ÄKTAxpress MAb

Cue Cards

System overview

Column block - for purification runs

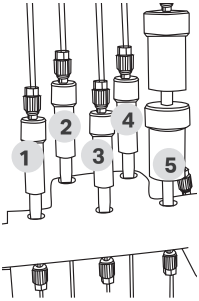
1 – Affinity column 1

2 – Affinity column 2

3 – Affinity column 3

4 – Affinity column 4 *or*
 – Desalting column[1] *or*
 – Gel filtration column[1]

5 – Desalting column *or*
 – Gel filtration column



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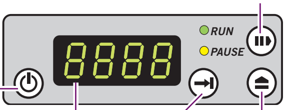
[1] *When using a protocol with one desalting or gel filtration column per sample.*

Control panel

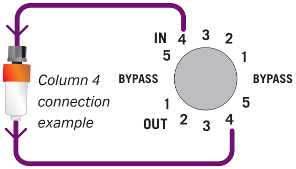


Sled for microplate

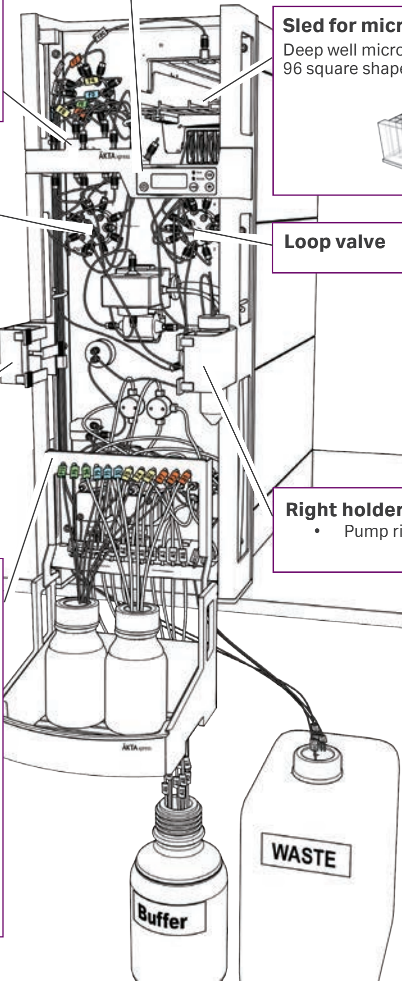
Deep well microplate, 96 square shaped wells

Column valve

Column connection



Loop valve



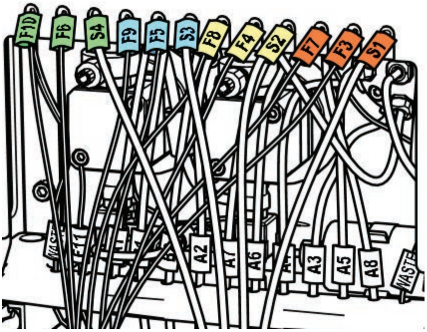
Left holder

- Gel filtration column
- HiPrep™ Desalting column

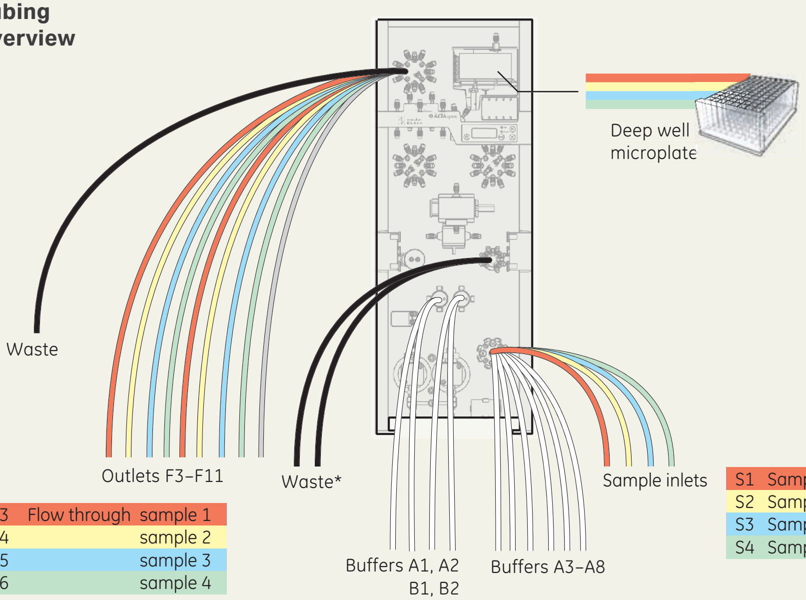
Right holder

- Pump rinsing solution

Recommended tubing positions



Tubing overview



F3	Flow through	sample 1
F4		sample 2
F5		sample 3
F6		sample 4

F7	Nonselected peaks,	sample 1
F8		sample 2
F9		sample 3
F10		sample 4

F11	Not used

S1	Sample 1
S2	Sample 2
S3	Sample 3
S4	Sample 4

Grouping buffer containers

If the same buffer is used for several systems, one buffer container can feed several systems.

For more information about buffer inlets, see page 7.

*	Waste	From injection valve port 2 (manual sample injection)
*	Waste	From injection valve port 3 (e.g. system wash)

Detailed flow charts

See "Flow charts" on page 18.

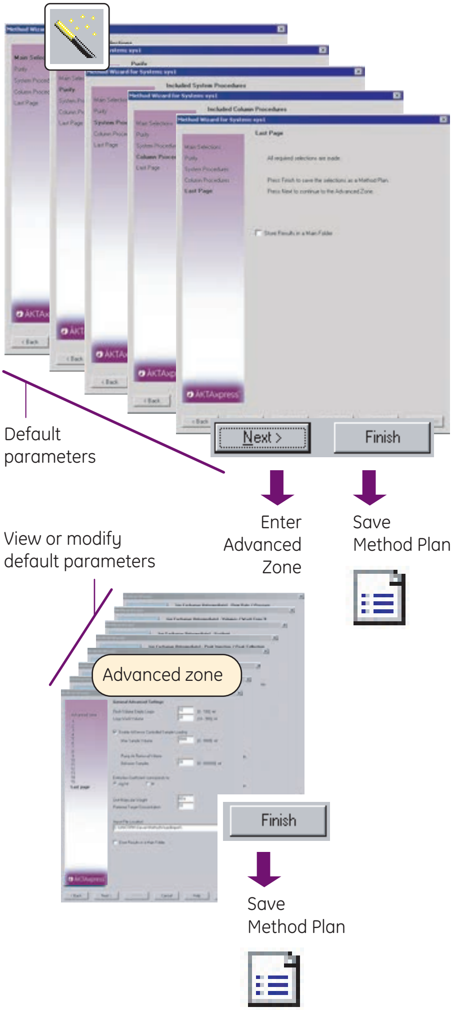
Safety

Read the ÄKTAxpress Operating Instructions before using the system.

UNICORN for ÄKTAxpress MAb, overview

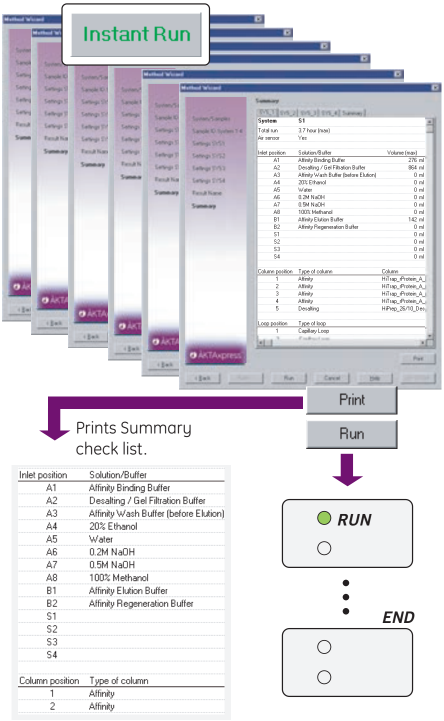
Method Editor wizard

The Method Editor wizard is used to create Method plans.



System Control wizard

The System Control wizard is used to start runs based on Method Plans. Input information: Systems to use, number of samples per system and sample information.

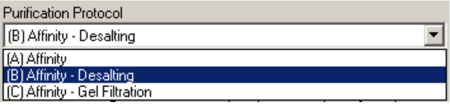


Inlet position	Solution/Buffer
A1	Affinity Binding Buffer
A2	Desalting / Gel Filtration Buffer
A3	Affinity Wash Buffer (before Elution)
A4	20% Ethanol
A5	Water
A6	0.2M NaOH
A7	0.5M NaOH
A8	100% Methanol
B1	Affinity Elution Buffer
B2	Affinity Regeneration Buffer
S1	
S2	
S3	
S4	

Column position	Type of column
1	Affinity
2	Affinity

1 Selecting protocols, columns and buffers

Protocols supported



Purification Protocol

(B) Affinity - Desalting

(A) Affinity

(B) Affinity - Desalting

(C) Affinity - Gel Filtration

For all protocols, individual affinity columns for each sample are always used.

For protocol B and C it is possible to select either

- the same desalting/gel filtration column for all samples (max 4 samples), or
- an individual desalting/gel filtration column for each sample (max 2 samples).

In addition to the basic method steps, it is possible to add system and/or column procedures in the purification method, e.g. system and column CIP.

System and column procedures can also be run as separate preparation or maintenance methods.

Columns supported

Column	**Volume** [ml]
HiTrap™ Protein A HP	1 or 5
HiTrap Protein G HP	1 or 5
HiTrap rProtein A FF	1 or 5
HiTrap MabSelect SuRe™	1 or 5
Any HiTrap[1]	1 or 5
2 × HiTrap Desalting	10
HiPrep 26/10 Desalting	53
HiLoad™ 16/600 Superdex™ 75 or 200 prep grade	120
HiLoad 26/600[2] Superdex 75 or 200 prep grade	320

[1] *Any HiTrap means that any HiTrap Affinity column can be used.*

[2] *An optional large column holder is available for the HiLoad 26/600 gel filtration column.*

For ordering numbers see "Ordering information" on page 25.

Column selection

Affinity columns

1 or 5 ml column

- Depends on required yield.
- For large sample volumes, use a 5 ml column to save time.
- To increase protein purity, overload the column.

Desalting columns

HiTrap or HiPrep

- Depends on loading volumes:
  - Up to 3 ml: Use 2 × HiTrap Desalting.
  - Up to 13 ml[3]: Use HiPrep Desalting.

Gel filtration columns

Superdex 75 or 200

- Separation range:
  - 3–70 kDa: Use Superdex 75 prep grade.
  - 10–600 kDa: Use Superdex 200 prep grade.

HiLoad 16/600 or 26/600[4]

- Loading volume:
  - up to 5 ml: Use HiLoad 16/600.
  - up to 13 ml[3]: Use HiLoad 26/600[4].

[3] *If you are expecting peaks larger than 7.5 ml from the affinity step, it is recommended to use a double loop, see "Option for large peaks" on page 10*

[4] *An optional large column holder is available for the HiLoad 26/600 gel filtration column.*

Column positions

	Column position					**Max no. of**
	1	**2**	**3**	**4**	**5**	**samples**
Protocol						
A	AC	AC	AC	AC	-	4
B (same DS for all samples)	AC	AC	AC	AC	DS	4
B (individual DS for all samples)	AC	AC	-	DS	DS	2
C (same GF for all samples)	AC	AC	AC	AC	GF	4
C (individual GF for all samples)	AC	AC	-	GF	GF	2

AC	*Affinity chromatography*
DS	*Desalting*
GF	*Gel filtration*

Typical run times

To estimate the total run time, add the typical run time for the selected protocol and the selected add-on procedure/s.

Typical run times for the different protocols

Protocol	No. of samples	Typical run time[1] RT[2] (h)	 CR[2] (h)
A	- Affinity (AC)	1	1.0
1.0			
	4	2.5	3.0
B - AC-DS	1	1.0	1.0
common DS-columns	4	3.5	4.0
B - AC-DS	1	1.0	1.0
individual DS-columns	2	2.0	2.5
C - AC-GF	1	2.5	3.0
common GF-columns	4	9.5	11.5
C - AC-GF	1	2.5	3.0
individual GF-columns	2	5.0	6.0

[1] *Run times are approximate and with default settings. Sample loading time is not included.*

[2] *RT=Room Temperature, CR=Cold Room*

Typical run times for add-on system procedures

SYSTEM PROCEDURES	Typical run time[1] RT[2] (min)	 CR[2] (min)
CIP system between samples	50	50
(4 samples)		
Fill system with ethanol	10	10
CIP system, then fill with ethanol20	20	
CIP system, then fill with buffer	10	10

Typical run times for add-on column procedures

COLUMN PROCEDURES	Typical run time[1] RT[2] (min)/ column	 CR[2] (min)/ column
Preparation		
Affinity column equilibration	5	10
Affinity blank run, 1 ml column	25	30
Affinity blank run, 5 ml column	15	20
Remove EtOH before preparation	5	5
HiPrep Desalting,		
column equilibration	15	15
remove EtOH before preparation	20	25
2 × HiTrap Desalting,		
column equilibration	5	10
remove EtOH before preparation	5	5
Gel filtration column equilibration	2.5 h	3.5 h
Between samples		
Affinity column regeneration	10	10
Affinity column reequilibration	5	5
CIP HiPrep Desalting	50	60
CIP 2 × HiTrap Desalting	15	20
CIP Gel filtration column	5.5 h	6 h
Post run		
Fill columns with ethanol		
HiTrap Affinity	10	10
HiPrep Desalting	30	35
2 × HiTrap Desalting	10	10
Gel filtration	3 h	3.5 h
CIP columns, then fill with ethanol		
HiTrap MabSelect SuRe	20	20
HiPrep Desalting	1.5 h	1.5 h
2 × HiTrap Desalting	20	20
Gel filtration	8 h	9.5 h
CIP columns, then reequilibrate		
HiTrap MabSelect SuRe	10	10
HiPrep Desalting	45	1 h
2 × HiTrap Desalting	10	10
Gel filtration	5 h	6 h

Buffers and solutions

AC buffer suggestions

When performing...	*using AC columns...*	*suggested buffer:*
binding *te*	*HiTrap MabSelect SuRe*	*20 mM sodium phospha-* *150 mM NaCl, pH 7.2*
binding *te*	*HiTrap Protein A HP* *HiTrap rProtein A FF* *HiTrap Protein G HP*	*20 mM sodium phospha-* *pH 7.0*
binding *te* *with high salt binding buffer*	*HiTrap MabSelect SuRe* *HiTrap Protein A HP* *HiTrap rProtein A FF*	*20 mM sodium phospha-* *3 M NaCl, pH 7.0*
elution	*HiTrap MabSelect SuRe* *HiTrap Protein A HP* *HiTrap rProtein A FF*	*0.1 M sodium citrate, pH 3-5*
elution	*HiTrap Protein G HP*	*0.1 M glysine-HCl, pH 2.7*
CIP[1]	*HiTrap MabSelect SuRe*	*0.2/0.5 M NaOH*
storage	*HiTrap MabSelect SuRe* *HiTrap Protein A HP* *HiTrap rProtein A FF* *HiTrap Protein G HP*	*20% ethanol*

[1] *CIP of other columns than HiTrap MabSelect SuRe must be performed in a maintain method, see page 17. CIP of affinity*

Extra wash before elution

An extra wash step can be peformed to remove weakly bound contaminants before elution . Use a buffer with lower pH than the binding buffer, but not too close to elution pH, since this might cause pre-elution of the target protein.

Regeneration after elution

Strong binders could be removed by regeneration of the affinity column with extra low pH buffer.

Tip

Use high purity liquids

For best purification results, use high purity deionized water and chemicals. Filtering of liquids through a 0.45 µm filter and degassing the liquids is recommended.

Tip

Buffer volumes required for a run

See “Buffer volumes required” on page 12.

DS buffer suggestions

If for example...	*suggested buffer:*
preparing the protein for further studies	PBS, pH 7.4[2]
performing CIP	0.2 M NaOH
column storage	20% ethanol

GF buffer suggestions

If for example...	*suggested buffer:*
preparing the protein for further studies	PBS, pH 7.4[2]
performing CIP	0.5 M NaOH
column storage	20% ethanol

[2] *The buffer should be selected to meet stability requirements for the specific antibody.*

Tip

Neutralise eluted samples

If using protocol A, the deep well microplate in the fraction collector can be prefilled with a neutralizing buffer so that the final pH of the eluted sample is approximately neutral.
Add e.g. 60-200 µl of 1M Tris-HCl, pH 9.0 per well before starting the run.

Note: *Decrease the fractionation volume in the Advanced zone to avoid overfilling of the microplate.*

Inlets

Buffer inlets	
A1	Affinity Binding Buffer
A2	Desalting/Gel Filtration Buffer
A3	Affinity Wash Buffer (before elution)
A4	20% Ethanol
A5	Water
A6	0.2 M NaOH
A7	0.5 M NaOH
A8	100% Methanol
B1	Affinity Elution Buffer
B2	Affinity Wash Buffer (after Elution)

Sample inlets	
S1	Sample 1
S2	Sample 2
S3	Sample 3
S4	Sample 4

2 System and column preparations

Before starting

- Fix the tubing, for example, by:
  – Fitting the inlet filter weights on the tubing, or
  – Running the tubing through the rubber cap included in the accessory kit.

Purging procedures

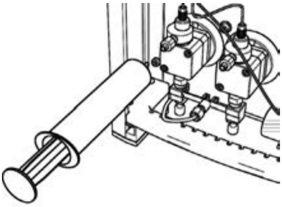
Before starting a run, the pump and inlet tubing must be filled with liquid.
There are diffrent ways to purge the pump:

- If the inlet tubing is empty, *Manual filling of the inlet tubing* is necessary (see below).
- If the system has been unused for a week or longer, or the pump has been run dry, it is recommended to *Purge pump with methanol*. This procedure can be included in a **Prepare** method (see below).
- To remove small amounts of air, or to change buffer, include *Fill buffer inlet tubing* procedures in
  - a **Prepare** method, or
  - a **Purify** method (see "Creating a method plan for the protein purification" on page 11).

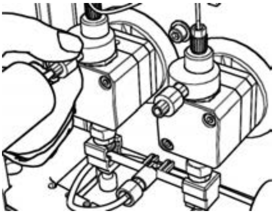
Manual filling of the inlet tubing

Filling inlet tubing A1–A8, S1–S4 and B1-B2

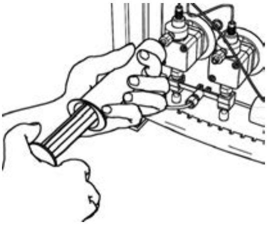
- Immerse tubing in the liquid containers.
- Set the inlet valve to the appropriate position in **System Control : Manual : Flowpath**.
- Connect a syringe to the purge valve.



- Turn the purge valve to open it.



- Slowly draw solution into the syringe. When fluid starts to enter the syringe, continue to draw a few milliliters before closing the purge valve. Check that there is no visible air left in the tubing.



- Repeat the procedure for **A2–A8** and **S1–S4**.
- To fill inlet **B1** and **B2**:
  - Start a low flow in **System Control : Manual : Pump : Flow**.
  – Set inlet valve to **B1** or **B2**:
  In **System Control : Manual : Pump : Gradient** select **Target 100%B** and **Mode A1/B1** to fill **B1** or **Mode A2/B2** to fill **B2**. Wait for a clicking sound before starting the purging procedure.
- When all inlets are filled, click **End**.

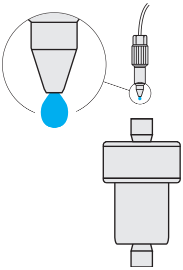
Connecting columns

For column positions, see "Column positions" on page 5

Air remaining in the system may be removed by purging the pump and system manually by selecting **Pump Wash** and **System Wash**.

- Immerse A1 tubing in the buffer to be used.
- Select **System Control : Manual : PumpWash** or **SystemWash**.

Drop-to-drop



To avoid air bubbles, use the drop-to-drop procedure when connecting columns:

- Start the pump with a low flow rate.
- Set the column valve to the required position.
- Fix the tubing to the column drop-to-drop.

For specification of buffers and inlets, see "Buffers and solutions" on page 7.

Using a Prepare method

Note: *All preparations, except **Purge pump with methanol** and **Rinse outlets**, can also be included in a **Purify** method.*

Creating the method plan

- Click the **Method Wizard** icon in the **Method Editor** module.

- Select **New** or open an existing method plan to edit.
- Select **Prepare : System and/or Column Preparations**.
- In **Prepare System**, select as appropriate (see example below).
- In **Prepare Columns**, select as appropriate (see below).
- On the last page, click **Finish** (or to enter the Advanced zone, click **Next**).
- In the **Save As** page, type the name of the method plan and click **OK**.

Performing the run

- Run the Prepare method plan as any other run using Instant Run. See "Select run parameters" on page 13.

Instant Run

System preparation

- **Purge pump with methanol**

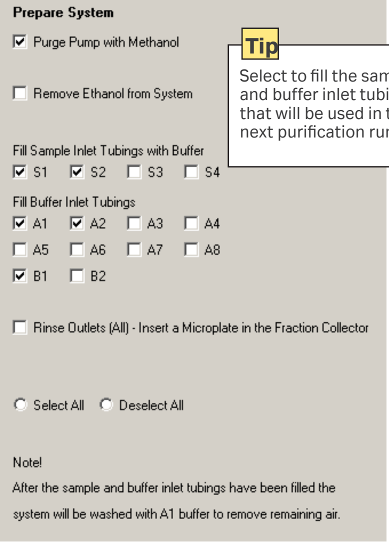
(Select in **Prepare System** page.)

Both pump heads must be purged with methanol if:

- The system has been left unused for a week, or longer, or
- The pump has been run dry.

Purging will maintain the pumping capacity and protect the pump piston seals.

Approximately 150 ml degassed 100% methanol from inlet A8 and 200 ml water from inlet A5 is needed.



Prepare System

☑ Purge Pump with Methanol

☐ Remove Ethanol from System

Fill Sample Inlet Tubings with Buffer

☑ S1 ☑ S2 ☐ S3 ☐ S4

Fill Buffer Inlet Tubings

☑ A1 ☑ A2 ☐ A3 ☐ A4
☐ A5 ☐ A6 ☐ A7 ☐ A8
☑ B1 ☐ B2

☐ Rinse Outlets (All) - Insert a Microplate in the Fraction Collector

○ Select All ○ Deselect All

Note!
After the sample and buffer inlet tubings have been filled the system will be washed with A1 buffer to remove remaining air.

Tip

Select to fill the sample and buffer inlet tubings that will be used in the next purification run.

Column preparation

- **Equilibration**
  (select in **Prepare columns** page)

AC **DS** **GF**

Columns can be equilibrated (according to the table below) with buffer before starting a purification run. If **Remove ethanol** is selected, EtOH will be washed out with water before equilibration. Different column types can be selected in the same run.

Column	Inlet	Volume	Flow [ml/min]	
			RT	CR
Any HiTrap, HiTrap Protein A HP, HiTrap Protein G HP, HiTrap rProtein A FF, HITrap MabSelect SuRe (1 ml or 5 ml column)	A1	5 CV[1]	1 or 10	0.8 or 8
2 × HiTrap Desalting	A2	2.5 CV	10	8
HiPrep 26/10 Desalting	A2	2.5 CV	10	8
Hi Load 16/600 Superdex 75/200 pg	A2	2 CV	1.5	1.2
HiLoad 26/600 Superdex 75/200 pg	A2	2 CV	4	3.2

[1] *CV=column volume*

Tip

Check for leakage during column equilibration.

AC

- **Blank run** (select in **Prepare columns** page)

A blank run can be performed on Affinity columns according to the table below. It is recommended to run a blank run prior to using an affinity column for the first time.

Affinity blank run (1 ml or 5 ml column)	Inlet	Volume	Flow [ml/min]	
			RT	CR
Step 1 Buffer A	A1	5 CV[1]	1 or 5	0.8 or 4
Step 2 Buffer B	B1	5 CV	1 or 5	0.8 or 4
Step 3 Buffer A	A1	10 CV	1 or 5	0.8 or 4

Customized equilibration

AC **DS** **GF**

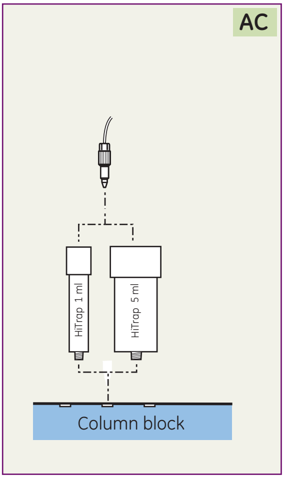
In the Method Wizard, select
Prepare : Customized Column Equilibration.

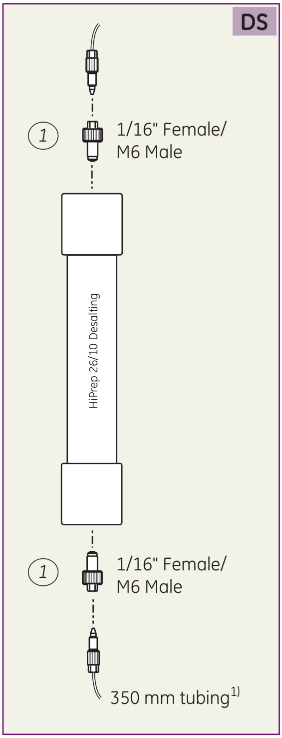
Customized equilibration is a procedure that equilibrates the column with up to 9 different solutions. Only one column type can be included at a time, but up to 5 columns of each type may be selected.

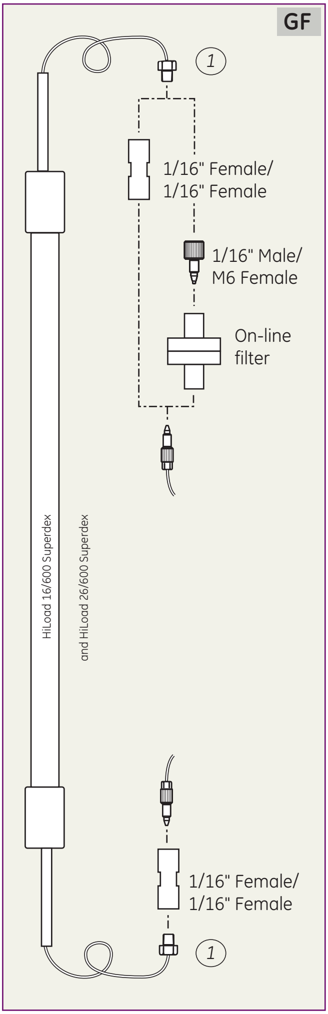
Customized system preparations

In the Method Wizard, select
Prepare : Customized System Preparations.

Customized system preparations is a procedure that cleans the system with up to 5 different cleaning solutions. The selected parts of the system will be washed with one cleaning solution at a time. The system will pause and a message will appear when the inlets should be inserted into a new solution.







(1) *New models of columns have Valco fittings.*

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[1] *The standard system tubing should be replaced by the 350 mm capillary tubing available in the accessory kit.*

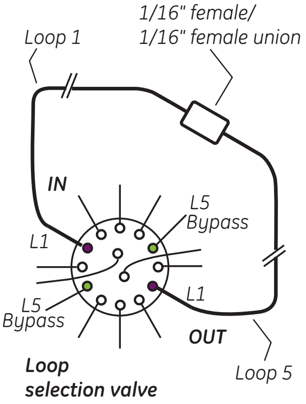
Tip

If connecting 2 loops, cut a suitable lenght of tubing, included in the ÄKTAxpress MAb kit, to use as a bypass for the unused loop position.

Tip

Option for large peaks

If a large affinity peak is expected (for example, if loading a large amount of sample) it is possible to connect two loops together. The total loop volume will then be 20 ml.



Change the following in the method:

- On **Affinity peak collection** page:
  - Set **Max volume in each loop** to e.g. 10 ml.
  - Set **Allow Collection of Single Peaks in Several Loops** to No.
- If CIP system is selected:
  - On **Advanced : Included System Procedure** page, set **Loop Wash Volume : Remove NaOH** to 50 ml.
- On **General Advanced Settings** page:
  - Set **Max number of loops** to 1.
  - Set **Loop Wash Volume** to 40 ml.

3 Run preparations

Creating a method plan for the protein purification

Use the Method Editor wizard to create a new method plan or to change an existing method plan.

Create the method plan

- Click the **Method Wizard** icon in the **Method Editor** module.
- Select **New** or open an existing method plan to edit.
- Select **Purify**.
- Select **Purification Protocol**.
- Make appropriate selections on each page.
- If desired, include system and/or column procedures.
- If desired, on the last page, select **Store Results in a Main Folder** and enter the name of a new or existing folder in **Main Folder Name**. Then click **Finish**
  (or to enter the Advanced zone, click **Next**).
- Finally, in the **Save As** page, type the name of the method plan and click **OK**.

Procedures possible to include

For each protocol it is possible to include different system and column procedures in the purification run.
The procedures, listed below, are further explained in the Method Wizard help texts and in User Manual.

Preparations:

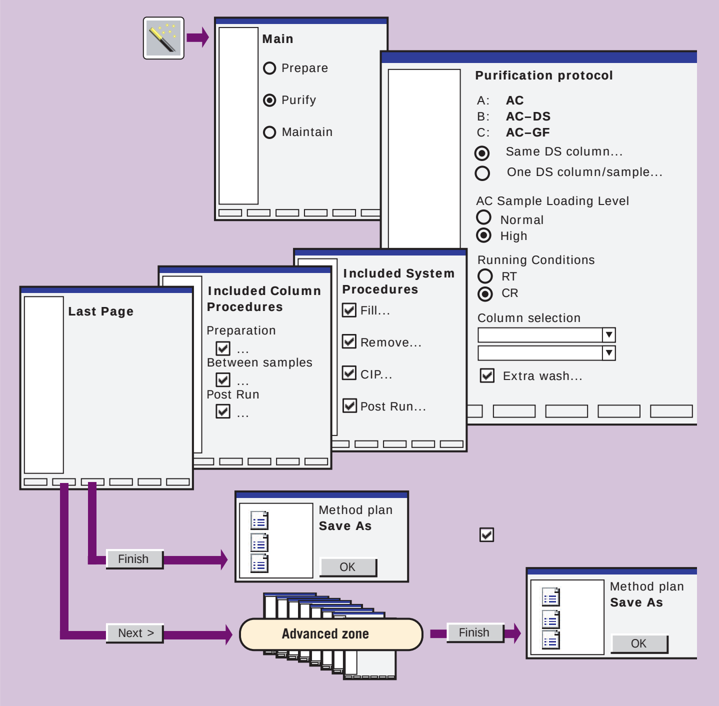
- Fill sample inlet tubings with buffer
- Remove ethanol from system
- Column equilibration/blank run
- Remove ethanol from column before preparation

Between samples:

- CIP system between samples
- CIP desalting / gel filtration columns
- Regenerate affinity columns
- Re-equilibrate affinity columns

Post run:

- Fill system with ethanol
- CIP system, then fill with ethanol
- CIP system, then fill with buffer
- Fill columns with ethanol
- CIP columns, then fill with ethanol
- CIP columns, then re-equilibrate



Main
○ Prepare
● Purify
○ Maintain

Purification protocol
A: **AC**
B: **AC–DS**
C: **AC–GF**
● Same DS column...
○ One DS column/sample...
AC Sample Loading Level
○ Normal
● High
Running Conditions
○ RT
● CR
Column selection
☑ Extra wash...

Included System Procedures
☑ Fill...
☑ Remove...
☑ CIP...
☑ Post Run...

Included Column Procedures
Preparation
☑ ...
Between samples
☑ ...
Post Run
☑ ...

Last Page

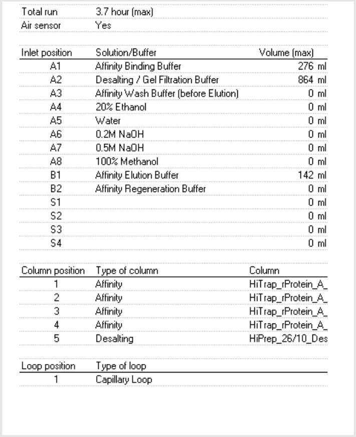
Method plan
Save As

Tip

Buffer volumes required

It is possible to print out a Summary check list to get a list of the buffer volumes and inlet positions for the run before preparing the system and columns.

To do this, perform steps 1 to 5 in *"Start the run" on page 13*, and then press **Cancel** instead of starting the run.



Total run	3.7 hour (max)
Air sensor	Yes

Inlet position	Solution/Buffer	Volume (max)
A1	Affinity Binding Buffer	276 ml
A2	Desalting / Gel Filtration Buffer	864 ml
A3	Affinity Wash Buffer (before Elution)	0 ml
A4	20% Ethanol	0 ml
A5	Water	0 ml
A6	0.2M NaOH	0 ml
A7	0.5M NaOH	0 ml
A8	100% Methanol	0 ml
B1	Affinity Elution Buffer	142 ml
B2	Affinity Regeneration Buffer	0 ml
S1		0 ml
S2		0 ml
S3		0 ml
S4		0 ml

Column position	Type of column	Column
1	Affinity	HiTrap_rProtein_A_
2	Affinity	HiTrap_rProtein_A_
3	Affinity	HiTrap_rProtein_A_
4	Affinity	HiTrap_rProtein_A_
5	Desalting	HiPrep_26/10_Des

Loop position	Type of loop
1	Capillary Loop

Prepare system and columns

System and column preparation can be included in the method plan. Only *Purge pump with methanol* and *Rinse outlets* has to be performed in a separate prepare run.

1 Prepare the buffer, inlets, outlets and waste

- Prepare buffers required for the run.
- Immerse all inlet tubing in the appropriate liquid containers as described on page 3 and, make sure the system is prepared as described in *"System preparation" on page 9*.
- Immerse unused tubing in e.g. 20% ethanol to avoid air entering the tubing.
- If **Fill sample inlets** is not included in the method plan to use, make sure the sample inlet tubing is filled with affinity binding buffer, see *"System preparation" on page 9*.

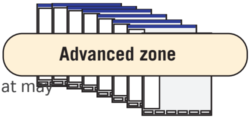
2 Connect columns

- Connect selected columns as described on page 5 and 10.

Advanced zone

General

In the Advanced zone, all main parameter values can be viewed, and modified if required. Examples of parameters that may be modified:



- Wash volumes
- Flow rates
- Watch parameters for peak detection

Note: *Do not change default parameter values in a method plan* ***unless the result is clearly understood****.*

Flow rates and pressure limits

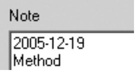
No warning will be given if the flow rate or pressure limit is set higher than the values recommended for the columns used.

Getting help

To get help about parameter setting, click **Help** in the wizard boxes.

Tip

Use the Note field in the **Save As** page to specify changes made to the default settings.



Note

2005-12-19
Method

Specify path to import file

If an import file is going to be used for specification of sample information, the file path can be set in **Import File Location** In the **General Advanced Settings** page.

Tip

If the import files are saved in the default folder **MethodWizardImport**, there is no need to specify the path. For further information, see User Manual.

4 Starting a run

Select run parameters

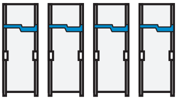
1 Select method plan


Instant Run

- In UNICORN **System Control**, click **Instant Run**.
- Select the required **Method plan** from the list.


Method plan
AC DS

2 Select systems for the run



- Select on which **System**(s) the method plan will be run and the **Number of Samples** on each system.

3 Specify samples

- Enter identification names for the samples, either via the keyboard or using a bar code reader.

- For each sample, enter:
  – isoelectric point, **pI**,
  – extinction coefficient for the protein, **Ext Coeff**,
  – molecular weight of the protein, **MW**.

  The data is automatically imported from an import file, if it has been prepared and placed in the specified folder. (See User Manual for further information.)
- Enter optional text, e.g. culture batch number.

4 Edit result file location and names

- If required, edit the folder path and file names of the result files to be created.

5 Print Summary check list

- Print out the *Summary check list* by clicking **Print**.

Final preparations

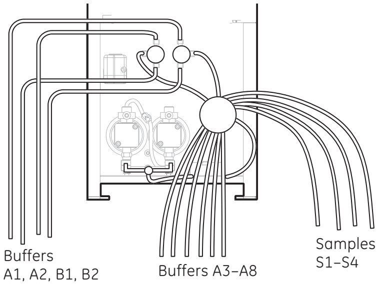
Use the *Summary check list* during the final inspection.

6 Check the flow path

Buffer and sample tubing inspection

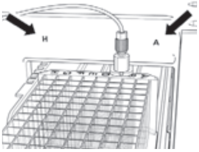
- Check that the tubings are fully immersed in the container and that the tubings are filled with liquid.
- Immerse the waste and outlet tubings in appropriate containers.


Method Editor: UN... | System Control... | Evaluation



- Make sure:
  –there is enough buffer available
  –the correct inlet is placed in each buffer
  –the columns are placed in correct positions.

7 Prepare the microplate



- Place an empty deep well microplate on the sled and check that the labelling **H** and **A** match the labelling on the system.

8 Prepare the samples

- Prepare the samples and clarify them using centrifugation and/or filtration through a 0.45 µm filter.
- Place the sample tubes in the tube or (optional) flask holder .
- If **Fill sample inlets** is included in the method plan,
  the inlets should be placed in affinity A buffer. During the run, the system will pause after initial buffer filling, and a message will appear requesting each sample inlet tubing to be moved to its sample.
- If **Fill sample inlets** is not included, gently move the sample inlet tubing to each sample before starting the run.

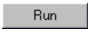
  Make sure that no air enters the tubing. Place the tubing *close to* the bottom of the liquid container but not too tight against the bottom.
- Secure the tubing with the tubing holder.

Start the run

9 Final check

- Peform a final check that tubing, columns and solu-tions are placed according to the *Summary check list*.

10 Start the run

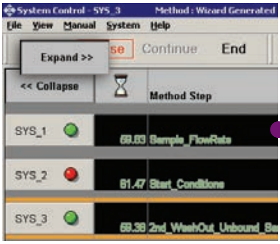

Run

- Click **Run** to start the run on the selected systems.

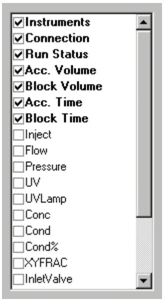
5 Monitoring a run

Monitoring a run is performed in System Control.

View single or all systems
The run data of all systems can be viewed simultaneously by clicking **Expand >>**. Return to single system view by clicking **<< Collapse.**

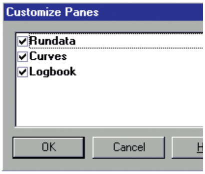


Run data
Right-click the view pane and select **Properties** to select run data to be displayed.



☑ Instruments
☑ Connection
☑ Run Status
☑ Acc. Volume
☑ Block Volume
☑ Acc. Time
☑ Block Time
☐ Inject
☐ Flow
☐ Pressure
☐ UV
☐ UVLamp
☐ Conc
☐ Cond
☐ Cond%
☐ XYFRAC
☐ InletValve

Panes
Click Panes to select panes to display.



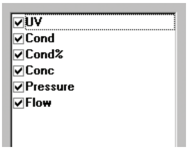
☑ Rundata
☑ Curves
☑ Logbook

Zoom
Use the mouse drag-and-drop box to zoom in and out the curve. Right-click to reset zoom.

Curves
Right-click to select which curves to be displayed.

Log book
Right-click to select log book properties to be displayed.

☑ Alarm/Warning
☑ Method
☑ Manual
☑ Error
☑ System



☑ UV
☑ Cond
☑ Cond%
☑ Conc
☑ Pressure
☑ Flow

Run status
Each system's status indicator shows the run status:

	Status	*Color*	*Example of events*
SYS_1 ○	**End**	White	
SYS_1 🟢	**Run** or **Manual**	Green	
SYS_1 🟡	**Hold**	Yellow	Sample loading
SYS_1 🔴	**Pause**	Red	Pump-wash
SYS_1 🔴	**Error**	Yellow, flashing	Air detected (e.g. running out of buffer)

Tip

The chapter Troubleshooting in the User Manual can be useful if something unexpected happens.

6 Evaluating the results

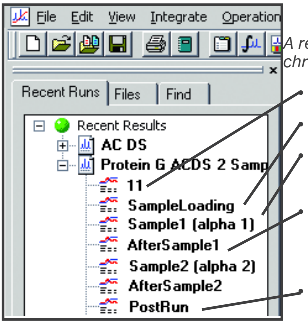
When a system has entered **End** status the result can be evaluated and the samples can be pooled.

1 Find and open the result files

- Use the **Recent Runs** or the **Find** tab, in the Evaluation module to locate the result file.

Note: Automatic pooling is not performed if the result file is opened via the ***Files*** *tab.*

- Click ⊞ to expand the list for the result file.
- Double-click a sample chromatogram to open it.



A result file includes chromatograms of:

equilibration

sample loading

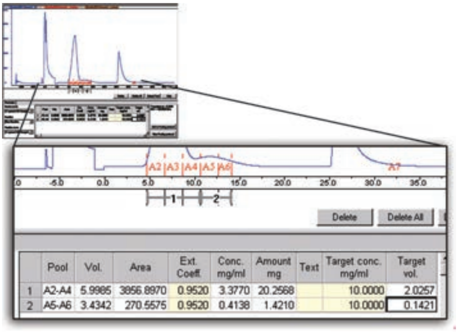
purification of sample(s)

system/column CIP performed between samples

CIP/fill with ethanol etc. performed after the purification run

2 Adjust pooling and add to Pooling protocol

The chromatogram is displayed and UNICORN™ will automatically display a suggested pooling of the fractions. The pooled fractions are listed in a table below the chromatogram and the pooled peaks are numbered sequentially in the chromatogram.



	Pool	Vol.	Area	Ext. Coeff.	Conc. mg/ml	Amount mg	Text	Target conc. mg/ml	Target vol.
1	A2-A4	5.9985	3856.8970	0.9520	3.3770	20.2568		10.0000	2.0257
2	A5-A6	3.4342	270.5575	0.9520	0.4138	1.4210		10.0000	0.1421

Only adjacent fractions will be pooled. The fraction numbers for each pool are listed in the table as a range in retention order, e.g. A6–A7 etc.

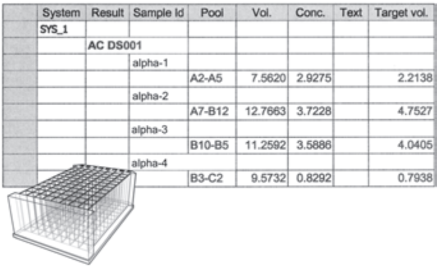
If the pooling suggestion is not performed, choose **Operations : Pool**.

- If necessary, adjust the suggested pooling.
- If the extinction coefficient has been entered in the wizard, concentration and amount are automatically calculated. *Otherwise*: enter the extinction coefficient manually by marking a pool and then typing the value in the extinction coefficient field. The concentration is given in mg/ml, and amount is given in mg.
- The linearity of the UV monitor is limited. For more information, see Technical specification in the User Manual.
- Click the **Add to Pooling Protocol** button to add the adjusted pools to the Pooling protocol.
- Repeat the procedure for other chromatograms from the same, or other, result files.
- Click the **View Pooling Protocol** button.

3 Print or save the Pooling protocol

The Pooling protocol can be used as a help when making the physical pooling of the purified samples from the microplate.

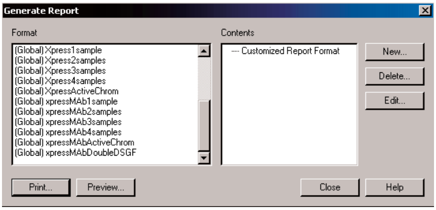
- To print the Pooling protocol:
  Click the **Print** button to print the protocol on the default printer.
- To save the Pooling protocol as a file:
  Click **Export** and save the protocol in one of the following formats: text (.txt), Excel® (.xls), HTML (.htm).



System	Result	Sample Id	Pool	Vol.	Conc.	Text	Target vol.
SYS_1							
	AC DS001						
		alpha-1					
			A2-A5	7.5620	2.9275		2.2138
		alpha-2					
			A7-B12	12.7663	3.7228		4.7527
		alpha-3					
			B10-B5	11.2592	3.5886		4.0405
		alpha-4					
			B3-C2	9.5732	0.8292		0.7938

4 Print the Report

- Select **File : Report** or click the **Report** icon.
- Select **xpressMAb1sample**, **xpressMAb2samples**, **xpressMAb3samples,** or **xpressMAb4samples** report format, depending on how many samples are included in the result file.
- The report format **xpressMAbActiveChrom** can be selected if only the active zoomed window should be included in the report.
- The report format **xpressMAbDoubleDSGF** can be selected if individual desalting/gel filtration columns have been used for each sample .


Generate Report

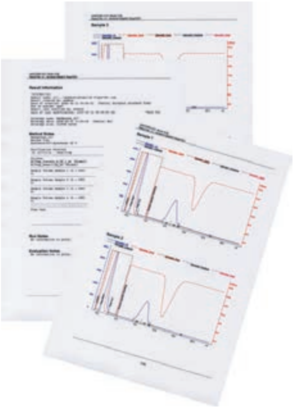
Format

(Global) Xpress1sample
(Global) Xpress2samples
(Global) Xpress3samples
(Global) Xpress4samples
(Global) XpressActiveChrom
(Global) xpressMAb1sample
(Global) xpressMAb2samples
(Global) xpressMAb3samples
(Global) xpressMAb4samples
(Global) xpressMAbActiveChrom
(Global) xpressMAbDoubleDSGF

Contents

Customized Report Format

- Click **Edit** or **Preview** to inspect and modify the report format if needed.
- Click the **Print** button.
- Choose which pages and how many copies to print and click **OK.**



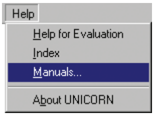
Cleaning after a run

Cleaning of inlets and outlets have to be performed using a maintain method, see *Maintaining columns and system*, page 17.

Getting help

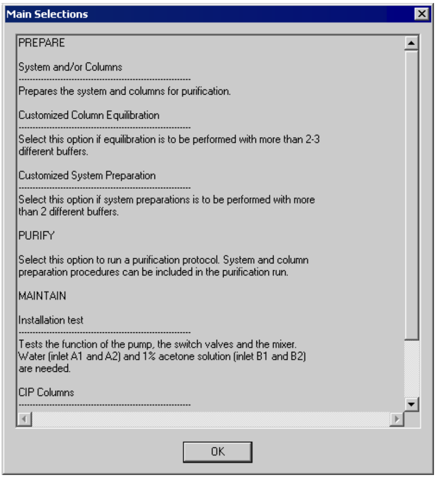
On-line Manuals and Cue Cards

- Open the **Help** menu and select **Manuals** to access the on-line instructions:
  - – UNICORN User Manual
  - – ÄKTAxpress MAb User Manual
  - – Cue Cards



Context-specific Help

- Click the **Help** button in the dialog box, or press **F1**.
  Example of Method Editor wizard help:


Main Selections

PREPARE

System and/or Columns

Prepares the system and columns for purification.

Customized Column Equilibration

Select this option if equilibration is to be performed with more than 2-3 different buffers.

Customized System Preparation

Select this option if system preparations is to be performed with more than 2 different buffers.

PURIFY

Select this option to run a purification protocol. System and column preparation procedures can be included in the purification run.

MAINTAIN

Installation test

Tests the function of the pump, the switch valves and the mixer. Water (inlet A1 and A2) and 1% acetone solution (inlet B1 and B2) are needed.

CIP Columns

7 Maintaining columns and system

CIP Column (Cleaning-In-Place)

Purpose

To clean contaminated columns. For column cleaning procedures and column storage instructions, please refer to the instructions supplied with each column or to the web page:
cytiva.com/protein-purification

Tip

CIP with NaOH can be included in the purification runs.

Creating the method plan

- Click the **Method Wizard** icon in the **Method Editor** module.
- Select **New** or open an existing method plan to edit.
- Select **Maintain**.
- Select **CIP columns.**

  CIP columns is a procedure that cleans the column with up to 9 different solutions. Only one column type can be included at a time but up to 5 columns of each type may be selected.
- On each page, select the parameter values.
- On the last page, click **Finish**
  (or to enter the Advanced zone, click **Next**).
- In the **Save As** page, type the name of the method plan and click **OK.**

Performing a CIP run

Note: Make sure all inlet tubing is filled before start. See "System preparation" on page 9.

- Run the CIP run as any other run using Instant Run. See "Select run parameters" on page 13.

Instant Run

Suggested CIP solutions

- **HiTrap Protein A HP (1 and 5 ml)**

Step 1: 0.1% non-ionic detergent (e.g. Triton X-100), 37°C
Step 2: Binding buffer, pH 8, at least 5 CV

- **HiTrap Protein G HP (1 and 5 ml)**

Step 1: 4-6 M guanidine hydrochloride, 4°C, 30-60 min
Step 2: Binding buffer, at least 5 CV

- **HiTrap rProtein A FF (1 and 5 ml)**

Step 1: 6 M guanidine hydrochloride, 2 CV
Step 2: Binding buffer, pH 7-8, at least 5 CV

- **HiTrap MabSelect SuRe (1 and 5 ml)**

Step 1: 0.1-0.5 M NaOH, 10-15 min, at least 2 CV
Step 2: Binding buffer, pH 7-8, at least 5 CV

- **HiTrap Desalting/HiPrep Desalting**

Step 1: 0.2 M NaOH, 2 CV
Step 2: Equilibration buffer, at least 5 CV

- **HiLoad Superdex 75/200 pg**

Step 1: 0.5 M NaOH, 0.5-1 CV, flow rate 0.8 ml/min for HiLoad 16/600 and 2.2 ml/min for HiLoad 26/600
Step 2: Equilibration buffer, at least 2 CV

Clean System

Purpose

To wash all used tubing within the system, including the loops. Up to 5 cleaning solutions can be used, one at a time.

Creating the method plan

- Click the **Method Wizard** icon in the **Method Editor** module.
- Select **New** or open an existing method plan to edit.
- Select **Maintain**.
- Select **Clean System**.
- Select the parts to be cleaned.
- On the last page, click **Finish**
  (or to enter the Advanced zone, click **Next**).
- In the **Save As** page, type the name of the method plan and click **OK**.

Manual preparation

- Prepare a sufficient volume of the wash solutions.
- Wash the outside of the inlet tubing with deionized water and/or ethanol.
- Immerse all tubings to be cleaned (inlet A1 is always used for cleaning outlets, loops and column valves) in a container of cleaning solution.
- *If the column valve is to be cleaned:*
  Remove the columns and reconnect the tubing to use as bypass column connections.

Instant Run

Performing a Clean System run

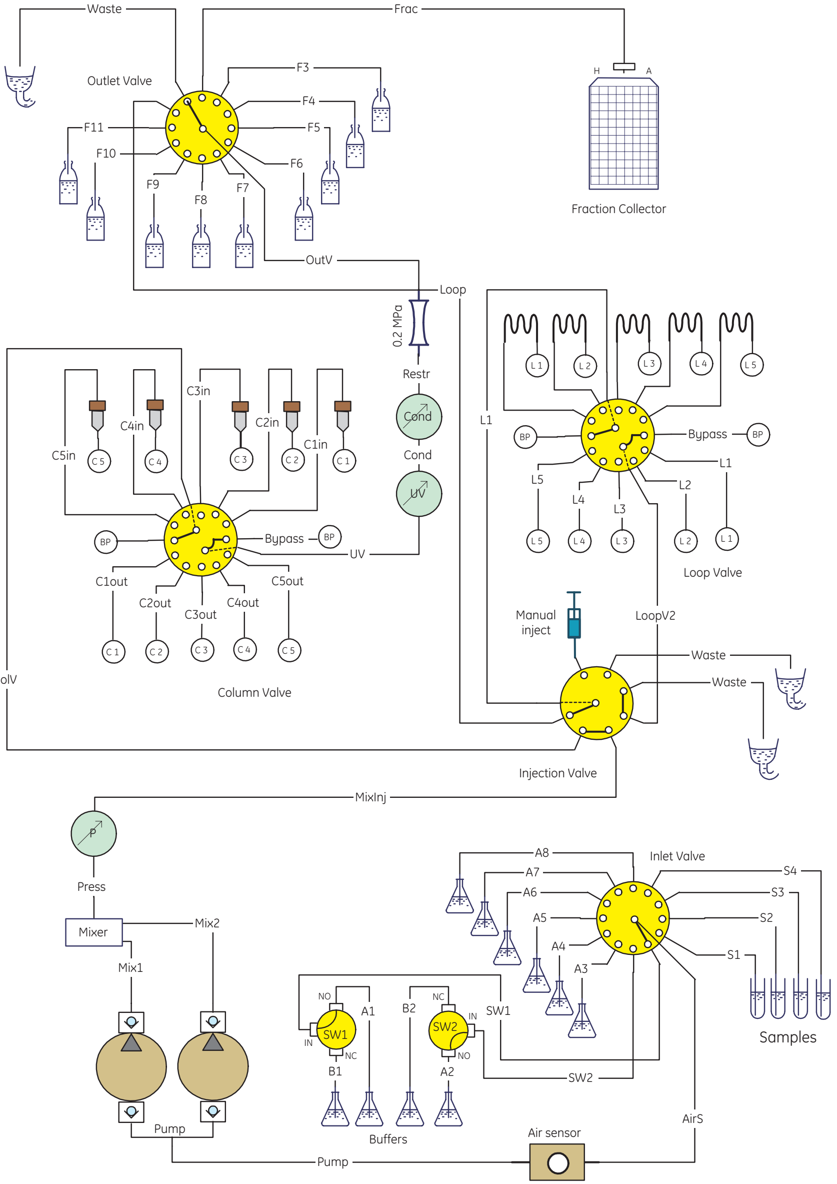
- Run the Clean System run as any other run using Instant Run. See "Select run parameters" on page 13.
- A message will be displayed when to manually change the cleaning solutions. Inlet A1 is used for cleaning outlets, loops and column valve.

Leaving the system for some days

If the system is not going to be used for some days:
Use a bacteriostatic solution, for example deionized water and 20% ethanol, as the two final wash solutions.

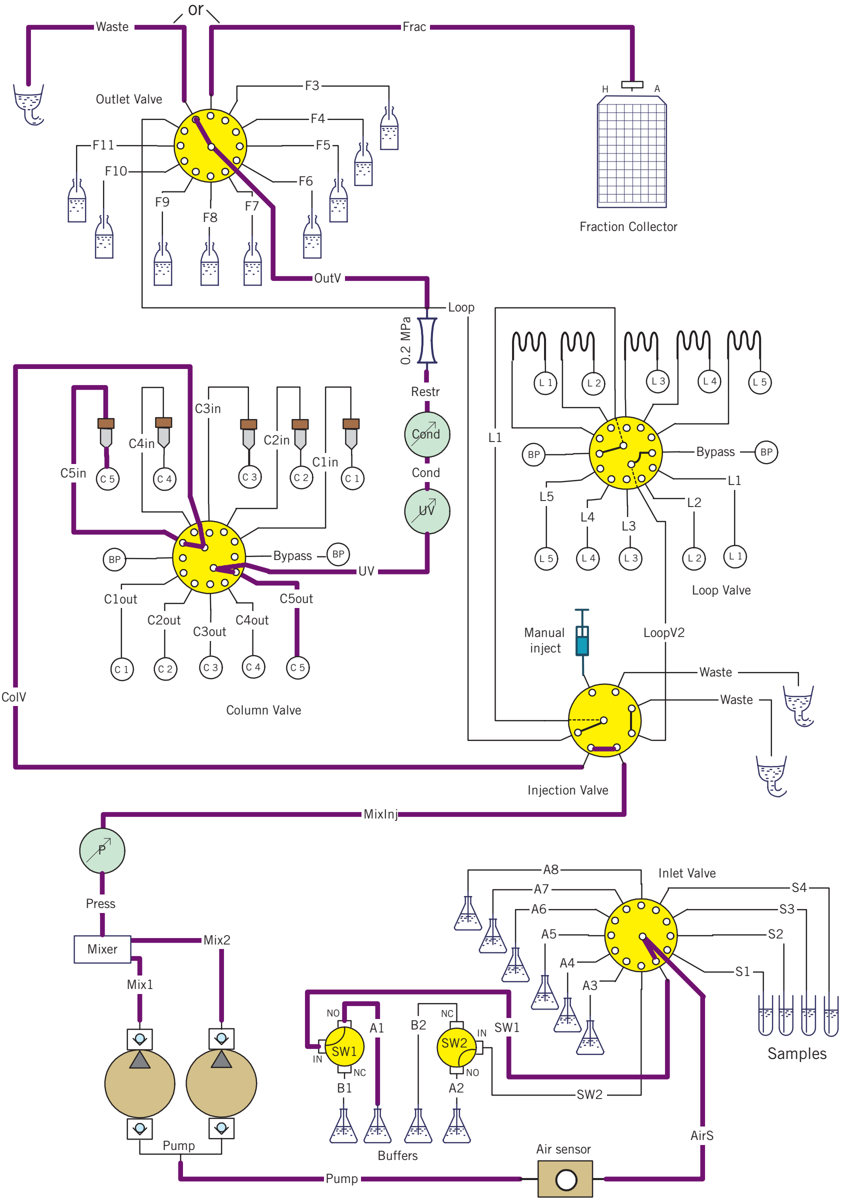
Flow charts

- **Valve positions after reset (END)**

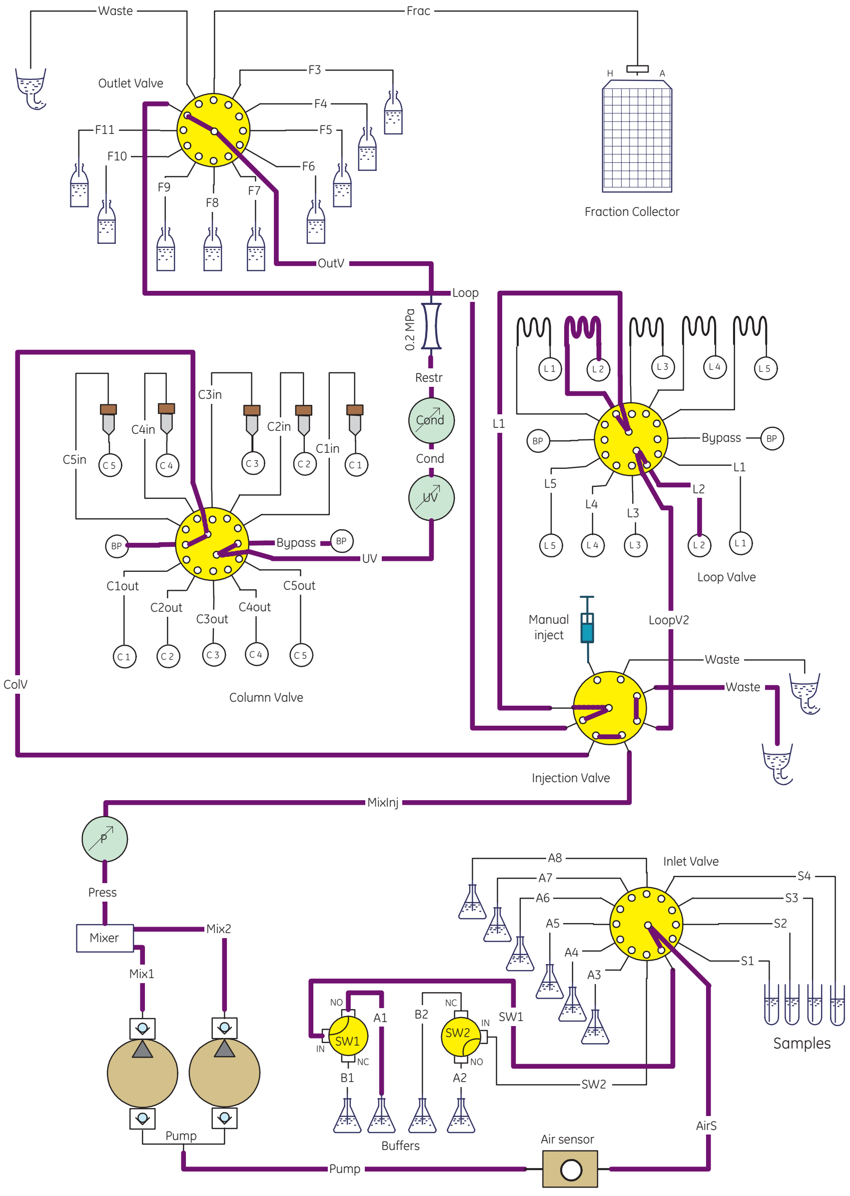


- **Column equilibration**
- **Sample loading**

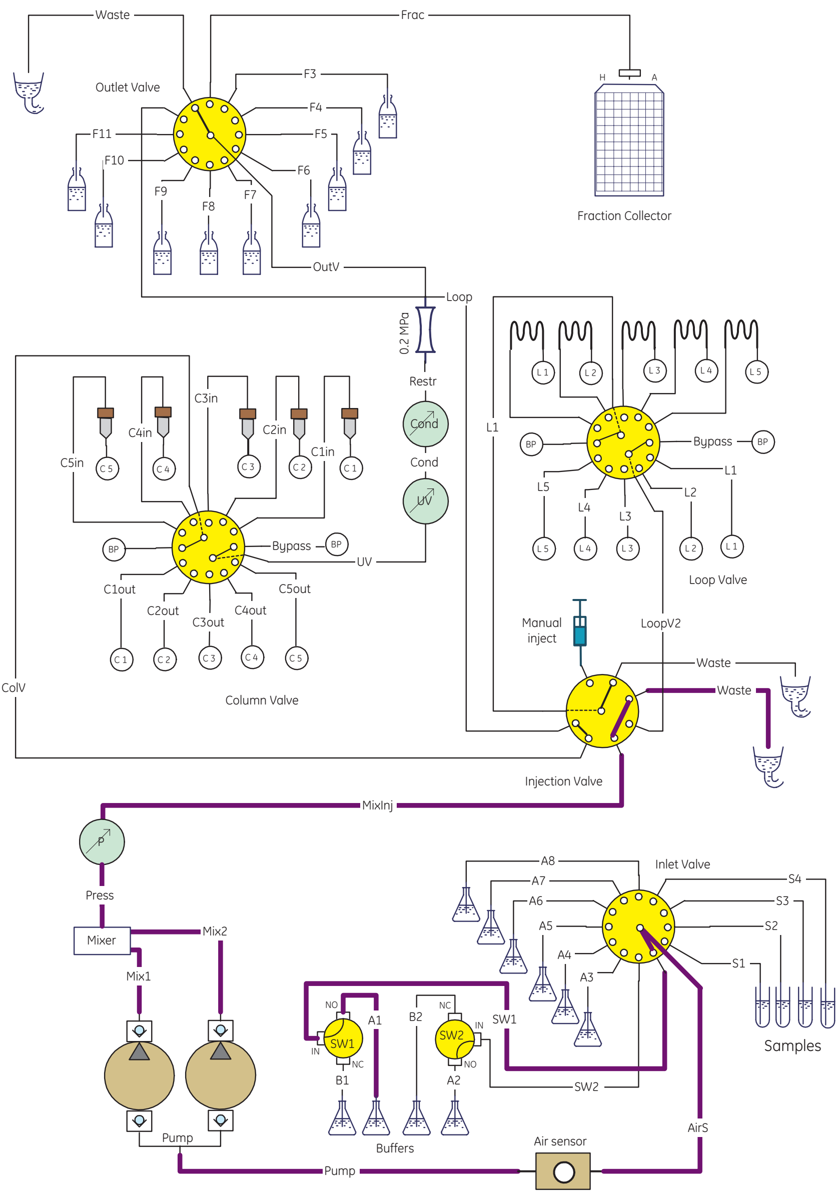
- **Wash out unbound sample**
- **Elution**



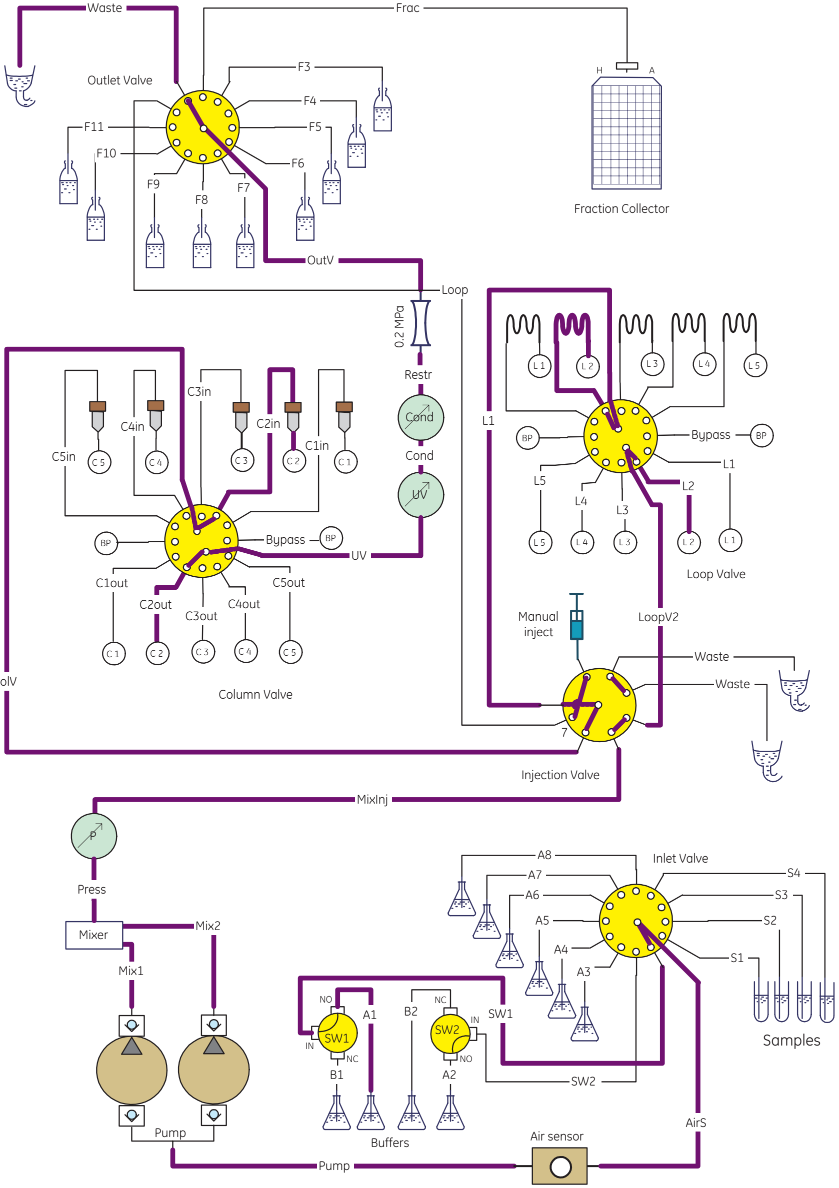
- **Intermediate peak collection in capillary loops**
- **System wash**



- **Pump wash**


Waste
Frac
Outlet Valve
F3
F4
F5
F6
F7
F8
F9
F10
F11
H
A
Fraction Collector
OutV
Loop
0.2 MPa
Restr
Cond
Cond
UV
UV
L 1
L 2
L 3
L 4
L 5
BP
Bypass
BP
L1
L2
L3
L4
L5
L1
Loop Valve
C3in
C4in
C2in
C1in
C5in
C 5
C 4
C 3
C 2
C 1
BP
Bypass
BP
C1out
C2out
C3out
C4out
C5out
C 1
C 2
C 3
C 4
C 5
Column Valve
ColV
Manual inject
LoopV2
Waste
Waste
Injection Valve
MixInj
P
Press
Mixer
Mix2
Mix1
Pump
Pump
A8
A7
A6
A5
A4
A3
Inlet Valve
S4
S3
S2
S1
Samples
NO
A1
IN
SW1
NC
B1
B2
NC
IN
SW2
NO
A2
SW1
SW2
Buffers
Air sensor
AirS

- **Sample loading from loops**
- **Loop wash**



For your own notes:

For your own notes:

Ordering information

Product	Pack Size	Code
System and software		
ÄKTAxpress	1	18-6645-01
USB/CAN device[1]	1	28-9692-01
ÄKTAxpress software & manuals	1	28-9053-45
Accessories		
Flask holder	1	18-1177-79
Large column holder ÄKTAxpress	1	28-4007-37
Supported columns		
Affinity chromatography		
HiTrap Protein A HP	1 x 1 ml	29-0485-76
HiTrap Protein A HP	5 x 1 ml	17-0402-01
HiTrap Protein A HP	1 x 5 ml	17-0403-01
HiTrap Protein A HP	5 x 5 ml	17-0403-03
HiTrap Protein G HP	1 x 1 ml	29-0485-81
HiTrap Protein G HP	5 x 1 ml	17-0404-01
HiTrap Protein G HP	1 x 5 ml	17-0405-01
HiTrap Protein G HP	5 x 5 ml	17-0405-03
HiTrap rProtein A FF	2 x 1 ml	17-5079-02
HiTrap rProtein A FF	5 x 1 ml	17-5079-01
HiTrap rProtein A FF	1 x 5 ml	17-5080-01
HiTrap MabSelect SuRe	1 x 1 ml	29-0491-04
HiTrap MabSelect SuRe	5 x 1 ml	11-0034-93
HiTrap MabSelect SuRe	1 x 5 ml	11-0034-94
HiTrap MabSelect SuRe	5 x 5 ml	11-0034-95
Desalting		
HiPrep 26/10 Desalting	1 x 53 ml	17-5087-01
HiPrep 26/10 Desalting	4 x 53 ml	17-5087-02

Product	Pack Size	Code Number
HiTrap Desalting	5 x 5 ml	17-1408-01
HiTrap Desalting	100 x 5 ml[2]	11-0003-29
Gel filtration		
HiLoad 16/600 Superdex 75 prep grade	1 x 120 ml	28-9893-33
HiLoad 16/600 Superdex 200 prep grade	1 x 120 ml	28-9893-34
HiLoad 26/600 Superdex 75 prep grade	1 x 320 ml	28-9893-35
HiLoad 26/600 Superdex 200 prep grade	1 x 320 ml	28-9893-36
Documents		
UNICORN manual set	1	11-0003-68
ÄKTAxpress MAb User Manual	1	11-0036-98
ÄKTAxpress Operating instructions	1	28-9579-08
ÄKTAxpress Installation Guide	1	18-1178-01
Affinity Chromatography Handbook	1	18-1022-29
Gel Filtration Handbook	1	18-1022-18
Strategies for Protein Purification Handbook	1	28-9833-31
Recombinant Protein Purification Handbook	1	18-1142-75
Antibody Purification Handbook	1	18-1037-46
ÄKTA Laboratory-scale Chromatography Systems - Instrument Management Handbook	1	29-0108-31

[1] *Needed between the first system and the computer.*

[2] *100-packs are special packs delivered on customer order. Includes connector package, domed nuts and instructions.*

Code numbers for more accessories and user replaceable spare parts can be found in the ÄKTAxpress MAb User Manual.

cytiva.com

For local office contact information, visit cytiva.com/contact.

11003699 AC 05/2021