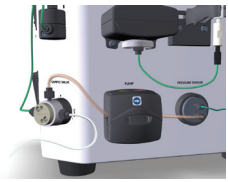
Instruction

1. Depending on column dimension, choose the appropriate location to place the column. The column holder rails are indicated in the image below.
2. Attach an appropriate column holder to the column holder rail on the instrument.
3. Mount the column on the union connector if the column type requires a union.
4. Fix the column to the column holder, then connect the tubing to the top and to the bottom of the column.

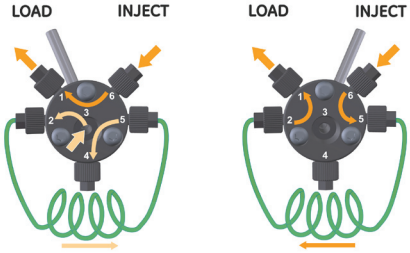
Sample application

The sample can be applied to the column in two ways:

- **via Pump**: The sample can be applied directly from a sample container through the **Sample valve** as shown in the image below. It allows application of large volume of sample (> 5 ml) and facilitates unattended operation.

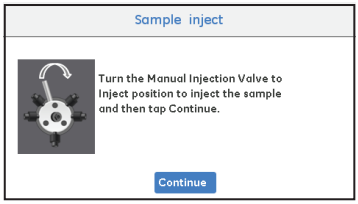


- **via Loop**: A sample loop allows the injection of small sample volumes (25 µl - 5 ml) onto the column. For larger sample volumes (10 - 150 ml), use a Superloop™. The loop has to be connected to the **Injection valve** (ports **2** and **5**) as depicted below.

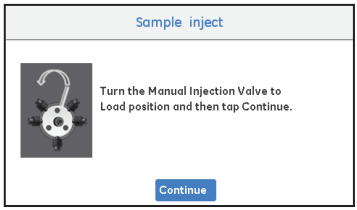


To apply the sample from a loop:

- Fill the loop with sample through port **3** of the **Injection valve** (in **Load** position).
- Switch the **Injection valve** to **Inject** position when prompted by the message below. Tap ***Continue***. The sample is loaded onto the column only when the **Injection valve** is in **Inject** position during the run.



- Switch the **Injection valve** to **Load** position when prompted by the message below. Tap ***Continue***.



Note: *The system is in hold state while injecting the sample from loop. To ensure that the injection mark coincides with the injection event, acknowledge the message immediately after the action is performed.*

Note: *For AC/IEX methods, when loading the sample through a loop it is advisable to empty the loop with 3× the loop volume to achieve good sample recovery.*

Cold room operations

Preparation

1. Place the ÄKTA start instrument in the cold room.
2. Allow the instrument to stabilize at the temperature of the cold room.

3. Tighten all connections and pump DM water through the system to check for leaks.
4. Tighten any leaking connector.

Removal from cold room

1. Switch off the instrument and disconnect power cable.
2. Loosen all connections to prevent them sticking when the system returns to room temperature.
3. Allow the instrument to stabilize at room temperature.
4. Tighten all connections and pump DM water through system to check for leaks.

Column preparation

The ***Column preparation*** method is used for:

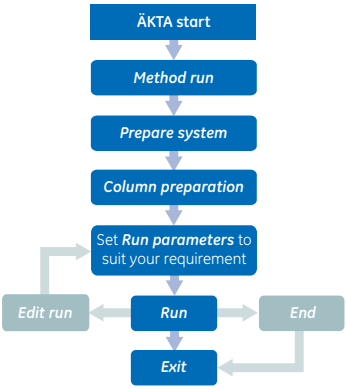
- Preparing a new column
- Equilibrating the column.

Note: *Equilibrate columns before starting a new run.*

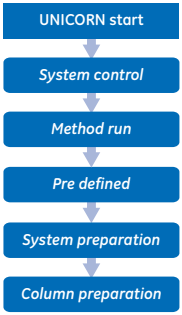
Instruction

1. Immerse the buffer inlet tubing into the buffer used in the chromatographic run.
2. Connect the column in the flow path.

Operation from the Instrument Display



Operation from UNICORN start



Starting a run

Chromatography runs can be performed either by running predefined methods, or by using manual operations.

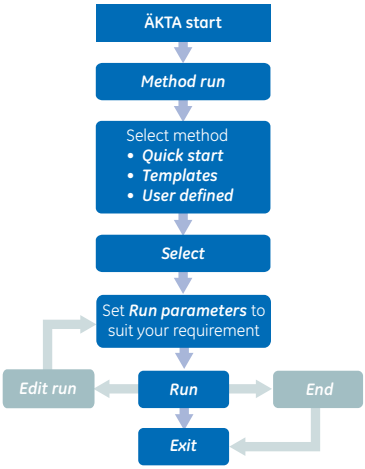
Perform a method run

The following method types are available with ÄKTA start to perform chromatography runs:

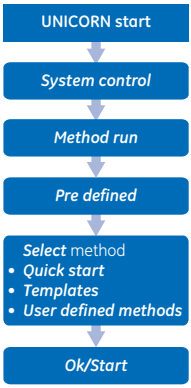
- ***Quick start***
- ***Templates***
- ***User defined***

Follow the workflows below to run predefined methods.

Operation from the Instrument Display



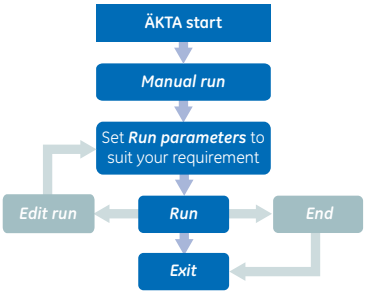
Operation from UNICORN start



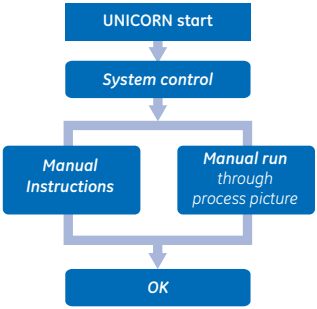
Perform a manual run

Follow the workflows below to run the system manually.

Operation from the Instrument Display

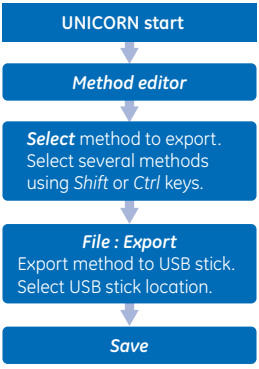


Operation from UNICORN start



Export a method from UNICORN start

Follow the workflow below to export a method from UNICORN start to a USB memory stick.

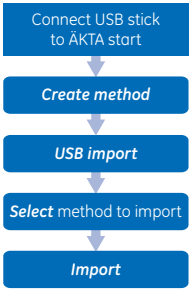


For detailed instructions on exporting methods, refer to the *UNICORN start 1.0 User Manual*.

Import a method to ÄKTA start

Transfer the UNICORN method to USB memory stick, then perform the following operations to import a method.

The imported methods are saved in **User defined** methods menu on the ÄKTA start.



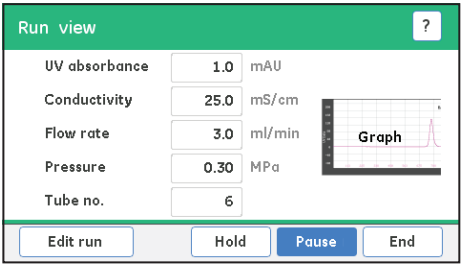
Monitoring a run

The user can monitor/control the ongoing run either from the ÄKTA start Instrument Display or from UNICORN start.

Operation from the Instrument Display

The following options are available from **Run view** screen:

- View real time values: UV absorbance, Conductivity, Flow rate, Pressure, Tube number
- ***Graph*** (view the chromatogram)
- ***Edit run*** (edit the run parameters of ongoing run)
- ***Hold*** (temporarily holds the run, with set flow rate, valve positions, and B concentration sustained)
- ***Pause*** (pauses the run by stopping the pump)
- ***End*** (terminates the current run)



Operation from UNICORN start

System Control module is used to start, view, and control a run. The instrument is controlled via simple clicks on the flow path depicted in the *process picture*, e.g. to turn the valves, set flow rates, change B concentration and start/stop fractionation.

Evaluating a run

Save Result as BMP image

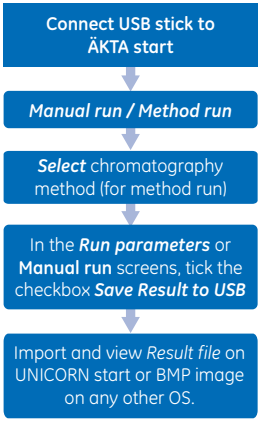
The system generates a BMP file of the result which can be viewed under any OS that has an image viewer supporting BMP. The chromatogram is plotted as UV abs (mAU) vs Volume (ml), along with Fractionation marks.

Note: *BMP file is generated only for the last 4 hours of run.*

Evaluating a run using UNICORN start

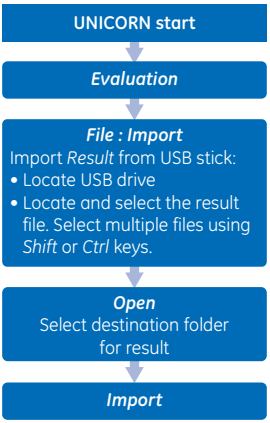
Evaluation module of UNICORN start is used to manage and evaluate the results from chromatography runs. For instructions to evaluate results that are generated using ÄKTA start, refer to the *UNICORN start 1.0 User Manual*.

Save Result to USB memory stick



Import Result to UNICORN start

The result file stored on the USB stick can be imported to UNICORN start for analysis, printing and generating a report.



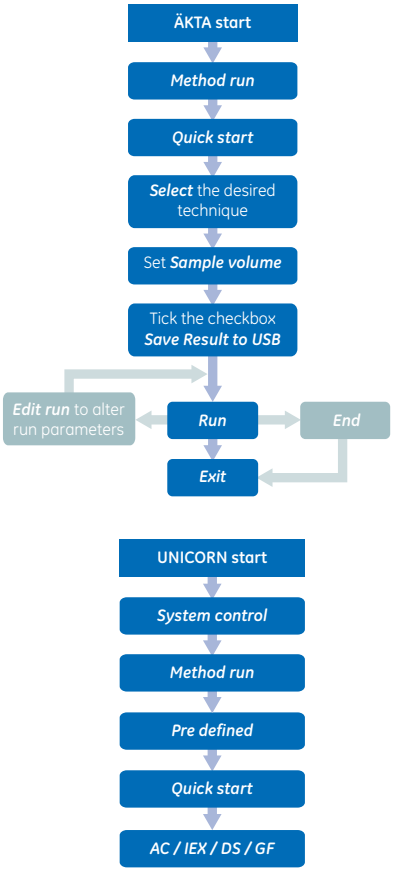
Note: *The result files will be saved in the GE folder which is automatically created by the instrument once the USB memory stick is plugged in. Only 10 results can be stored in the GE folder. To save further results, transfer the result files to another folder, PC or rename the GE folder.*

Quick start methods

Quick start contains "ready to run" methods like Affinity, Ion exchange, Gel filtration and Desalting. The run parameters are pre-set in the method. Details of *sample volume* need to be provided before starting the run.

- If required, the run parameters can be changed (***Edit run*** in **Run view** screen) when the run is in progress.
- Use appropriate columns as indicated in the template names. E.g., use 1 ml HiTrap™ column in case of selecting the Quick start *AC/IEX step 1 ml HiTrap*, or 5 ml column when selecting *AC/IEX step 5 ml HiTrap*.

Quick start workflow



The pre-set run parameters for each technique are listed in the table below.

Parameter	AC step 1 ml HiTrap	AC step 5 ml HiTrap	DS 5 ml HiTrap	DS 53 ml HiPrep™	IEX step 1 ml HiTrap	IEX step 5 ml HiTrap	IEX gradient 1 ml HiTrap	IEX gradient 5 ml HiTrap	GF 16/60 HiPrep
Column Volume (ml)	1.0	5.0	5.0	53.0	1.0	5.0	1.0	5.0	120
Flow rate (ml/min)	1.0	5.0	3.0	5.0	1.0	5.0	1.0	5.0	1.0
Pressure Limit (MPa)	0.3	0.3	0.3	0.2	0.3	0.3	0.3	0.3	0.2
Save Result to USB *	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Sample From	Pump	Pump	Pump	Pump	Pump	Pump	Pump	Pump	Pump
Sample Volume (ml) **	0.1**	0.1**	0.1**	0.1**	0.1**	0.1**	0.1**	0.1**	0.1**
Equilibration Volume (CV)	5.0	5.0	3.0	3.0	5.0	5.0	5.0	5.0	0.2
Wash Unbound Volume (CV)	15.0	15.0	NA	NA	10.0	10.0	10.0	10.0	NA
Elution Option	Isocratic	Isocratic	Isocratic	Isocratic	Isocratic	Isocratic	Gradient	Gradient	Isocratic
Target B Conc (%)	100	100	NA	NA	100	100	100	100	NA
Elution Volume (CV)	5.0	5.0	1.2	1.2	5.0	5.0	20.0	20.0	1.2
Hold at 100% B (CV)	NA	NA	NA	NA	NA	NA	5.0	5.0	NA
Re-Equilibration Volume (CV)	5.0	5.0	NA	NA	5.0	5.0	5.0	5.0	NA
Fractionation Volume (ml)	1.0	3.0	0.5	3.0	1.0	3.0	1.0	5.0	4.0

** the results will be saved only if the USB memory stick is inserted before opening the method.*
*** variable parameter*

Note: *The sample can be loaded* ***only*** *through Pump.*

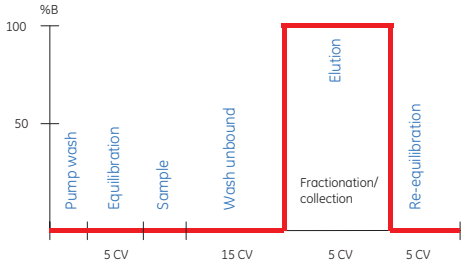
Quick start methods description

The "ready to run" methods available with ÄKTA start are presented below. The length of each step or phase is indicated on the diagrams as column volumes (CV).

Sample: variable volume to be set by the user.
Pump wash:
- for AC/IEX: 5 ml Buffer B, then 5 ml of Buffer A wash.
- for DS/GF: 5ml of Buffer A wash.

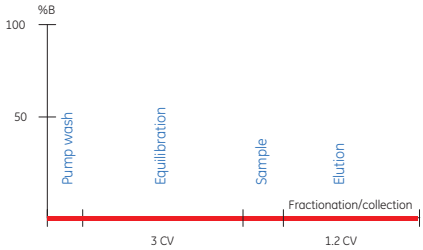
AC Step 1 ml/5 ml HiTrap - Affinity Chromatography

- Commonly used for purification of tagged proteins, e.g., His tag, GST tag, Mab tagged proteins, etc.
- The bound proteins are eluted in a single step using single elution buffer.



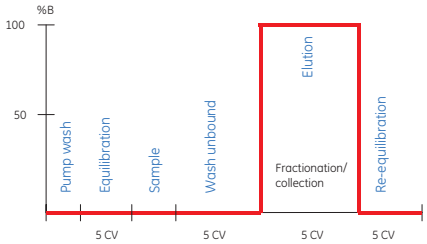
DS 5 ml HiTrap/53 ml HiPrep - Desalting/Buffer Exchange Chromatography

- The proteins are eluted in a single step using single elution buffer.
- Make sure to load the recommended sample volume.



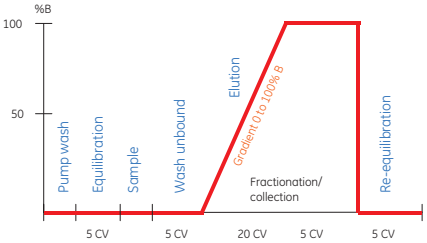
IEX Step 1 ml/5 ml HiTrap - Ion Exchange Chromatography

The bound proteins are eluted in a single step using single elution buffer.



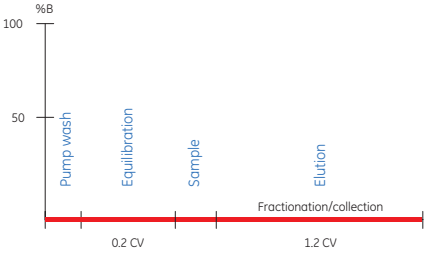
IEX Gradient 1 ml/5 ml HiTrap - Ion Exchange Chromatography

The bound proteins are eluted using two buffers with linear increase in the concentration of buffer B over a specified time, followed by a step with 100% B concentration.



GF 16/60 HiPrep - Gel Filtration Chromatography

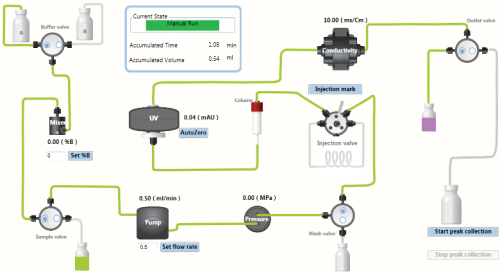
- The proteins are eluted in a single step using single elution buffer.
- The column needs to be pre-equilibrated before the start of the run.



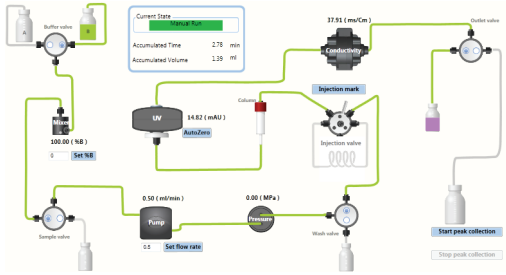
Flow charts

The active flow path is depicted in Green color.

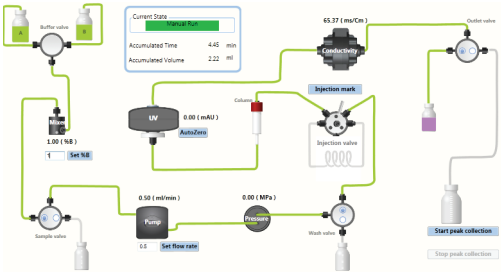
A. Flow path details for flow of 100% buffer A through Outlet valve to waste



B. Flow path details for flow of 100% buffer B through Outlet valve to waste

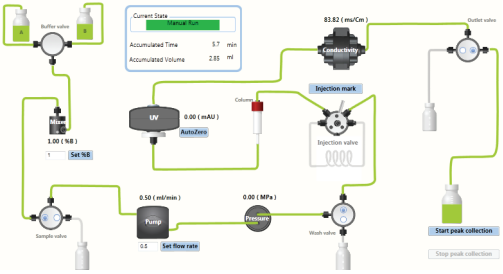


C. Flow path details during gradient operation at specified B concentration



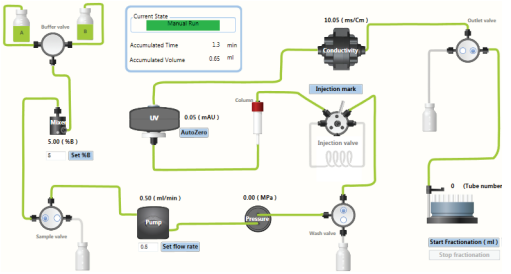
D. Flow path details, Outlet valve collection

Note: *Fraction collector is disabled.*

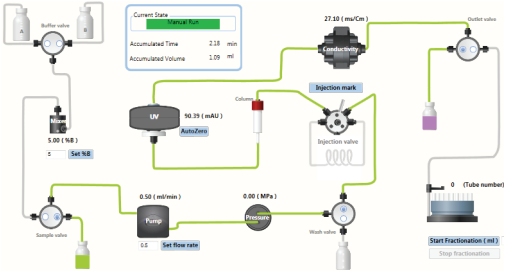


E. Flow path details, fraction collection

Note: *Fraction collector is enabled.*



F. Flow path details while loading Sample through the Pump



Getting help

ÄKTA start Instrument Display Help

- Accessible from every screen on the Instrument Display by tapping the *question mark* [?] located in the upper right corner.
- The Display Help text provides information about the content of the current screen or refers to more detailed documentation.

UNICORN start Online Help

- Dialog descriptions of instructions needed to operate UNICORN start and analyze chromatography run data.
- Accessible within UNICORN start by pressing ***F1*** or using the ***Help*** menu.
- The online help covers the system control, method editor and result evaluation functions in UNICORN start.

The user documentation available with ÄKTA start is listed in section *Reference information*.

Reference information

Ordering information

For ordering information visit www.gelifesciences.com/AKTA.

Selecting protocols, columns and buffer

Column supported	Volume (ml)	Method
HisTrap™ FF	1 or 5	AC
HisTrap FF crude	1 or 5	
HisTrap HP	1 or 5	
GSTrap™ FF	1 or 5	
GSTrap HP	1 or 5	
GSTrap 4B	1 or 5	
HiTrap MabSelect™	1 or 5	
HiTrap MabSelect SuRe™	1 or 5	
HiTrap rProtein A FF	1 or 5	
HiTrap Protein A HP	1 or 5	
HiTrap Protein G HP	1 or 5	
HiTrap Protein L	1 or 5	
HiTrap IgM Purification HP	1	
HiTrap IgY Purification HP	5	
HiTrap Heparin HP	1 or 5	
HiTrap Blue HP	1 or 5	
HiTrap Con A 4B	1 or 5	
HiTrap TALON® crude	1 or 5	
HiTrap IMAC HP	1 or 5	
HiTrap IMAC FF	1 or 5	
HiTrap Chelating HP	1 or 5	
HiTrap Streptavidin HP	1	
HiTrap Benzamidine FF	1 or 5	
HiTrap NHS-Activated HP	1 or 5	
StrepTrap™ HP	1 or 5	
MBPTrap™ HP	1 or 5	
HiPrep 16/60 Sephacryl S-100 HR	120	GF
HiPrep 16/60 Sephacryl S-200 HR	120	
HiPrep 16/60 Sephacryl S-300 HR	120	
HiPrep 16/60 Sephacryl S-400 HR	120	
HiPrep 16/60 Sephacryl S-500 HR	120	
HiTrap Desalting	5	DS
HiPrep 26/10 Desalting	53	
HiTrap IEX Selection Kit	1	IEX
HiTrap SP HP	1 or 5	
HiTrap Q HP	1 or 5	
HiTrap DEAE FF	1 or 5	
HiTrap CM FF	1 or 5	
HiTrap Q FF	1 or 5	
HiTrap SP FF	1 or 5	

Buffer selection

Buffer types needed

For AC:	• Binding buffer • Elution buffer • Wash buffer (optional)
For DS:	• One buffer type per run
For IEX:	• Binding buffer • Elution buffer • Wash buffer (optional)
For GF:	• One buffer type per run

AC buffer suggestions for His-tagged proteins

If performing...	*suggested buffer*
binding using HisTrap or HiTrap Chelating	50 mM Tris-HCl pH 7.5, 0.5 M NaCl, 20-40 mM imidazole[1]
wash	50 mM Tris-HCl pH 7.5, 0.5 M NaCl, 100 mM imidazole
step elution	50 mM Tris-HCl pH 7.5, 0.5 M NaCl, 500 mM imidazole

[1] *The imidazole concentration is protein dependent.*

AC buffer suggestions for GST-tagged proteins

If performing...	*suggested buffer*
binding using GSTrap 4B, HP or FF	50 mM Tris-HCl pH 7.5, 150 mM NaCl, 1 mM DTT
step elution	50 mM Tris-HCl, 10 mM reduced gluthathione, pH 8

DS buffer suggestions

If performing...	*suggested buffer*
Buffer exchange/ desalting	50 mM Tris-HCl pH 8.0

IEX buffer suggestions

If performing...	*suggested buffer (depends on the pI of the protein)*
binding to AIEX	50 mM Tris-HCl pH 8.0
binding to CIEX	20 mM MES pH 6.0
elution from AIEX	50 mM Tris-HCl, pH 8.0, 1 M NaCl
elution from CIEX	20 mM MES pH 6.0, 1 M NaCl

GF buffer suggestions

If performing...	*suggested buffer*
Buffer exchange/ size exclusion chromatography	a suitable buffer, e.g. 50 mM Tris-HCl pH 7.5, 150 mM NaCl

Health and Safety Declaration Form

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 *ÄKTA start Operating Instructions*, *Section 10.4 Health and Safety Declaration Form*, or print it from the PDF file available on the User Documentation CD.

User documentation for ÄKTA start

Documentation	Book	PDF	Web	Online
Operating Instructions	-	•	•	-
Maintenance Manual	-	•	•	-
System Cue Card	•	•	•	-
Maintenance Cue Card	•	•	•	-
UNICORN start 1.0 User Manual	-	•	•	-
UNICORN start Online Help	-	-	-	•
Display Help	-	-	-	•

For local office contact information, visit
www.gelifesciences.com/contact

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