# ÄKTAxpress

## Cue Cards

**Contents**

# System overview

## Column block - for purification runs

**1** – AC or IEX column for sample 1

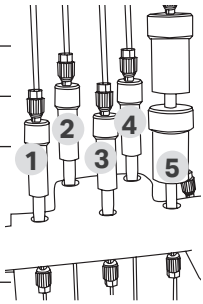
**2** – AX or IEX column for sample 2

**3** – AC or IEX column for sample 3

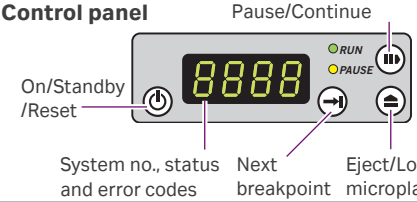
**4** – AC or IEX column for sample 4 *or*
- AC or IEX column for 2nd AC/IEX step *or*
- DS column for 1st DS step out of 2 DS-steps

**5** – Desalting column for first or last DS step *or*
- Gel filtration column

See also "A2 Column positions" on page 20

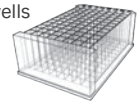



## Control panel



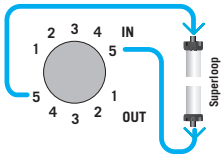

## Sled for microplate

Deep well microplate, 24 or 96 square shaped wells



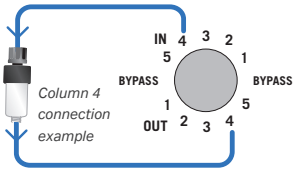
## Loop valve

Superloop™ connection




## Column valve

Column connection




## Right holder

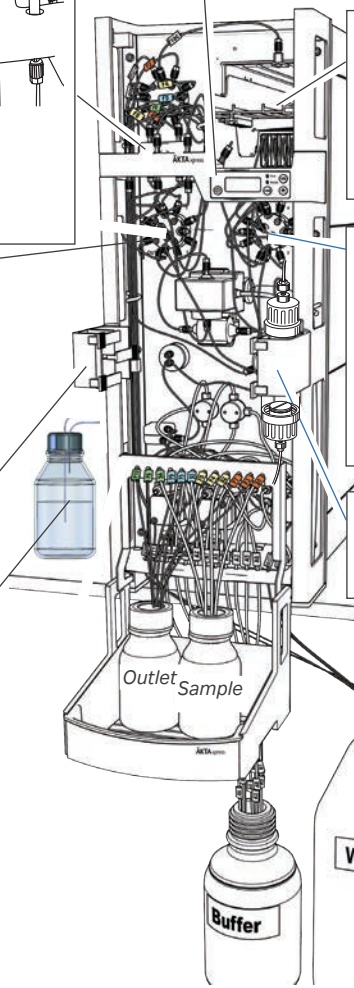
- Superloop

## Left holder

- Gel filtration column
- HiPrep™ Desalting column

## On bench

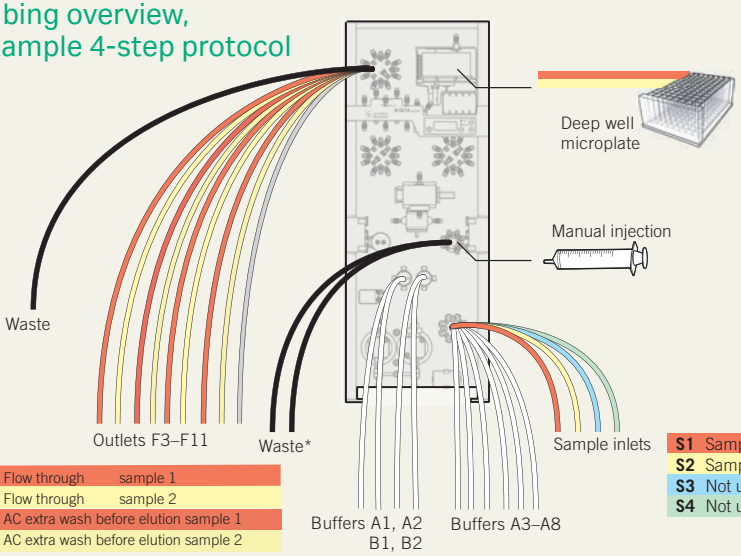
- Pump rinse solution



## Tubing overview

Detailed information on inlets and outlets for a specific run need to be checked in the "2.1 Print out a summary" on page 9. The below image is an example, showing inlets and outlets for a 4-step protocol with 2 samples.

### Tubing overview, example 4-step protocol




**F3** Flow through sample 1
**F4** Flow through sample 2
**F5** AC extra wash before elution sample 1
**F6** AC extra wash before elution sample 2

**F7** Non selected peaks from AC sample 1
**F8** Non selected peaks from AC sample 2
**F9** Non selected peaks from DS/IEX sample 1
**F10** Non selected peaks from DS/IEX sample 2

**F11** Metal ion waste

**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 9.

**S1** Sample 1
**S2** Sample 2**
**S3** Not used**
**S4** Not used**

* Waste From injection valve port 2 (manual sample injection)
* Waste From injection valve port 3 (e.g., system wash)
** S2, S3 and S4 can be used for other buffers/solutions than sample.

**Detailed flow charts**

*See "Appendix B Flow charts" on page 24*

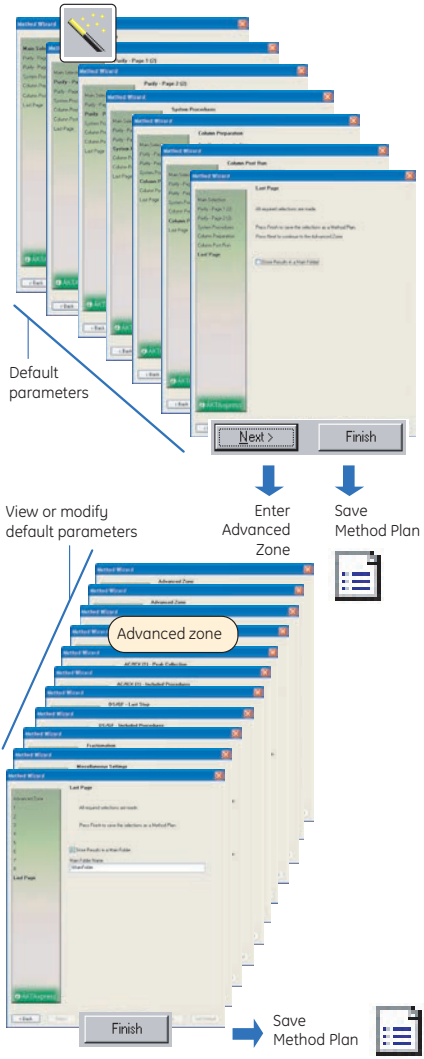
## Safety

Read the ÄKTAxpress Operating Instructions before using the system.

# UNICORN for ÄKTAxpress, overview


## Method Editor wizard

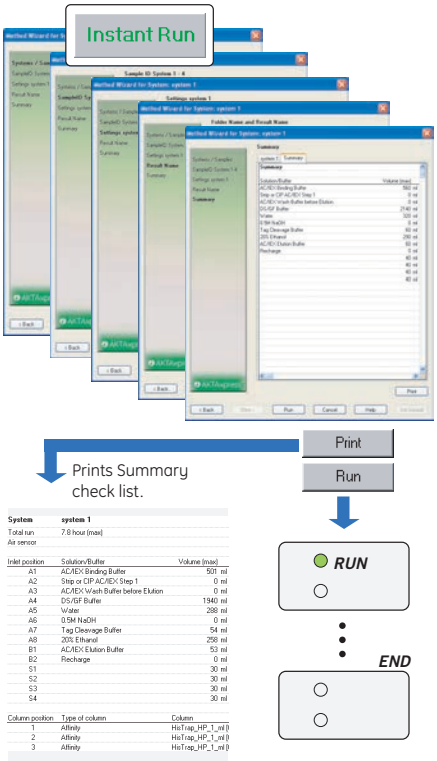
The Method Editor wizard is used to create Method Plans for Purify or Prepare and Maintain. Input information: protocol type, columns, system and column procedures.





## 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.




| System | system 1 |
|---|---|
| Total run | 7.8 hour (max) |
| Air sensor | |

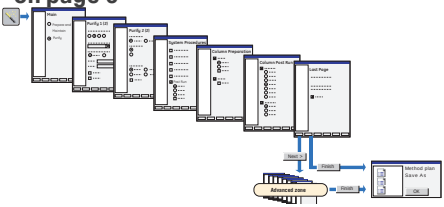
| Inlet position | Solution/Buffer | Volume (max) |
|---|---|---|
| A1 | AC/IEX Binding Buffer | 501 ml |
| A2 | Strip or CIP AC/IEX Step 1 | 0 ml |
| A3 | AC/IEX Wash Buffer before Elution | 0 ml |
| A4 | DS/GF Buffer | 1940 ml |
| A5 | Water | 288 ml |
| A6 | 0.5M NaOH | 0 ml |
| A7 | Tag Cleavage Buffer | 54 ml |
| A8 | 20% Ethanol | 258 ml |
| B1 | AC/IEX Elution Buffer | 53 ml |
| B2 | Recharge | 0 ml |
| S1 | | 30 ml |
| S2 | | 30 ml |
| S3 | | 30 ml |
| S4 | | 30 ml |

| Column position | Type of column | Column |
|---|---|---|
| 1 | Affinity | HisTrap_HP_1_ml () |
| 2 | Affinity | HisTrap_HP_1_ml () |
| 3 | Affinity | HisTrap_HP_1_ml () |

# ÄKTAxpress workflow, overview

## “1 Creating a method plan for purification” on page 6

- Select Prepare and Maintain or Purify
- Select Columns
- Include system and column procedures (page 7 and page 15)
  - Preparation
  - During Purification run
  - Post run



## “2 Preparing solutions, system and columns” on page 9

- Print out a summary
- Prepare buffers and inlets
- Connect columns and Superloop

| System | system 1 | |
|---|---|---|
| Total run | 7.8 hour (max) | |
| Air sensor | | |
| | | |
| Inlet position | Solution/Buffer | Volume (max) |
| A1 | AC/IEX Binding Buffer | 501 ml |
| A2 | Strip or CIP AC/IEX Step 1 | 0 ml |
| A3 | AC/IEX Wash Buffer before Elution | 0 ml |
| A4 | DS/GF Buffer | 1940 ml |
| A5 | Water | 288 ml |
| A6 | 0.5M NaOH | 0 ml |
| A7 | Tag Cleavage Buffer | 54 ml |
| A8 | 20% Ethanol | 258 ml |
| B1 | AC/IEX Elution Buffer | 53 ml |
| B2 | Recharge | 0 ml |
| S1 | | 30 ml |
| S2 | | 30 ml |
| S3 | | 30 ml |
| S4 | | 30 ml |
| | | |
| Column position | Type of column | Column |
| 1 | Affinity | HisTrap_HP_1_ml [I |
| 2 | Affinity | HisTrap_HP_1_ml [I |
| 3 | Affinity | HisTrap_HP_1_ml [I |

## “3 Starting a run” on page 12

- Run setup
  - Select method plan
  - Select number of systems and samples
  - Add sample IDs and sample information
  - Prepare microplate
  - Prepare samples


- Start the run 

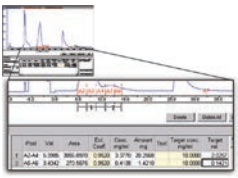
- Be prepared for any manual actions (Load samples into loops, protease or sample into Superloop, move sample inlets)

## “4 Monitoring a run” on page 14

## “5 Column and system procedures” on page 15

- Column procedures (page 15)
  - Equilibrate/re-equilibrate
  - Affinity blank run
  - Ion exchange blank run
  - Metal Ion Stripping (chelating columns)
  - Metal Ion charging/re-charging
  - CIP columns
  - Remove/fill columns with ethanol
- 
- System procedures (page 16)
  - Manual filling of the inlet tubing
  - Purge pump with methanol
  - Clean sample inlet tubing
  - Clean pump
  - Wash frac tubing
  - Rinse all outlet tubing
  - Remove ethanol from the system
  - Fill system with ethanol
  - Clean system

## “6 Evaluating the results” on page 18



# 1 Creating a method plan for purification

## 1.1 Overview

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

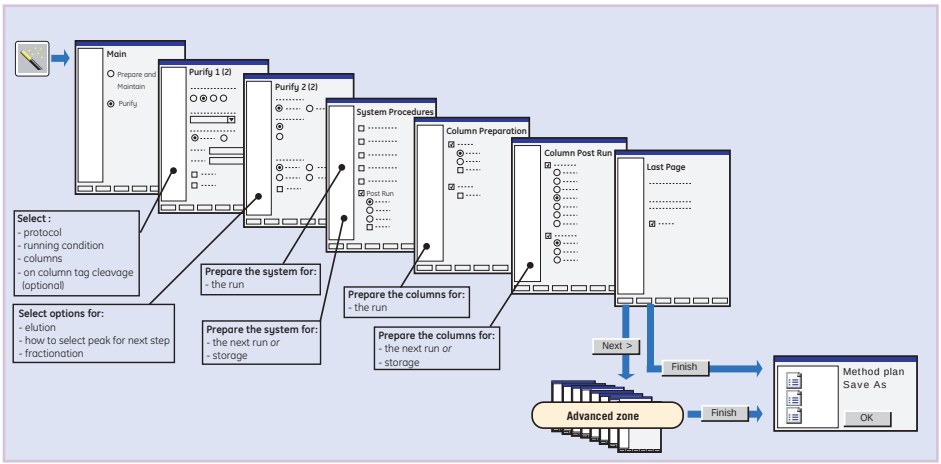

- Click the Method Wizard icon in the Method Editor module. 
- Select New or open an existing method plan to edit.
- Select Purify and click Next to proceed.

In addition to the basic method steps, it is possible to include most system and/or column procedures in the purifiction method, (e.g., system and column CIP).

System and column procedures can also be run as separate prepare and maintain methods.

For more guidance on protocol selection, see the User Manual.

For guidance on run times, see “A3 Typical run times” on page 20.




## 1.2 Protocols supported

| For purification of | use either of these protocols |
|---|---|
| tagged proteins[1] | AC<br>AC-DS/GF<br>AC-DS[2]-IEX<br>AC-DS[2]-IEX-DS/GF |
| double-tagged proteins[1] | AC-DS[2]-AC<br>AC-DS[2]-AC-DS/GF |
| tagged proteins in need of desalting or ion exchange first[1] | DS-AC<br>DS-AC-DS/GF<br>IEX-DS[2]-AC<br>IEX-DS[2]-AC-DS/GF |
| proteins with removed tag | AC[3]/DS/IEX/GF<br>DS-IEX<br>IEX-DS/GF<br>DS-IEX-DS/GF<br>IEX-DS[2]-IEX-DS/GF |

[1] *Automated on-column tag cleavage can be performed in protocols starting with AC or IEX.*

[2] *Optional desalting step.*

[3] *Collect the flowthrough fraction as the target protein will not bind to the AC-column.*

## 1.3 Automated on-column tag cleavage

Automated on-column tag cleavage can be performed in protocols starting with AC or IEX.

- In the Purify method plan, check the On-column tag cleavage box on the Purify 1 page to include the cleavage in the method plan.
- Choose Incubation time and select Fill Columns with Cleavage buffer if preferred. To condition the column before the tag cleavage, it can be filled with cleavage buffer before protease is injected.

## 1.4 Column selection

For guidance on column selection and position, see "A1 Columns supported (examples)" on page 20, "A2 Column positions" on page 20 and the User Manual.

### Affinity columns

***1 or 5 ml column***

- Depends on required yield.
- If many purification steps are used, start with a good amount of protein and probably with a 5 ml column.
- For large sample volumes, use a 5 ml column to save time.
- To increase protein purity, overload the column.

### Ion exchange columns

***Required resolution***

The smaller bead size the higher resolution:

- HiTrap™ Q HP and SP HP: 34 µm
- RESOURCE™ Q and S: 15 µm
- Mono Q™ and Mono S™ 5/50 GL: 10 µm

The longer column the higer resolution.

***Anion exchange, e.g., RESOURCE Q***

- The pH of the buffer should be at least 1 pH unit *above* the pI of the protein(s).

***Cation exchange, e.g., RESOURCE S***

- The pH of the buffer should be at least 1 pH unit *below* the pI of the protein(s).

### Desalting columns

***HiTrap or HiPrep***

- Depends on loading volumes:
  - Up to 3 ml: Use 2 × HiTrap Desalting.
  - Up to 13 ml[1]: 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.

***Sephacryl™ S-100, S-200 or S-300***

- Separation range:
  - 1-100 kDa: Use Sephacryl S-100.
  - 5-250 kDa: Use Sephacryl S-200.
  - 10-1500 kDa: Use Sephacryl S-300.

***HiLoad™ and HiPrep 16/60[2] or 26/60[3]***

- Loading volume:
  - up to 5 ml: Use HiLoad or HiPrep 16/60.
  - up to 13 ml[3]: Use HiLoad or HiPrep 26/60.

*[1] If you are expecting peaks larger than 7.5 ml from the affinity step, it is recommended to use a double loop, see "Appendix B Flow charts" on page 24.*

*[2] An optional column holder is available.*

*[3] An optional column holder is available.*

**Tip**

**Grouping samples**

Considerations when running several separation systems in parallel:

To maximize the success rate, run similar proteins on the same system, for example:

- To maximize GF performance, run small proteins <70 kD on one system and large proteins >70kD on another system.
- To maximize IEX performance, run proteins with low pI on one system and proteins with high pI on another system.

## 1.5 Buffer selection

For guidance on buffer selection, see "A4 Buffers" on page 22

## 1.6 Procedures possible to include

It is possible to include many system and column procedures in the purification run, see the list below.
Further explanations can be found in the Method Wizard help texts and in the User Manual.

**Preparations:**

- Fill sample inlet tubing with buffer
- Remove ethanol from system/columns
- Column equilibration
- Column blank run (affinity and ion exchange columns)
- Guided loading of Superloop

**During Purification run:**

- Wash frac tubing between samples
- Clean sample inlet tubing after sample loading
- Clean pump between samples

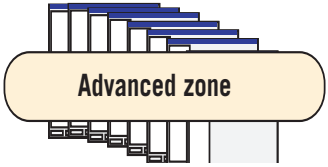
**Post run (prepare for next run or for storage):**

- Fill system/columns with ethanol
- CIP system, then fill with ethanol/buffer
- CIP columns, then fill with ethanol/re-equilibrate
- Re-equilibrate affinity or ion exhange columns
- Strip and recharge AC chelating columns
- Clean sample inlet tubing

## 1.7 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:




- Volumes
- Flow rates
- Gradient and slopes
- 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**

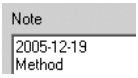
No warning will be given if the flow rate or pressure limit is set higher than the values recommended for the columns used.

### 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.




### 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 **Miscellaneous 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.

### More information about Advanced zone

See ÄKTAxpress User Manual.

# 2 Preparing solutions, system and columns

## 2.1 Print out a summary

A *Summary* can be printed from System Control and it is useful during the preparation of the system, columns and all solutions. The list includes:

- Buffers/solutions, volumes and inlets
- Sample ID, sample volume loaded and inlet position
- Usage of outlet and outlet position
- Type of column, column and column positions
- Loop positions
- Microplate type


### a Select method plan


- In UNICORN™ **System Control**, click **Instant Run**.


- Select the required **Method plan** from the list.

### b Select systems for the run

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

### c Print Summary

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

**Note**: It is also possible to copy the Summary information and paste into, for example, Microsoft® Excel®.

## 2.2 Prepare buffers, solutions and inlets

- Prepare buffers and solutions required for the run (see "A4 Buffers" on page 22).
- Immerse all inlet tubing in the appropriate liquid containers as described in the summary check list.

### Inlets

| | |
|---|---|
| **A1-A4** | See the Summary page for the specific run |
| **A5** | Water |
| **A6** | 0.5 M NaOH |
| **A7** | See the Summary for the specific run |
| **A8** | 20% EtOH |
| **B1-B2** | See the Summary for the specific run |
| **S1-S4** | See the Summary for the specific run |

- Fill the pump and inlet tubing with liquid:

| If... | then... |
|---|---|
| *small amounts of air need to be removed* **or** *buffer needs to be changed* | perform **Fill buffer inlet tubing** *in a* **Prepare and Maintain** *method:* **Standard System and Column Procedures** |
| *inlet tubing is empty* | *perform* **Fill inlets manually using syringe** *in a* **Prepare and Maintain method,** *see page 16* |
| *the system has been unused for a week or longer* **or** *the pump has been run dry* | *perform* **Purge pump with methanol** *in a* **Prepare and, Maintain method,** *see page 16.* |

- Immerse unused tubing in (e.g., 20% ethanol) to avoid air entering the tubing.
- If **Fill Sample inlet Tubings with Buffer** is not included in the method plan to use, make sure the sample inlet tubing is filled with affinity binding buffer or ion exchange buffer, see "5.3 System procedures Manual filling of the inlet tubing" on page 16.

## 2.3 Connect columns and superloop

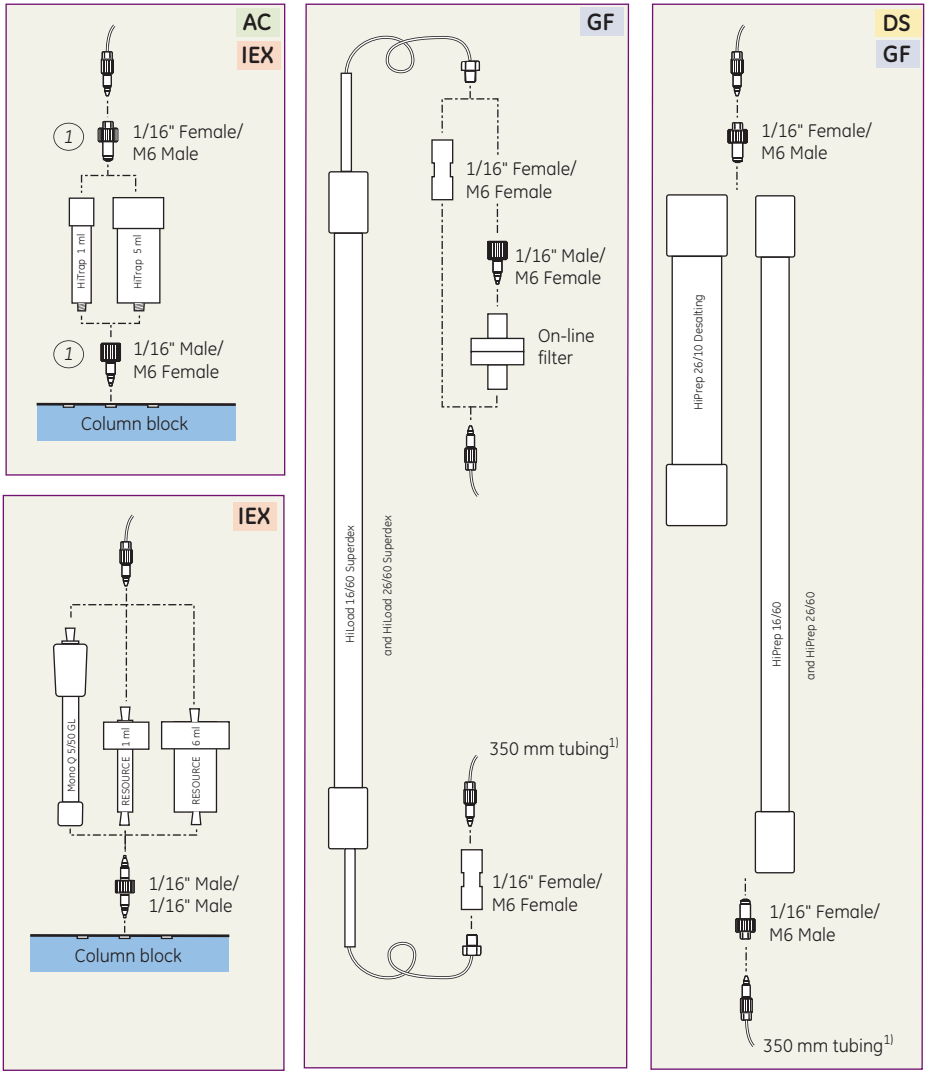
For column positions, see the Summary and "A2 Column positions" on page 20.

### a Remove air before connecting columns

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**.

### *b Connecting tubing to columns*




1 *New models of HiTrap columns do not require the unions.*

**Tip**

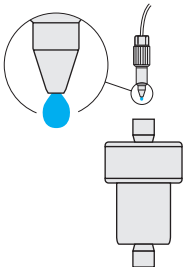
Replace the union's internal capillary with a 1 mm peek tubing (brown) to reduce backpressure, if required.

*1) The standard system tubing (190 mm) should be replaced by the 350 mm capillary tubing (available in the accessory kit) when using a HiPrep 26/10 desalting column or gel filtration column in position 4 or 5.*

## c Column Attachment drop-to-drop

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

- In **Prepare and Maintain,** select **Column Attachment** to create a method plan which starts the pump with a low flow rate in the first column position.
- Click **Continue** to start filling Column position 1.
- Fix the tubing to the column drop-to-drop.
- Click **Next Breakpoint** on system to start filling the next column position.



**Note**: It is also possible to Attach Columns manually by starting a low flow and selecting **System Control:Manual:Flowpath:ColumnPosition**.

## d Column preparations

- If preparations of the columns are not included in the method, prepare them according to the "5.2 Column procedures" on page 15.

  Note: Uncharged IMAC columns must be loaded with metal ions in a Prepare and Maintain run before use to be functional.

## e Superloop connection

### Pre-filling the Superloop with buffer

To avoid pressing air into the system, pre-fill the Superloop with buffer manually before fitting it to the system.

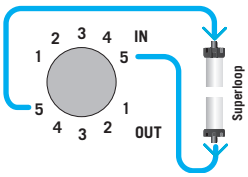
- Remove the upper end pieces.
- Fill the upper chamber with buffer.
- Replace the end pieces and ensure there is no air trapped.
- Turn the Superloop upside down and fill the lower chamber the same way.
- Reinsert the inner end piece and turn it until the slot lines up with the countersink in the glass tube. Replace the other end pieces and ensure there is no air trapped.
- For further information, see Superloop Instruction.

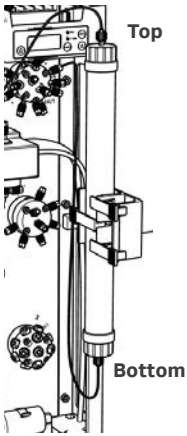
### Connecting the Superloop

- Fit the buffer-filled Superloop in the right hand holder.

**Note**: Make sure the Superloop is not hindering the ejection of the microplate.

- Connect the Superloop to the Loop Valve:
  - Top to **OUT 5**
  - Bottom to **IN 5**




### Filling the Superloop

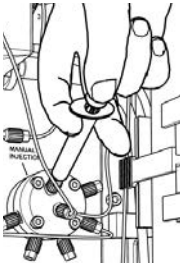
The Superloop is filled with protease/sample with assistance from a **Purify** method plan, if **Guided loading of Superloop** has been selected. To avoid filling before every run, it is also possible to include the filling in a **Prepare and Maintain** method (**Standard System**, and **Column Procedures**).

**Guided loading of Superloop:**

During **Guided loading of Superloop**, dialog boxes will give guidance to filling the Superloop at the beginning of the run. The filling is performed via a syringe connected to the injection valve.

- Follow the dialog boxes and press **Continue** between each step.

  1 Fill syringe with buffer and insert syringe in the manual inject port in injection valve.
  2 Inject buffer.
  3 Fill syringe with protease solution, or sample, and insert syringe in the manual inject port in injection valve.
  4 Inject protease solution, or sample.



Manual filling off-line:

The protease can be injected directly into the Superloop before starting the run.

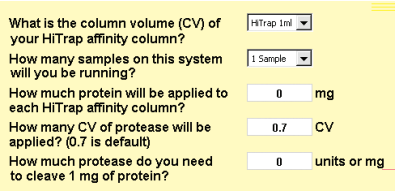
- Connect a syringe to the lower port of the Superloop and fill it. Avoid introducing air bubbles.

### f Filling capillary loop

The capillary loops are filled in the same way as Superloop.

### g Calculating protease amount

- Calculate the volume and concentration of protease needed. Use, for example, the Microsoft Excel file available on the ÄKTAxpress strategy CD.




### h Optional loop volume

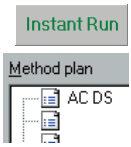
It is possible to increase the loop volume by prolonging each loop with an extra loop tubing. Each total loop volume will then be 20 ml. Following parameters have to be changed in Advanced Zone:

- Max Volume in Each Loop: 15 ml
- Loop Wash Volume: 40 ml
- Flush Volume Empty Loops: 20 ml
- Loop Wash Volume Empty Loops: 40 ml

# 3 Starting a run

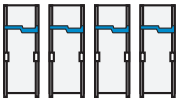
## 3.1 Select method plan

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



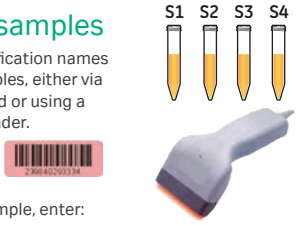

## 3.2 Select systems for the run

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



## 3.3 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 and the location must be specified in **Import File Location** in Advanced zone settings pages, see p8. (See User Manual for further information.)
- Enter optional text, for example culture batch number.

## 3.4 Edit result file location and names

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

## 3.5 Print Summary

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

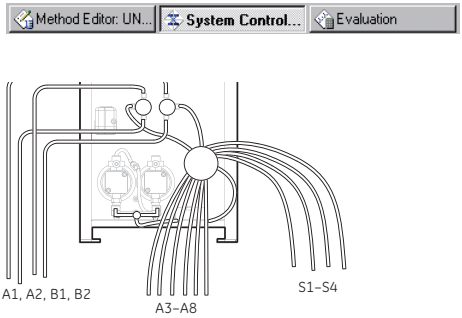
## 3.6 Preparations completed?

Ensure that the preparations according to “2 Preparing solutions, system and columns” on page 9 has been performed

## 3.7 Check the flow path

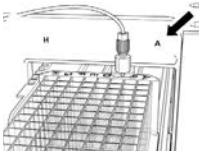
Check against the summary:

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




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

## 3.8 Prepare the microplate



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

## 3.9 Prepare the samples

- The samples should have been prepared and clarified using centrifugation and/or filtration through a 0.45 µm filter. If using crude columns, clarification is not needed.
- Place the sample tubes in the tube or (optional) flask holder *or* inject the sample into the Superloop.
- If **Fill sample inlets** is included in the method plan,
  the inlets should be placed in affinity binding buffer.
  During the run, the system will pause after initial buffer filling, and a message will appear requesting each sample inlet tubing to be gently moved to its sample.
- If **Fill sample inlets** is not included, gently move the
  already filled 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.

## 3.10 Final check


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

## 3.11 Start the run

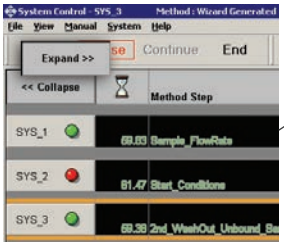
- Click **Run** to start the run on the selected systems.

Be prepared for manual interactions (fill loops, guided loading of Superloop, fill sample inlets etc.).

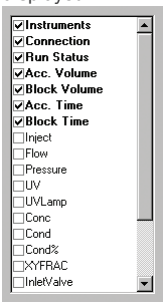
# 4 Monitoring a run


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.**

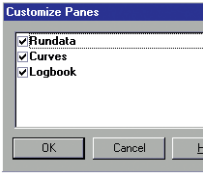



**Run data**
Right-click the view pane and select **Properties** to select run data to be displayed.



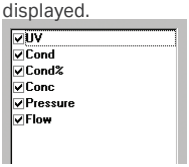

**Panes**

Click Panes to select panes to display.




**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.


**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.

# 5 Column and system procedures

## 5.1 Using a Prepare and Maintain method

**Note**: All preparations, except ***Column Attachment, Fill Inlets Manually Using Syringe, Fill Buffer Inlets, Purge Pump with Methanol*** and ***Rinse All Outlets***, can also be included in a ***Purify*** method.

### 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 **Prepare and Maintain:Standard System and Column Procedures**.
- In **System Procedures**, select as appropriate.
- In the **Column Procedures** pages, select as appropriate. Each column type has its own page.
- 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**.

### Perform the run

- Start the run using Instant Run.

Instant Run

## 5.2 Column procedures

### Equilibrate/re-equilibrate

AC DS IEX GF

Equilibration can be included in a Purification run. The procedure equilibrates the columns with buffer before the samples are loaded onto the columns. All four column types can be equilibrated.

An Equilibration method can also be created in **Prepare and Maintain:Standard System and Column Procedures**.

Customized equilibration can be performed in **Prepare and Maintain:Customized Column Procedures**. The procedure equilibrates up to 5 columns, of one column type, with up to 9 different solutions.

### Affinity blank run

AC

Affinity blank run can be included in a Purification run. If **Remove ethanol** is selected, EtOH will be washed out with water before equilibration.

It is recommended to include a blank run:

- Prior to a first time usage of an affinity column, or
- After metal ion charging.

### Ion exchange blank run

IEX

Ion exchange blank run can be included in a Purification run. The procedure provides ion exchange columns with exchangable counterions.

It is recommended to include a blank run:

- After long-term storage, or
- Prior to a first time usage of an ion exchange column.

AC

### Metal ion stripping (chelating columns)

To remove metal ions before regenerating the HiTrap chelating, HiTrap IMAC and HisTrap™ affinity columns, Metal Ion Stripping can be included in **Purify** or in a **Prepare and Maintain:Standard System and Column Procedures** method plan.

Strip of HisTrap, HiTrap IMAC and HiTrap Chelating (1 ml/5 ml)

| | Step 1 | | Flow [ml/min] | |
|---|---|---|---|---|
| **Procedure** | **Solution** | **Volume** | **RT** | **CR** |
| Strip | His affinity binding buffer with 50mM EDTA | 5 CV | 1/5 | 0.8/4 |
| | **Step 2** | | | |
| Strip | Deionized water | 5–10 CV | 1/5 | 0.8/4 |

### Metal ion charging/re-charging

Metal ion charging/recharging of affinity columns can be performed in a Prepare and Maintain:Standard System and Column Procedures run or included in a Purify run as a post run (in combination with strip). The procedure is used to charge new or stripped HiTrap Chelating HP or HiTrap IMAC HP/FF, or stripped HisTrap HP columns with metal ions (e.g., Ni2+). In a Prepare and Maintain run several columns can be prepared simultaneously.

Note: Put the outlet tubing F11 in a liquid container for collection of any metal waste.

### CIP Column (Cleaning-In-Place)

The purpose of CIP column is to clean contaminated columns.

CIP is normally included in a **Purifiy** method plan as a **Column Post Run**. However, **Customized column procedures** can be performed in a **Prepare and Maintain run**. The procedure cleans up to 5 columns of one type with up to 9 different solutions.

**Note**: Chelating colums have to be stripped before CIP with NaOH

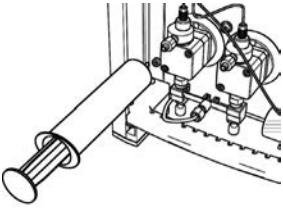
Recommended solutions and inlets are listed in "A5 Recommended solutions and inlets for CIP in Customized column procedures (Prepare and Maintain)" on page 23. For detailed column cleaning procedures and column storage instructions, please refer to the instructions supplied with each column or to the web page: cytiva.com/protein-purification-labresearch

## *5.3 System procedures* Manual filling of the inlet tubing

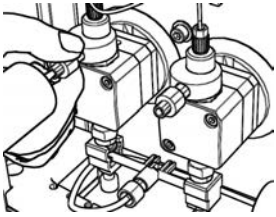
Either use the interactive procedure of a method plan **Fill Inlets Manually Using Syringe** in **Prepare and Maintain** or handle it manually in **System Control** .

Filling inlet tubing S1–S4, A1–A8, and B1-B2 using **Fill Inlets Manually Using Syringe** protocol

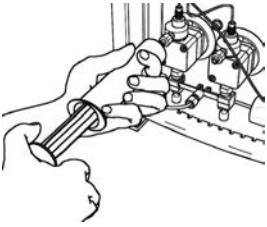
- Immerse tubing in the liquid containers.
- Start the **Fill Inlets Manually Using Syringe** protocol which will start with inlet **S1**.
- Connect a syringe to the purge valve.



- 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.



- Press the **Next Breakpoint** button to turn the inlet valve to the next position, **S2.** Repeat the procedure for **S3–S4, A1–A8** and **B1-B2**.

Filling inlet tubing manually in **System Control**

To fill the inlets manually in **System Control:**

- Select which system to fill by left-clicking on the symbol for the system to the left in System control.
- Set the inlet valve to the appropriate position in **System Control:Manual:Flowpath**.
- Connect the syringe to the purge valve and fill the inlets as described above.
- 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 the valve to turn (a clicking sound) before starting the purging procedure.
- When all inlets are filled, click **End**.

### Purge pump with methanol

(Select on the **Prepare and Maintain** 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.

### Clean sample inlets

Cleaning of the sample inlet tubing can be included in a **Purify** run (**Clean Sample Inlets after Sample Loading**),

**Note**: The procedure is only available for protocols starting with AC/IEX.

After the samples have been loaded, the system will pause and a message will appear on the screen. Sample inlet tubing should then carefully be immersed into a separate bottle containing the appropriate wash solution, (e.g., water or buffer).

### Clean pump

The procedure can be inluded in a **Purify** run (**System Procedures:Clean Pump with 0.5 M NaOH between Loading of Different Samples)**

After a sample has been loaded via the pump, the pump is cleaned with 0.5 M NaOH followed by water and AC/IEX binding buffer.

### Wash frac tubing

The procedure can be inluded in a **Purify** run (**System Procedures:Wash Frac Tubing Between Sample)**

The fraction collector tubing, FracCollF2, will be washed with 2 ml buffer between fractionation of samples. One microplate well will be used for each wash.

### Rinse all outlets

The procedure can be included in a **Prepare and Maintain** method by selecting **Standard System and Column Procedures:Rinse All Outlets.**

All outlet tubing F1-F11, including FracCollF2, will be washed with solution from inlet A1.

> **Note**: A microplate must be inserted in the fraction collector to collect solution from FracCollF2.

### Remove ethanol from the system

Removal of ethanol from the system can be included in a **Purify** run by selecting **Remove Ethanol From System** as **Preparation** on the **System Procedures** page.

In the beginning of the purification run, the system is washed with water. The loops to be used during purification will also be washed.

It is also possible to select the procedure in **Prepare and Maintain** by selecting **Standard System and Column Procedures:Remove Ethanol from System and Loops.**

### Fill system with ethanol

The procedure can be included in a **Purify** run by selecting **Fill System with Ethanol** as **Post Run** on the **System Procedures** page.

After the purification has been completed, the system and the loops used during purification are filled with 20% ethanol.

It is also possible to select the procedure in **Prepare and Maintain** by selecting **Standard System and Column Procedures:Fill System+Loops+Outlets with Ethanol.**

### Clean System

Cleaning of the system is usually named CIP System (Cleaning-In-Place). A CIP method can be created on different pages in the Wizard.

**a Included in a purification run**

A CIP System method can be included in a **Purify** run by selecting **CIP System then Fill with Ethanol** or **CIP System then Fill with Buffer** as **Post Run** on the **System Procedures** page.

**CIP System then Fill with Ethanol:** After the purification run, the system and the loops used during purification are cleaned with 0.5 M NaOH followed by water, affinity binding buffer, water and finally 20% ethanol.

**CIP System then Fill with Buffer:** After the purification run, the system and the loops used during purification are cleaned with 0.5 M NaOH followed by water and affinity binding buffer.

**b Standard method in Prepare and Maintain**

The same type of CIP System methods can be created on the **System Procedures** page in **Prepare and Maintain** by selecting **Standard System and Column Procedures**.

**c Customized method**

Customized system procedures can be performed in a **Prepare and Maintain** run. **Customized System Procedures** 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 inlet tubing should be inserted into a new solution.

> **Note**: Recommended cleaning solutions are 0.5 M NaOH, deoinized water and buffer. When the system will be unused for a longer time, fill the system with water after recommended cleaning solutions and then 20% ethanol as the last step.

# 6 Evaluating the results


When a system has entered **End** status 

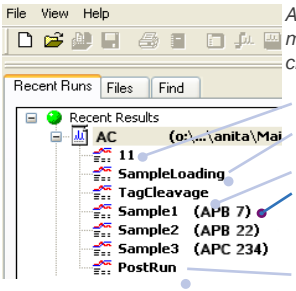
the result can be evaluated and the samples can be pooled.

## 6.1 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.

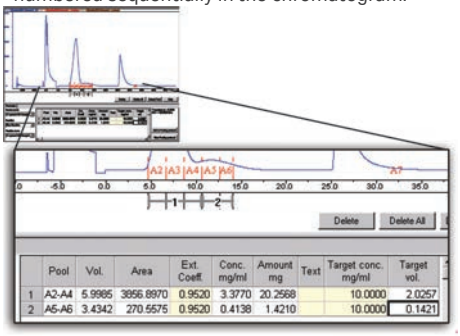



*A result file may includes chromatograms of:*

- equilibration
- sample loading
- tag cleavage
- **purification of sample(s)**
- CIP/fill with ethanol etc. performed after the purification run

## 6.2 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.




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.

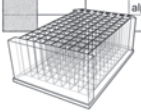
## 6.3 Print or save the Pooling protocol

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).

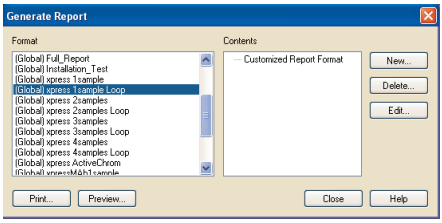
**Pooling protocol**

| 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 |

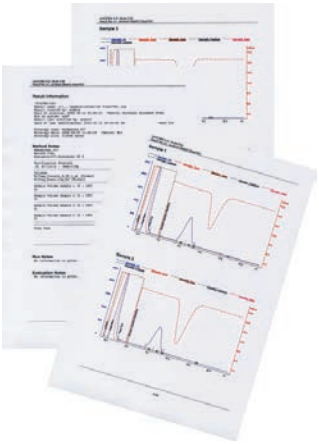


### 6.4 Print the Report

- Select **File:Report** or click the **Report** icon.



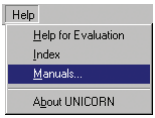

- Select **xpress 1sample...**, **xpress 2samples...**, **xpress 3samples...,** or **xpress 4samples...** report format, depending on how many samples are included in the result file.
- Select **xpress 1sample** when the sample has been loaded with the sample pump.
  Select **xpress 1sample Loop** when the sample has been loaded from a loop or Superloop.
- The report format **xpressActiveChrom** can be selected if only the active zoomed window in the Evaluation module should be included in the report.
- Click **Edit** or **Preview** to inspect and modify the report format if needed.
- Click the **Print** button. Select the printer from the Main UNICORN window.
- Choose which pages and how many copies to print and click **OK.**



# Getting help

## Manuals and Cue Cards

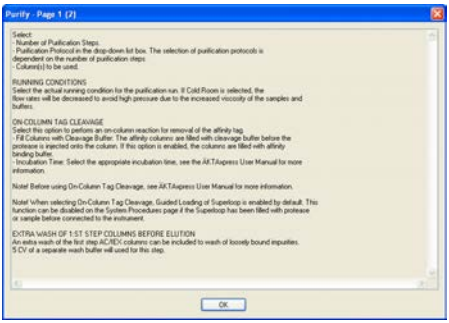
- Open the **Help** menu and select
  **Manuals** to access the :
  - UNICORN User Manual
  - ÄKTAxpress User Manual
  - ÄKTAxpress Cue Cards




## Context-specific Help

- Click the **Help** button in the dialog box, or press **F1**.


Example of Method Editor wizard help:



# Appendix A

## A1 Columns supported (examples)

| Column | Volume [ml] |
|---|---|
| Any HiTrap AC [1] | 1 or 5 |
| GSTrap™ FF | 1 or 5 |
| GSTrap 4B | 1 or 5 |
| GSTrap HP | 1 or 5 |
| HisTrap HP | 1 or 5 |
| HisTrap FF | 1 or 5 |
| HisTrap FF Crude | 1 or 5 |
| HiTrap Chelating HP | 1 or 5 |
| HiTrap IMAC HP | 1 or 5 |
| HiTrap IMAC FF | 1 or 5 |
| 2 × HiTrap Desalting | 10 |
| HiPrep 26/10 Desalting | 53 |
| Any HiTrap IEX [2] | 1 or 5 |
| HiTrap Q HP | 1 or 5 |
| HiTrap SP HP | 1 or 5 |
| MonoQ 5/50 GL | 1 |
| MonoS 5/50 GL | 1 |
| RESOURCE Q | 1 or 6 |
| RESOURCE S | 1 or 6 |
| HiLoad 16/600 Superdex 75 or 200 prep grade | 120 |
| HiLoad 26/600[3] Superdex 75 or 200 prep grade | 320 |
| HiPrep 16/60[4] Sephacryl S-100 or S-200 or S-300 HR | 120 |
| HiPrep 26/60 Sephacryl S-100 or S-200 or S-300 HR | 320 |

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

*[2] Any HiTrap IEX means that any HiTrap ion exchange column can be used.*

*[3] An optional column holder is available.*

*[4] An optional column wrap is available.*

## A3 Typical run times

To estimate the total run time, see the Summary. Examples of estimated run times for some different types of runs are shown below.

### A3.1 Typical run times for the different protocols

| Protocol | Max. No. of samples | Typical run time[1] (h) |
|---|---|---|
| **1 Step** | | |
| AC/IEX | 4 | 2.5 |
| DS | 4 | 0.5 |
| GF | 4 | 14 |
| **2 Step** | | |
| AC/IEX-DS | 4 | 4.5 |
| AC/IEX-GF | 4 | 18 |
| DS-AC/IEX | 2 | 3 |
| **3 Step** | | |
| AC/IEX-(DS)-AC/IEX | 3 | 7 |
| DS-AC/IEX-DS | 2 | 4 |
| DS-AC/IEX-GF | 2 | 11 |
| **4 Step** | | |
| AC/IEX-(DS)-AC/IEX-DS | 2 | 5.5 |
| AC/IEX-(DS)-AC/IEX-GF | 2 | 12.5 |

*[1] Run times are approximate and are valid at room temperature. Sample loading time is not included. The largest columns have been used. AC: 5 ml, DS: HiPrep 26/10 Desalting. IEX: 6 ml, GF: HiPrep 26/60 Sephacryl,*

## A2 Column positions

| *Protocol* | | *Column position* | | | | | *Max no of samples* | *On-column tag cleavage possible* |
|---|---|---|---|---|---|---|---|---|
| | | *1* | *2* | *3* | *4* | *5* | | |
| *1-step* | *AC/IEX* | *AC/IEX* | *AC/IEX* | *AC/IEX* | *AC/IEX* | *-* | *4* | *Yes* |
| | *DS/GF* | *-* | *-* | *-* | *-* | *DS/GF* | *4[1]* | *No* |
| *2-step* | *AC-DS/GF* | *AC* | *AC* | *AC* | *AC* | *DS/GF* | *4* | *Yes* |
| | *IEX-DS/GF* | *IEX* | *IEX* | *IEX* | *IEX* | *DS/GF* | *4* | *Yes[2]* |
| | *DS-AC/IEX* | *AC/IEX* | *-* | *-* | *-* | *DS* | *2* | *No* |
| *3-step* | *AC-DS-AC/IEX* | *AC* | *AC* | *AC* | *AC(2)/IEX* | *DS* | *3* | *Yes* |
| | *IEX-DS-AC/IEX* | *IEX* | *IEX* | *IEX* | *AC/IEX(2)* | *DS* | *3* | *Yes[2]* |
| | *DS-AC/IEX-DS/GF* | *AC/IEX* | *-* | *-* | *DS(1)* | *DS(2)/GF* | *2* | *No* |
| *4-step* | *AC-DS-IEX-DS/GF* | *AC* | *AC* | *IEX* | *DS(1)* | *DS(2)/GF* | *2* | *Yes* |
| | *AC-DS-AC-DS/GF* | *AC* | *AC* | *AC(2)* | *DS(1)* | *DS(2)/GF* | *2* | *Yes* |
| | *IEX-DS-AC-DS/GF* | *IEX* | *IEX* | *AC* | *DS(1)* | *DS(2)/GF* | *2* | *Yes[2]* |
| | *IEX-DS-IEX-DS/GF* | *IEX* | *IEX* | *IEX(2)* | *DS(1)* | *DS(2)/GF* | *2* | *Yes[2]* |

*[1] Or one sample injected four times when using Superloop*

*[2] Note: This may not be a useful protocol for on-column tag-cleavage if a tagged protease is used.*

## A3.2 Typical run times for Included System procedures

| SYSTEM PROCEDURES | Typical run time[1] (min) |
|---|---|
| **Preparation** | |
| Remove EtOH | 10 |
| **During Run** | |
| Clean Sample inlets (4 samples) | 5 |
| Wash Frac Tubing (4 samples) | 5 |
| **Post Run** | |
| Fill with EtOH | 10 |
| CIP then fill with EtOH | 35 |
| CIP then fill with Buffer | 25 |

[1] *Run times are approximate and are valid at room temperature.*

## A3.3 Typical run times for Included Column procedures

| COLUMN PROCEDURES | Typical run time[1] /column (min) |
|---|---|
| **Preparation** | |
| AC Equilibration | 5 |
| AC Blank run | 25 |
| AC Remove EtOH before preparation | 5 |
| DS Equilibration | 10 |
| DS Remove EtOH before preparation | 5 |
| IEX Equilibration | 5 |
| IEX Blank run | 25 |
| IEX Remove EtOH before preparation | 5 |
| GF Equilibration | 325 |
| GF Remove EtOH before preparation | 155 |
| **Post run** | |
| AC Fill with EtOH | 5 |
| AC CIP Fill with EtOH | 25 |
| AC CIP+ Re-equilibrate | 20 |
| AC Re-equilibrate | 5 |
| AC Strip+ Fill with EtOH | 10 |
| AC Strip+ Recharge+ Blank Run | 40 |
| AC Strip+ Recharge+ Fill with EtOH | 20 |
| DS Fill with EtOH | 25 |
| DS CIP Fill with EtOH | 50 |
| DS CIP+Re-equilibrate | 25 |
| IEX Fill with EtOH | 5 |
| IEX CIP Fill with EtOH | 30 |
| IEX CIP+Re-equilibrate | 20 |
| IEX Re-equilibrate | 5 |
| GF Fill with EtOH | 485 |
| GF CIP Fill with EtOH | 1130 |
| GF CIP+Re-equilibrate | 650 |

[1] *Run times are approximate and are valid at room temperature. Sample loading time is not included. The largest columns have been used. AC: 5 ml, DS: HiPrep 26/10 Desalting. IEX: 6 ml, GF: HiPrep 26/60 Sephacryl,*

## A4 Buffers

### Buffer types needed

| | | |
|---|---|---|
| for AC: | • binding buffer<br>• cleavage buffer (optional) | • elution buffer<br>• extra wash buffer (optional) |
| for DS: | • one buffer type per desalting step | |
| for IEX: | • binding buffer | • elution buffer |
| for GF: | • one buffer type per run | |

### AC buffer suggestions for some His-tagged proteins

| *If performing...* | *suggested buffer* |
|---|---|
| ***binding** using HisTrap HP/FF, HisTrap FF crude, HiTrap IMAC HP/FF* | *20 mM sodium phosphate, pH 7.4, 0.5 M NaCl, 20–40 mM imidazole [1)]* |
| ***binding** using HiTrap Chelating* | *20 mM sodium phosphate, pH 7.4, 0.5 M NaCl, 10-20 mM imidazole[1]* |
| ***extra wash*** | *20 mM sodium phosphate, pH 7.4, 0.5 M NaCl, 50 mM imidazole* |
| ***cleavage** using TEV protease on HisTrap HP or HiTrap Chelating HP[2]* | *20 mM sodium phosphate, pH 7.4, 0.5 M NaCl, 50 mM imidazole* |
| *step or gradient **elution*** | *20 mM sodium phosphate, pH 7.4, 0.5 M NaCl, 500 mM imidazole* |

### AC buffer suggestions for GST-tagged proteins

| *If performing...* | *suggested buffer* |
|---|---|
| ***binding** using GSTrap HP/FF/4B* | *10 mM sodium phosphate, pH 7.4, 140 mM NaCl, 1-10 mM DTT[3]* |
| ***cleavage** using PreScission™ protease on GSTrap HP or FF[3]* | *50 mM Tris-HCl pH 7.5, 150 mM NaCl, 1 mM EDTA, 1 mM DTT* |
| *step or gradient* | *50 mM Tris-HCl,* |

### DS buffer suggestions

| *If preparing for...* | *suggested buffer* |
|---|---|
| *AC* | *Use the AC binding buffer that will be used in the following step* |
| *IEX* | *Use the IEX binding buffer that will be used in the following step* |
| *protein storage* | *include e.g., 10% glycerol in a suitable buffer, (e.g., 50 mM Tris-HCl, pH 7.5, 150 mM NaCl).* |

### IEX buffer suggestions

| *If for example...* | *suggested buffer (depends on protein pI)* |
|---|---|
| *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 preparing for...* | *suggested buffer* |
|---|---|
| *further studies* | *a suitable buffer, e.g., 50 mM Tris-HCl pH 7.5, 150 mM NaCl* |
| *protein storage* | *include e.g.. 10% glycerol in a suitable buffer (e.g.. 50 mM Tris-HCl pH 7.5, 150 mM NaCl)* |

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

*[2] In the affinity tag removal protocols, the affinity columns can be equilibrated with either AC-binding/wash buffer or an alternative cleavage buffer prior to protease injection.*

*[3] DTT enhance GST-binding.*

*[4] Higher amounts of reduced glutathions may give sharper elution peaks.*

**Tip**

**Buffer volumes required for a run**

See the Summary for the specific run (see "2.1 Print out a summary" on page 9).

For other solutions (metal ion charging etc): see "5.2 Column procedures" on page 15.

**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.

## A5 Recommended solutions and inlets for CIP in Customized column procedures (Prepare and Maintain)

AC DS IEX GF

Note: Ensure that the columns are filled with water prior to CIP. If required, select **Wash Columns with Water before CIP**.
If the columns will not be used for some days, fill with 5 CV 20% ethanol (HiTrap SP and RECOURCE S: 20% ethanol, 0.2 M sodium acetate), as step 9.

| | Step 1 | | | Flow [ml/min] | | Step 2 | | | Flow [ml/min] | |
|---|---|---|---|---|---|---|---|---|---|---|
| Column examples | Solution | Inlet | Volume | RT | CR | Solution | Inlet | Volume | RT | CR |
| HisTrap HP/FF/FF crude, HiTrap Chelating/IMAC HP/IMAC FF (1 ml / 5 ml) | 1 M NaOH | A7 | 1 CV[1] | 1/5 | 0.8/4 | Deionized water | A5 | 10 CV | 1/5 | 0.8/4 |
| GSTrap HP/FF/4B (1 ml / 5 ml) | 6 M GuaHCl | A7 | 2 CV | 1/5 | 0.8/4 | GST A-buffer | A1 | 5 CV | 1/5 | 0.8/4 |
| HiTrap Desalting | 1mg pepsin[4]/ml in 0.1 M acetic acid, 0.5 M NaCl | A7 or | 1 CV[2] | 5 | 4 | 0.2 M NaOH | A3 | 2 CV | 5 | 4 |
| HiPrep 26/10 Desalting | 1mg pepsin[4]/ml in 0.1 M acetic acid, 0.5 M NaCl | A7 or | 1 CV[2] | 10 | 8 | 0.2M NaOH | A3 | 2 CV | 10 | 8 |
| HiTrap Q/SP HP (1ml / 5ml) | 2 M NaCl | B2 | 4 CV | 0.25/1.3 | 0.20/1 | Deionized water | A5 | 2 CV | 0.25/1.3 | 0.20/1 |
| RESOURCE Q/S (1 ml / 6 ml) | 1 M NaCl | A2 | 5 CV | 4/6 | 3.2/4.8 | Deionized water | A5 | 2 CV | 4/6 | 3.2/4.8 |
| Mono Q/S | 1 M NaCl | A2 | 4 CV | 0.5 | 0.4 | Deionized water | A5 | 2 CV | 0.5 | 0.4 |
| HiLoad Superdex[5] pg (16/600 / 26/600) | 1 M NaOH | A7 | 4 CV | 0.5/1.3 | 0.4/1 | Deionized water | A5 | 4 CV | 0.5/1.3 | 0.4/1 |
| HiPrep Sephacryl[6] HR (16/60 / 26/60) | 0.5 M NaOH | A6 | 0.25 CV | 0.3/0.8 | 0.2/0.6 | Deionized water | A5 | 4 CV | 0.3/0.8 | 0.2/0.6 |

| | Step 3 | | | | | Step 4 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| HisTrap HP/FF/FF crude, HiTrap Chelating/IMAC HP/IMAC FF (1 ml / 5 ml) | 30% isopropanol | A4 | 10 CV | 0.5/2.5 | 0.4/2 | Deionized water | A5 | 10 CV | 1/5 | 0.8/4 |
| GSTrap HP/FF/4B (1 ml / 5 ml) | 1% Triton X-100[3] | A7 | 2 CV | 1/5 | 0.8/4 | GST A-buffer | A1 | 5 CV | 1/5 | 0.8/4 |
| HiTrap Desalting | Deionized water | A5 | 5 CV | 7.5 | 6 | | | | | |
| HiPrep 26/10 Desalting | Deionized water | A5 | 5 CV | 15 | 12 | | | | | |
| HiTrap Q/SP HP (1ml / 5ml) | 1 M NaOH | A7 | 4 CV | 0.25/1.3 | 0.20/1 | Deionized water | A5 | 4 CV | 0.25/1.3 | 0.20/1 |
| RESOURCE Q/S (1 ml / 6 ml) | 1 M NaOH | A7 | 5 CV | 4/6 | 3.2/4.8 | Deionized water | A5 | 2 CV | 4/6 | 3.2/4.8 |
| Mono Q/S | 1 M NaOH | A7 | 4 CV | 0.5 | 0.4 | Deionized water | A5 | 2 CV | 0.5 | 0.4 |
| HiLoad Superdex[5] pg (16/600 / 26/600) | 30% isopropanol | A4 | 0.5 CV | 0.5/1.3 | 0.4/1 | Deionized water | A5 | 2 CV | 0.5/1.3 | 0.4/1 |
| HiPrep Sephacryl[6] HR (16/60 / 26/60) | 30% isopropanol | A4 | 0.5 CV | 0.3/0.8 | 0.2/0.6 | Deionized water | A5 | 4 CV | 0.3/0.8 | 0.2/0.6 |

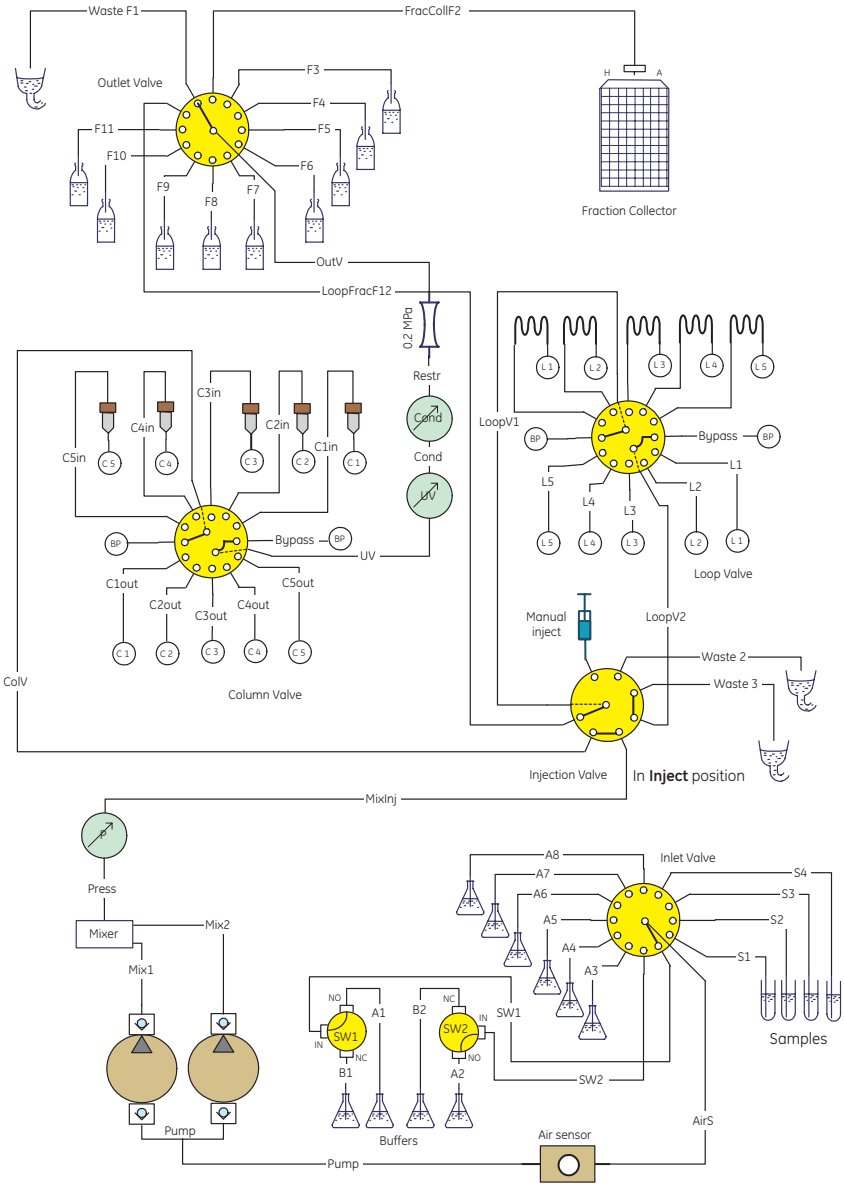
| | Step 5 | | | | | Step 6 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| HisTrap HP/FF/FF crude, HiTrap Chelating/IMAC HP/IMAC FF (1 ml / 5 ml) | 2 M NaCl | B2 | 1 CV | 1/5 | 0.8/4 | Deionized water | A5 | 10 CV | 1/5 | 0.8/4 |
| HiTrap Q/SP HP (1ml / 5ml) | 30% isopropanol | A4 | 2 CV | 0.25/1.3 | 0.20/1 | Deionized water | A5 | 4 CV | 0.25/1.3 | 0.20/1 |
| RESOURCE Q/S (1 ml / 6 ml) | 1 M HCl | A1 | 5 CV | 4/6 | 3.2/4.8 | Deionized water | A5 | 2 CV | 4/6 | 3.2/4.8 |
| Mono Q/S | 1 M HCl | A1 | 4 CV | 0.5 | 0.4 | Deionized water | A5 | 2 CV | 0.5 | 0.4 |

| | Step 7 | | | | | Step 8 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| RESOURCE Q/S (1 ml / 6 ml) | 1 M NaCl | A2 | 5 CV | 4/6 | 3.2/4.8 | Deionized water | A5 | 2 CV | 4/6 | 3.2/4.8 |
| Mono Q/S | 1M NaCl | A2 | 4 CV | 0.5 | 0.4 | Deionized water | A5 | 2 CV | 0.5 | 0.4 |

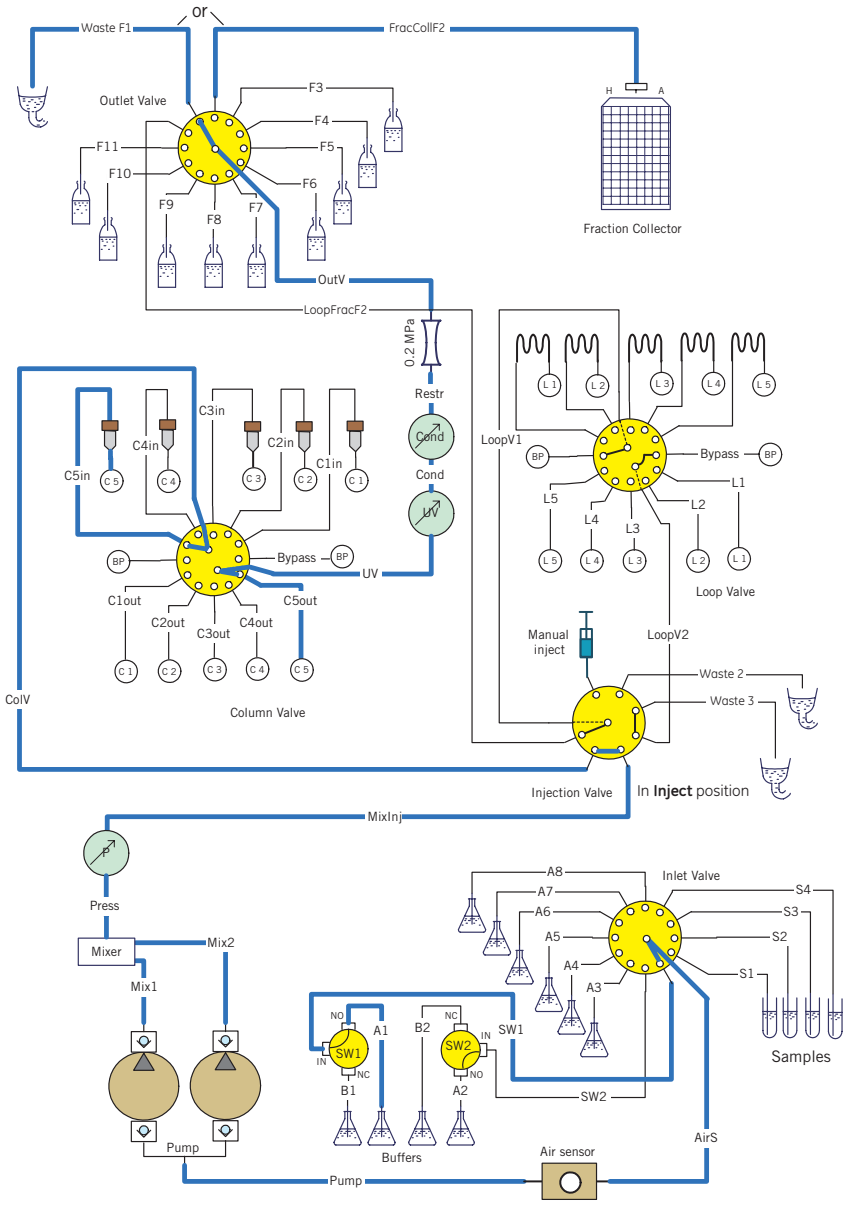
*[1] Pause 1 hour.*
*[2] Pause over night at RT.*
*[3] Alternatively 3–4 CV 70% ethanol.*
*[4] If pepsin is used, ensure it has been completely eluted or has been denatured by NaOH.*
*[5] HiLoad 16/600 and 26/600, Superdex 75 and 200 columns.*
*[6] HiPrep 16/60 and 26/60, S-100, S-200 and S-300 columns.*

# Appendix B Flow charts

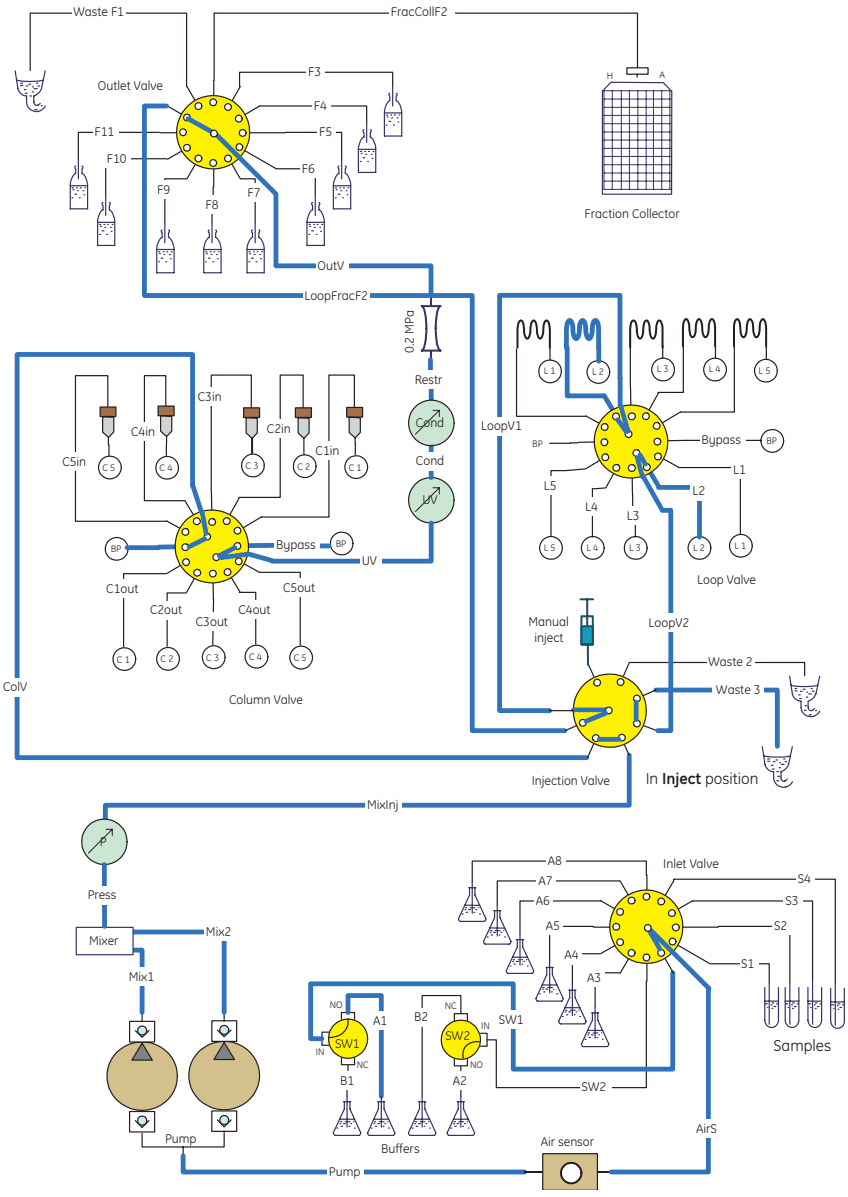
- Valve positions after reset (END), no flow



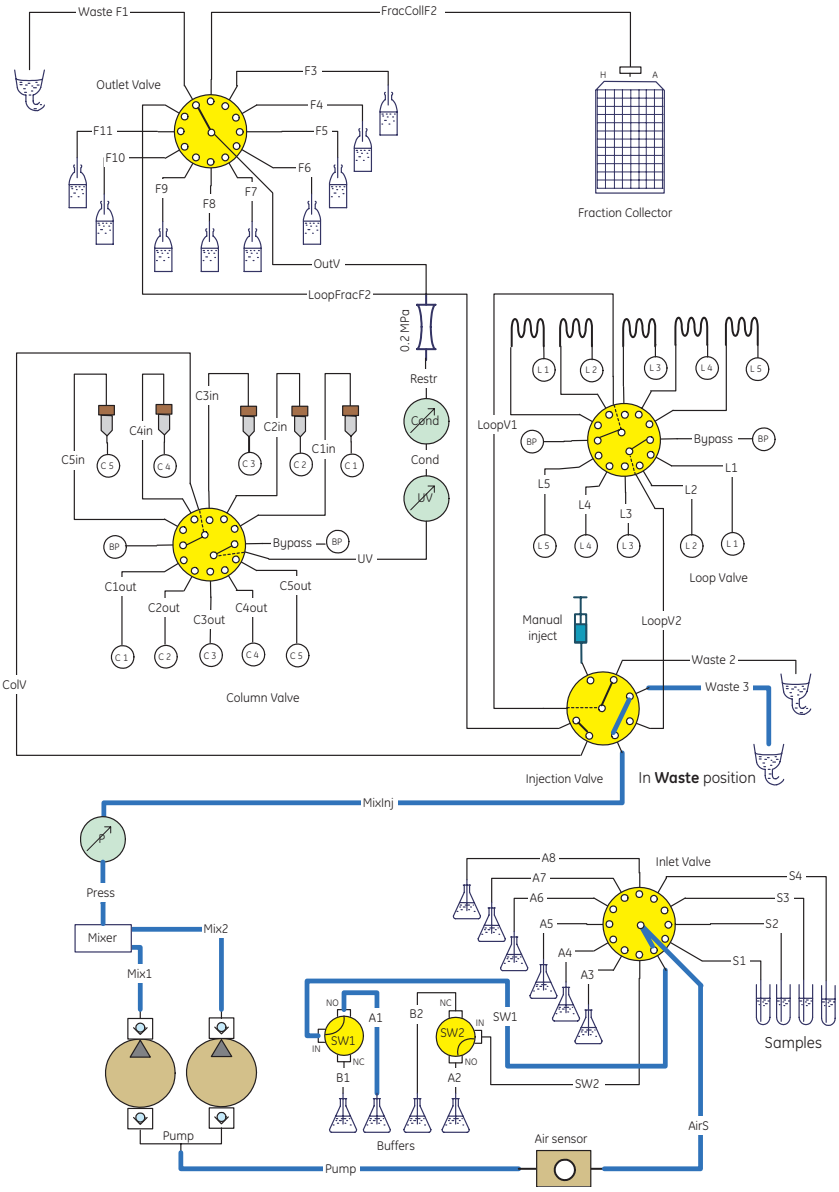
- Column equilibration
- Sample loading
- Wash out unbound sample
- Elution



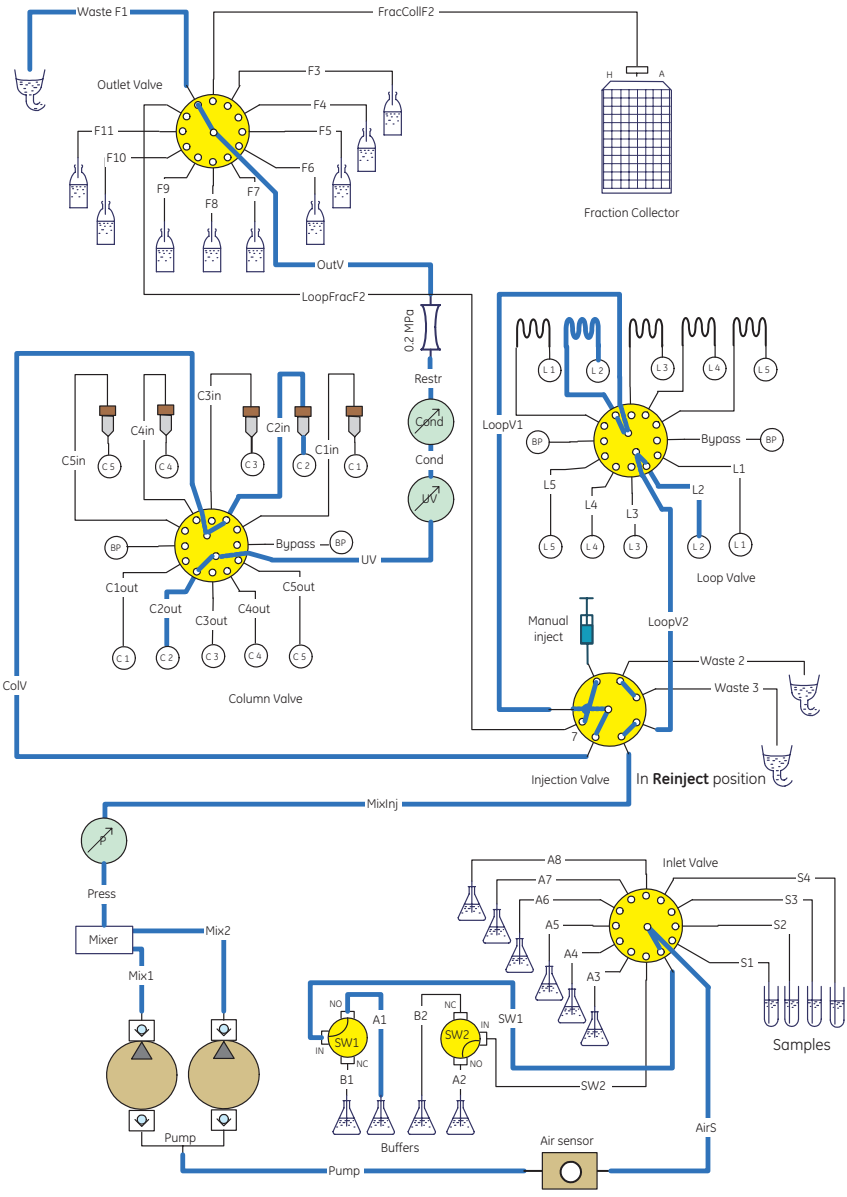
- Intermediate peak collection in capillary loops
- System wash

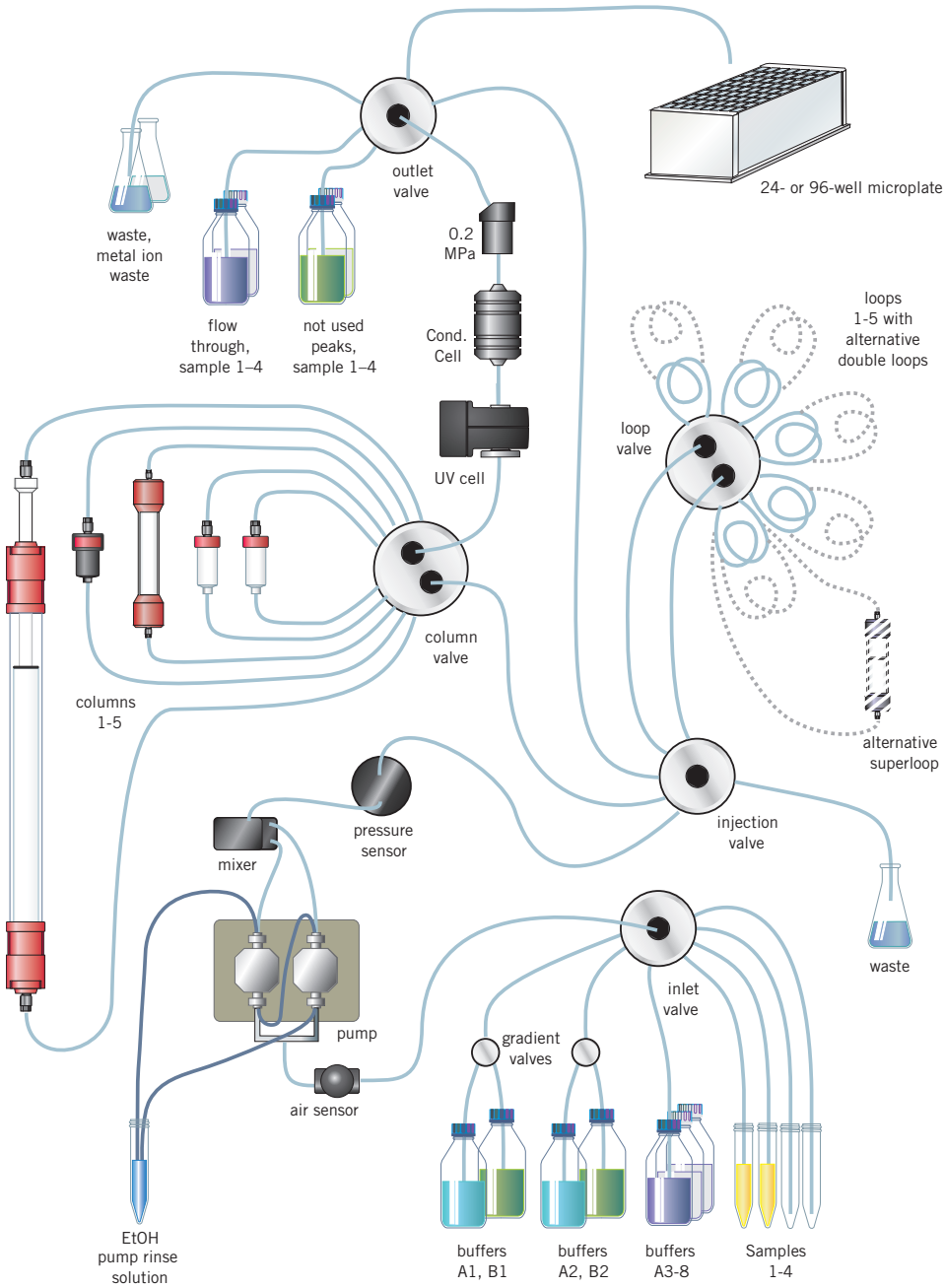


• Pump wash


Waste F1
FracCollF2
Outlet Valve
F3
F4
F5
F6
F7
F8
F9
F10
F11
Fraction Collector
OutV
LoopFracF2
0.2 MPa
Restr
Cond
UV
C5in
C4in
C3in
C2in
C1in
Bypass
C1out
C2out
C3out
C4out
C5out
Column Valve
ColV
LoopV1
Bypass
Loop Valve
Manual inject
LoopV2
Waste 2
Waste 3
Injection Valve
In Waste position
MixInj
Press
Mixer
Mix1
Mix2
Pump
Inlet Valve
Samples
SW1
SW2
Buffers
Air sensor
AirS
Pump

- Sample loading from loops
- Loop wash




outlet valve
waste, metal ion waste
flow through, sample 1–4
not used peaks, sample 1–4
0.2 MPa
Cond. Cell
UV cell
24- or 96-well microplate
loops 1-5 with alternative double loops
loop valve
alternative superloop
column valve
columns 1-5
injection valve
waste
pressure sensor
mixer
pump
air sensor
inlet valve
gradient valves
EtOH pump rinse solution
buffers A1, B1
buffers A2, B2
buffers A3-8
Samples 1-4

# Ordering information

| Product | Pack Size | Code Number |
|---|---|---|
| **Systems** | | |
| ÄKTAxpress | 1 | 18-6645-01 |
| USB/CAN device[3] | 1 | 28-9692-01 |
| ÄKTAxpress software & manuals[1] | 1 | 28-9053-45 |
| **Accessories** | | |
| Flask holder | 1 | 18-1177-79 |
| Large column holder ÄKTAxpress | 1 | 28-4007-37 |
| Capillary loop | 1 x 10 ml | 11-0003-02 |
| Red fingertights | 8 | 28-4010-81 |
| Tubing 1 mm diameter | 1 x 3 m | 18-1142-38 |
| Tubing 1.6 mm diameter | 1 x 3 m | 18-1121-16 |
| HiPrep 16/xx Column Wrap | 1 | 28-9021-50 |
| Union 1/16" female to 1/16" female | 1 | 11-0003-39 |
| Union M6 male to 1/16" female | 1 | 18-1112-57 |
| Union M6 female to 1/16" male | 1 | 18-1112-58 |
| Union M6 female to 1/16" female (PEEK) | 1 | 18-1123-94 |
| On-line filter | 1 | 18-1112-44 |
| **Supported columns** | | |
| ***Affinity chromatography*** | | |
| GSTrap FF | 5 x 1 ml | 17-5130-01 |
| GSTrap FF | 100 x 1 ml[2] | 17-5130-05 |
| GSTrap FF | 5 x 5 ml | 17-5131-02 |
| GSTrap FF | 100 x 5 ml[2] | 17-5131-05 |
| GSTrap 4B | 5 x 1 ml | 28-4017-45 |
| GSTrap 4B | 5 x 5 ml | 28-4017-48 |
| GSTrap 4B | 100 x 1 ml[2] | 28-4017-46 |
| GSTrap 4B | 100 x 5 ml[2] | 28-4017-49 |
| GSTrap HP | 5 x 1 ml | 17-5281-01 |
| GSTrap HP | 100 x 1 ml[2] | 17-5281-05 |
| GSTrap HP | 5 x 5 ml | 17-5282-02 |
| GSTrap HP | 100 x 5 ml[2] | 17-5282-05 |
| HisTrap excel | 5 x 1 ml | 17-3712-05 |
| HisTrap excel | 5 x 5 ml | 17-3712-06 |
| HisTrap HP | 5 x 1 ml | 17-5247-01 |
| HisTrap HP | 100 x 1 ml[2] | 17-5247-05 |
| HisTrap HP | 5 x 5 ml | 17-5248-02 |
| HisTrap HP | 100 x 5 ml[2] | 17-5248-05 |
| HisTrap FF | 5 x 1 ml | 17-5319-01 |
| HisTrap FF | 5 x 5 ml | 17-5255-01 |
| HisTrap FF | 100 x 1 ml[2] | 17-5319-02 |
| HisTrap FF | 100 x 5 ml[2] | 17-5255-02 |
| HisTrap FF Crude | 5 x 1 ml | 11-0004-58 |
| HisTrap FF Crude | 5 x 5 ml | 17-5286-01 |
| HisTrap FF Crude | 100 x 1 ml[2] | 11-0004-59 |
| HisTrap FF Crude | 100 x 5 ml[2] | 17-5286-02 |
| HiTrap Chelating | 5 x 1 ml | 17-0408-01 |
| HiTrap Chelating | 5 x 5 ml | 17-0409-03 |
| HiTrap Chelating | 100 x 5 ml[2] | 17-0409-05 |
| HiTrap IMAC HP | 5 x 1 ml | 17-0920-03 |
| HiTrap IMAC HP | 5 x 5 ml | 17-0920-05 |
| HiTrap IMAC FF | 5 x 1 ml | 17-0921-02 |
| HiTrap IMAC FF | 5 x 5 ml | 17-0921-04 |
| HiTrap TALON® crude | 5 x 1 ml | 28-9537-66 |
| HiTrap TALON crude | 5 x 5 ml | 28-9537-67 |
| HiTrap TALON crude | 100 x 1 ml[2] | 28-9538-05 |
| HiTrap TALON crude | 100 x 1 ml[2] | 28-9538-09 |
| ***Desalting*** | | |
| HiPrep 26/10 Desalting | 1 x 53 ml | 17-5087-01 |
| HiPrep 26/10 Desalting | 4 x 53 ml | 17-5087-02 |
| HiTrap Desalting | 5 x 5 ml | 17-1408-01 |
| HiTrap Desalting | 100 x 5 ml[2] | 11-0003-29 |
| ***Ion Exchange chromatography*** | | |
| HiTrap Q HP | 5 x 1 ml | 17-1153-01 |
| HiTrap Q HP | 5 x 5 ml | 17-1154-01 |
| HiTrap SP HP | 5 x 1 ml | 17-1151-01 |
| HiTrap SP HP | 5 x 5 ml | 17-1152-01 |
| Mono Q 5/50 GL | 1 x 1 ml | 17-5166-01 |
| Mono S 5/50 GL | 1 x 1 ml | 17-5168-01 |
| RESOURCE Q | 1 x 1 ml | 17-1177-01 |
| RESOURCE Q | 1 x 6 ml | 17-1179-01 |
| RESOURCE S | 1 x 1 ml | 17-1178-01 |
| RESOURCE S | 1 x 6 ml | 17-1180-01 |

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

[1] *Includes both ÄKTAxpress and ÄKTAxpress Mab software.*

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

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

| | | |
|---|---|---|
| ***Gel filtration*** | | |
| *HiLoad 16/600 Superdex 75 prep grade* | *1 x 120 ml* | *17-1068-01* |
| *HiLoad 16/600 Superdex 200 prep grade* | *1 x 120 ml* | *17-1069-01* |
| *HiLoad 26/600 Superdex 75 prep grade* | *1 x 320 ml* | *17-1070-01* |
| *HiLoad 26/600 Superdex 200 prep grade* | *1 x 320 ml* | *17-1071-01* |
| *HiPrep 16/60 Sephacryl S-100 HR* | *1 x 120 ml* | *17-1165-01* |
| *HiPrep 16/60 Sephacryl S-200 HR* | *1 x 120 ml* | *17-1166-01* |
| *HiPrep 16/60 Sephacryl S-300 HR* | *1 x 120 ml* | *17-1167-01* |
| *HiPrep 26/60 Sephacryl S-100 HR* | *1 x 320 ml* | *17-1194-01* |
| *HiPrep 26/60 Sephacryl S-200 HR* | *1 x 320 ml* | *17-1195-01* |
| *HiPrep 26/60 Sephacryl S-300 HR* | *1 x 320 ml* | *17-1196-01* |
| ***Superloop*** | | |
| *Superloop* | *1 x 10 ml* | *18-1113-81* |
| *Superloop* | *1 x 50 ml* | *18-1113-82* |

## Proteases

| | | |
|---|---|---|
| *PreScission Protease* | *500 units* | *27-0843-01* |
| *Thrombin* | *500 units* | *27-0846-01* |
| *Factor Xa* | *400 units* | *27-0849-01* |

## Documents

| | | |
|---|---|---|
| *UNICORN user manual* | *1* | *11-0003-68* |
| *ÄKTAxpress User Manual* | *1* | *28-4090-22* |
| *ÄKTAxpress Operating instructions* | *1* | *28-9579-08* |
| *ÄKTAxpress Installation Guide* | *1* | *28-4090-29* |
| *Superloop Instructions* | *1* | *56-3015-99* |
| *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* |
| *Ion Exchange Chromatography & Chromatofocusing* | *1* | *11-0004-21* |

*All documents can be viewed at:*
*cytiva.com*

cytiva

cytiva.com



28409023 AC 04/2021