Desalting using **ÄKTA** start

Training cue card

This protocol will help you understand the practical principles of desalting chromatography by taking you step-by-step through the desalting of Bovine Serum Albumin (BSA).

Requirements

- ÄKTA™ start system
- Frac30 fraction collector
- Desalting Buffer (DS buffer): 25 mM sodium phosphate, 150 mM NaCl, pH 7.5 (Prepare at least 200 mL of buffer)
- Sample: BSA 1 mg/mL in 25 mM sodium phosphate buffer, 0.5 M NaCl, pH 7.5. (Prepare 5 mL of sample)
- Column: HiTrap™ Desalting 5 mL
- Fraction tubes: 1.5 mL microcentrifuge tubes
- 1 mL Sample loop
- USB 2.0 memory stick

Checklist

- Ensure the Frac30 fraction collector is connected to the ÄKTA start instrument.
- Ensure the pump tube is properly inserted in the pump head and the pump cover is closed properly.
- Ensure there is no column connected in the flow path while preparing the system for a run.
- If the system or column is stored in ethanol, wash with water prior to starting the run.

Preparing the system

Step	Action
1	Place the bottle containing the DS buffer in the buffer tray on top of the instrument.
2	Immerse buffer inlet A in the bottle containing DS buffer.
3	Place the waste bottle on the right side of the instrument. ***Note:*** *The waste tubing (from Wash valve, Manual injection valve and Outlet valve) should be inserted into the waste bottle as shown in Fig. 1, on page 1.*

Step	Action

Fig 1. ÄKTA start instrument with Frac30 fraction collector.

4 Power ***ON*** the ÄKTA start instrument.

Note:
Enable Frac30 from the Fraction collector screen in the ***Settings and service*** *screen menu, if not previously enabled.*

5 Prime the entire flow path (buffer tubing to fractionation tubing) with DS buffer to ensure the tubing is filled with DS buffer before starting the chromatography run. Perform ***Washout fractionation tubing***:

a. Place the fractionation tubing in the waste bottle.

b. From ÄKTA start instrument display home screen (*Fig. 2, on page 1*), tap ***Method run***.

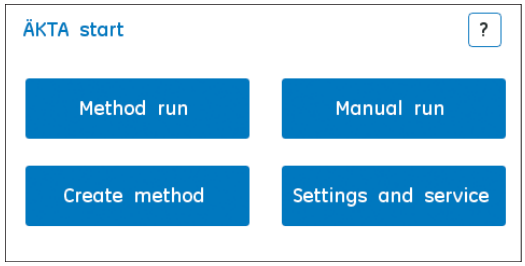

Fig 2. ÄKTA start display: Screenshot of the main menu.

c. From ***Method run*** screen, tap ***Prepare system***.

d. Select ***Washout fractionation tubing*** (*Fig. 3, on page 2*).

e. Select the run parameters. Tap ***Run*** to initiate the method.

Step	Action

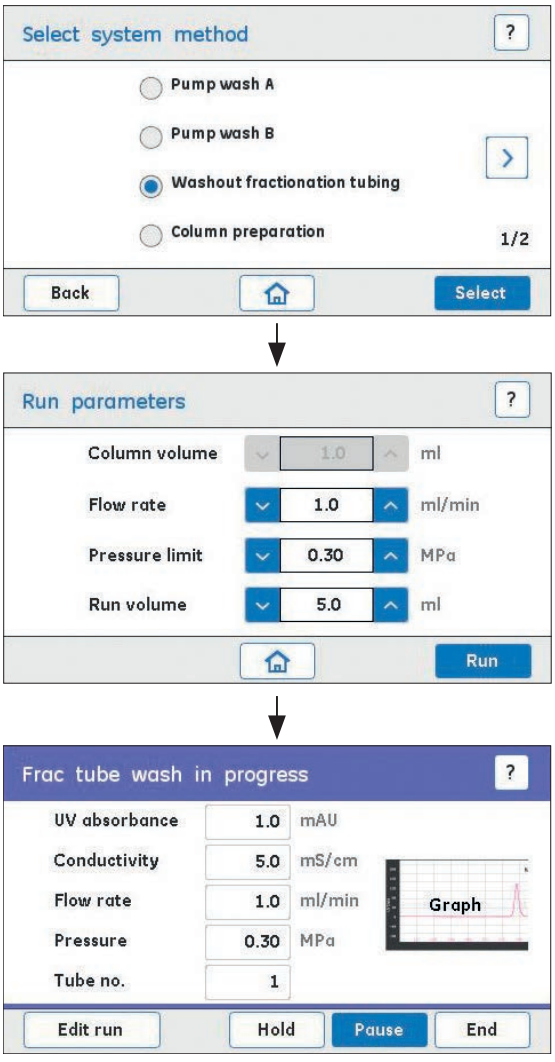

Fig 3. ÄKTA start display: Screenshots of the ***Prepare system methods***, ***Select parameters***, and ***Fractionation wash*** run screens.

6 Prepare Frac30 fraction collector (*Fig. 4, on page 2*).

a. Fill the inner row of holders with 1.5 mL microcentrifuge tubes (*Fig. 4, on page 2*).

b. Move the dispenser arm to the dispensing position.

c. Insert the fractionation tubing into the tubing holder.

Step	Action

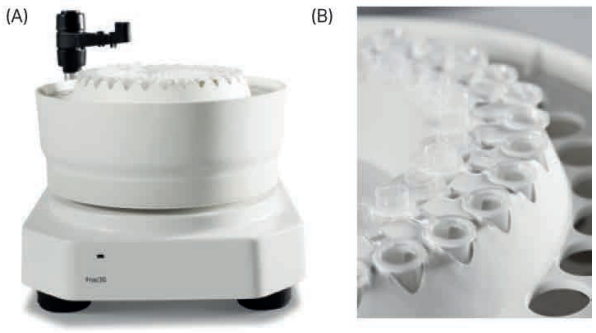

Fig 4. Frac30 fraction collector with collection tubes. A) Frac30 fraction collector. B) Fraction collector showing placement of the microcentrifuge tubes.

Connecting the column

Connect the HiTrap Desalting 5 mL column to the system (*Fig. 5, on page 2*). To avoid introducing air into the column, connect the column "drop to drop".

Step	Action
1	Attach a column clamp to the column holder rail on the instrument.
2	Remove the column stoppers and mount the column on the union connector.
3	Fix the column to the column clamp.

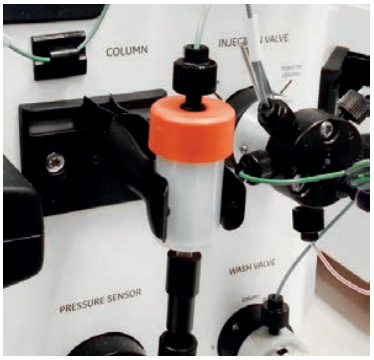

Fig 5. Image showing the column position.

4	Remove the G5 tubing from the union connector (Manual injection valve to the top/inlet of the column).
5	Start a manual run with 0.5 mL/min flow rate. Wait for the buffer to flow continuously from the tubing labeled G5 and then start filling the top part of the column with the buffer. When the top part of the column is filled with the buffer, connect the tubing to the top part of the column.
6	Connect the G6 tubing (column outlet to UV) to the bottom of the column holder/union connector.

Loading sample

Step	Action
1	Ensure that the 1 mL sample loop is connected to the Manual injection valve (ports 2 and 5).
2	Ensure the Manual injection valve is in LOAD position, as illustrated in *Fig. 6, on page 3*. Wash the sample loop with 5 mL DS buffer with a syringe (through port 3 of the Manual injection valve).

Fig 6. Image showing Manual injection valve in ***LOAD*** position and sample loop attached to ports 2 and 5.

3	Pre-fill the loop with 1.5 mL sample (port 3). ***Note:*** • *In order to avoid sample drainage do not remove the syringe until the sample is loaded onto the column* • *It is recommended to overload the loop with higher sample volume to make sure the loop is completely filled*

Starting the run

Step	Action
1	Insert a USB memory stick in the USB port of the instrument. ***Note:*** *The result files will be saved in the Cytiva folder which is automatically created by the instrument once the USB memory stick is plugged in.*
2	From ÄKTA start instrument display home screen, tap ***Method run***.
3	From the Method run screen, tap ***Templates*** (*Fig. 7, on page 3*).

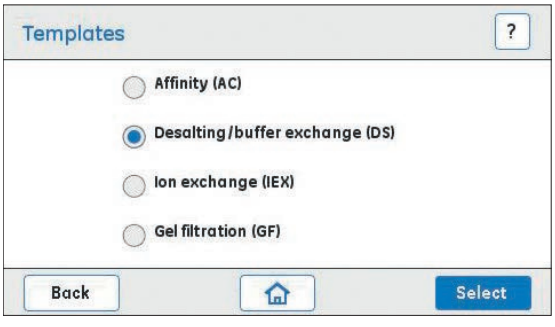

Fig 7. ÄKTA start display: Screenshot of the ***Templates*** screen.

4	Select ***Desalting/Buffer exchange***, and tap ***Select***.

Step	Action
5	The following run parameter screen (1/3) appears (*Fig. 8, on page 3*).

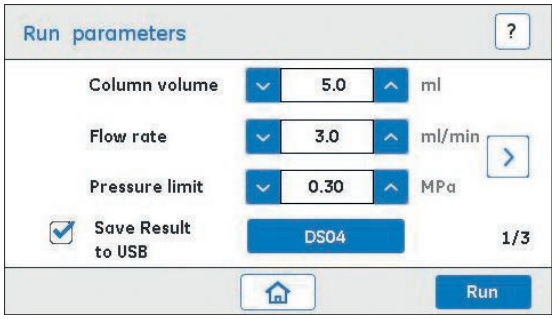

Fig 8. ÄKTA start display: Screenshot of ***Run parameters*** screen (1/3).

6	Tick the ***Save Result to USB*** check box.
7	Provide a result file name (e.g. DS04). Only the two digits of the result file name can be modified.
8	Tap the forward arrow to go to screen (2/3) (*Fig. 9, on page 3*).

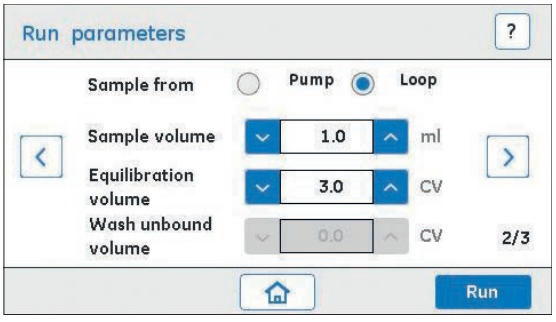

Fig 9. ÄKTA start display: Screenshot of ***Run parameters*** screen (2/3).

9	Select sample from ***Loop***.
10	Enter ***Sample volume*** = 1 mL.
11	Enter ***Equilibration volume*** = 3 CV. Tap the forward arrow to go to screen (3/3) (*Fig. 10, on page 3*).

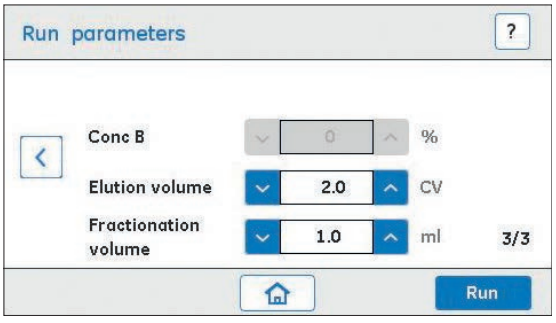

Fig 10. ÄKTA start display: Screenshot of ***Run parameters*** screen (3/3).

12	Enter ***Fractionation volume*** = 1 mL.
13	Tap ***Run*** to start the run (*Fig. 9, on page 3*)

Step	Action
	Note: *While the run is in progress, the real time UV curve can be observed by tapping on the graph icon. The run view screen also displays other real-time run parameters such as conductivity, pressure, flow rate, and tube number (Fig. 11, on page 4).*

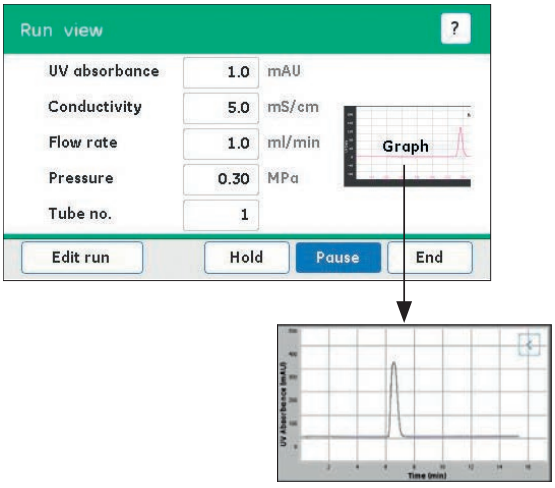

Fig 11. ÄKTA start display: Screenshot of ***Run view*** and real time graph screens.

During the run

Step	Action
1	When prompted on the screen (as depicted below in Fig. 12, on page 4), manually turn the injection valve to ***INJECT*** position. ***Note:*** *The system is in hold state while injecting the sample from loop. To ensure that the injection mark coincides with the injection event, acknowledge the message immediately after the action is performed.*

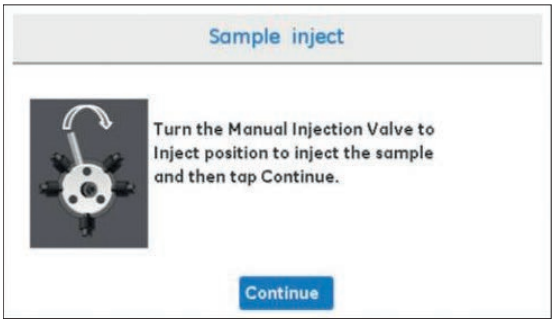

Fig 12. ÄKTA start display: Screenshot of ***Sample inject*** message screen.

2	After manually switching the position, acknowledge the message by tapping ***Continue*** (Fig. 12, on page 4). The sample is injected from the loop on to the column.
3	After 1 mL of sample has been injected, a prompt appears on the screen (depicted below in Fig. 13, on page 4).

Step	Action

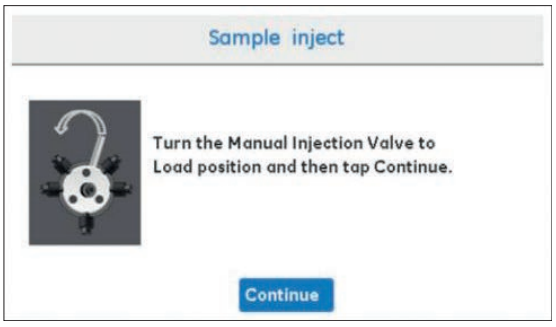

Fig 13. ÄKTA start display: Screenshot of ***Sample inject*** message screen prompt following sample injection.

4	Manually turn the injection valve to ***LOAD*** position.
5	After manually switching position, acknowledge the message by tapping ***Continue*** (Fig. 13, on page 4).
6	After the completion of the run tap ***Exit***.
7	Remove the USB memory stick from the ÄKTA start instrument.

Typical result

Step	Action
1	Insert the USB memory stick into a computer to open the chromatography result file (DS01) in any image viewing software (Microsoft® picture manager/ paint etc.). A representative chromatogram for the chromatography run is shown in Fig. 14, on page 4.

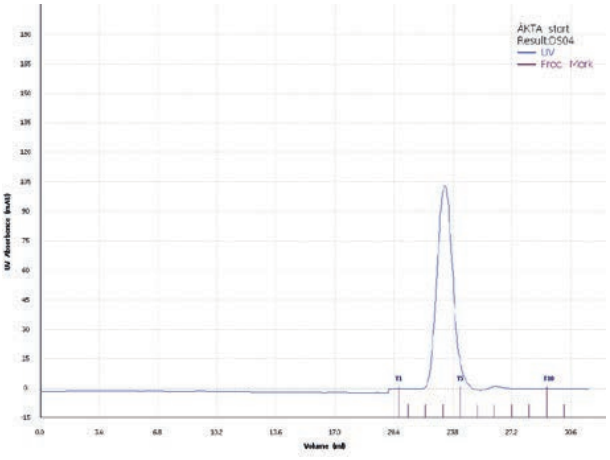

Fig 14. Chromatogram (.bmp image) of desalting on ÄKTA start.

Troubleshooting

High back pressure

- Column clogged: Clean the column according to instructions. Make sure the sample has been centrifuged and/or filtered through a 0.45 µm filter.
- System clogged: Replace the column with a piece of tubing. Check pressure using water at a flow rate of 5 mL/min. If backpressure is more than 0.3 MPa (3 bar, 43.5 psi), clean system according to instructions in manual.

No separation

- Check that the correct column is used.
- Check that the inlet tubing from each buffer is connected to the correct inlet port.
- Check that the composition and pH of the buffers are correct.
- Check that the sample contains target protein.

System maintenance and storage

For detailed description of maintenance and storage see ÄKTA start operating instructions.

Storage of column

For detailed description of column storage see HiTrap Desalting column instructions.

Check your knowledge

1. ***Where should the Desalting buffer be placed?***
   a. Under the ÄKTA start instrument
   b. On the bench
   c. On the buffer tray
2. ***How can you load your sample on the column using ÄKTA start?***
   a. Via the pump
   b. Via a sample loop
   c. All of the above
3. ***Why is it recommended to overload the sample loop?***
   a. To load more sample volume in the sample loop
   b. To make sure that the capillary loop is completely filled
   c. All of the above
4. ***Why should the "Sample inject" message be acknowledged as soon***
   a. To ensure that the injection mark coincides with the injection event
   b. To ensure that the injection of sample is performed
   c. All of the above
5. ***Why should the column be connected to the ÄKTA start instrumentusing "drop-to-drop"?***
   a. To equilibrate the column faster
   b. To avoid introducing air into the column
   c. To remove the storage solution
6. ***What can be viewed in the Run view screen?***
   a. Real-time UV absorbance and conductivity
   b. Real-time tube number
   c. Real-time flow rate and pressure
   d. Chromatogram
   e. All of the above
7. ***Where can the result file be found after the run is completed?***
   a. On the USB stick
   b. On the display
   c. There is no result file

Answers

Q1. c

Q2. c

Q3. b

Q4. a

Q5. b

Q6. e

Q7. a

Ordering information

Product	Quantity	Code number
HiTrap Desalting	1 × 5 mL	29048684
HiTrap Desalting	5 × 5 mL	17140801
Sample Loop 1 ml	1 × 1 mL	18111401
Column Clamp	1	28956319
Union 1/16" female-1/16" female	1	11000339

Reference information

Document	Code number
ÄKTA start System cue card	29024042
ÄKTA start Maintenance cue card	29024043
ÄKTA start Operating instructions	29027057
HiTrap Desalting instructions	71715400

Related literature

Product	Code number
Application notes	
Purification of N-terminal histidine-tagged protein using ÄKTA start	29064277
Purification of GST-tagged protein using ÄKTA start	29064298
Purification of antibodies using ÄKTA start and HiTrap Protein G HP column	29064302
Depletion of albumin from serum samples using ÄKTA start	29064295
Training cue cards	
Gel filtration using ÄKTA start	29112091
Affinity purification using ÄKTA start	29115058
Anion exchange purification using ÄKTA start	29110759

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