# Anion exchange purification using **ÄKTA** start

## Training cue card

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

### Requirements

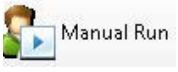
- ÄKTA™ start system
- Frac30 fraction collector
- Computer installed with UNICORN™ start 1.0 control software
- Binding buffer (Buffer A): 25 mM sodium phosphate, pH 7.5 (Prepare at least 200 mL of buffer).
- Elution buffer (Buffer B): 25 mM sodium phosphate, 1 M NaCl, pH 7.5 (Prepare at least 200 mL of buffer).
- Sample: BSA 1 mg/mL in Buffer A (Prepare 5 mL of sample).
- Column: HiTrap™ DEAE FF 1 mL
- 2 mL Sample loop
- Fraction tubes: 1.5 mL microcentrifuge tubes

### Checklist

- Ensure that the PC Connection cable is connected between the connector marked as **PC Connection** at the back of ÄKTA start and a USB port on the computer.
- 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.


**Fig 1.** ÄKTA start instrument with Frac30 fraction collector and UNICORN start software.

### Preparing the system

| Step | Action |
|---|---|
| 1 | Place the bottles containing Buffer A and Buffer B in the buffer tray on top of the instrument. |
| 2 | Immerse both buffer inlets (A and B) in the corresponding bottle. |
| 3 | Place the waste bottle on the right side of the instrument.<br>***Note:***<br>*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.* |
| 4 | Power **ON** the ÄKTA start instrument .<br>***Note:***<br>*Enable Frac30 from the Fraction collector screen in the **Settings and service** screen menu, if not previously enabled.* |
| 5 | Start the computer and launch UNICORN start and connect to ÄKTA start. |
| 6 | Prime the flow path (buffer tubing to wash valve) with Buffer B to ensure the tubing to the pump is filled with B buffer before starting the chromatography run.<br>**a.** Place the fractionation tubing in the waste bottle.<br>**b.** From UNICORN start ***System Control*** module (*Fig. 2, on page 2*), click ***Manual run***.<br>  <br>**c.** The ***Manual run*** dialog box will open. |

| Step | Action |
|---|---|
| | **d.** Set the flow rate to 5 mL/min and click ***OK***. |

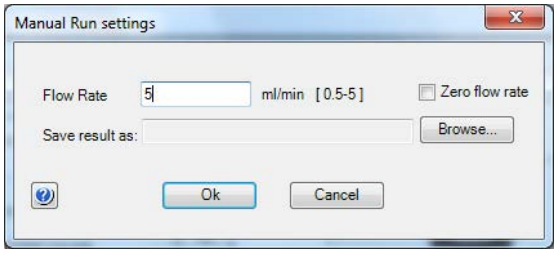



**Fig 2.** UNICORN start Manual Run settings dialog with flow rate set to 5 mL/min.

| | |
|---|---|
| | **e.** In the process picture (*Fig. 3, on page 2*) set Buffer valve to B. Wash the flow path using Buffer B for 2 minutes (see green highlighted part in the process picture). |

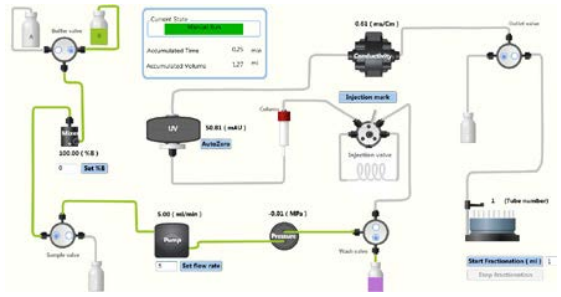


**Fig 3.** UNICORN start process picture illustrating the Buffer B tubing wash in-progress.

| | |
|---|---|
| | **f.** Prime the entire flow path (buffer tubing to outlet fractionation tubing) with Buffer A to ensure the flowpath is filled with buffer before starting the chromatography run. From the process picture, set the Buffer valve to position A, Wash valve to position Column and Outlet valve to position fraction collector (*Fig. 4, on page 2*). Prime the entire flow path for 5 minutes. |
| | ***Note:*** *Ensure that there is no column in line before switching the wash valve.* |
| | **g.** End the manual run. |

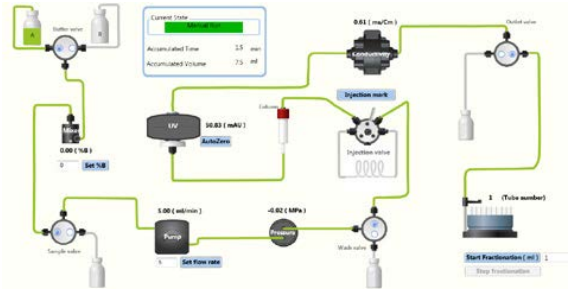


**Fig 4.** UNICORN start process picture illustrating priming of the entire flow path.

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| 7 | Prepare Frac30 fraction collector (*Fig. 5, on page 2*). |
| | **a.** Fill the inner row of holders with 1.5 mL microcentrifuge tubes (*Fig. 5, on page 2*). |
| | **b.** Move the dispenser arm to the dispensing position. |

| Step | Action |
|---|---|
| | **c.** Insert the fractionation tubing into the tubing holder. |

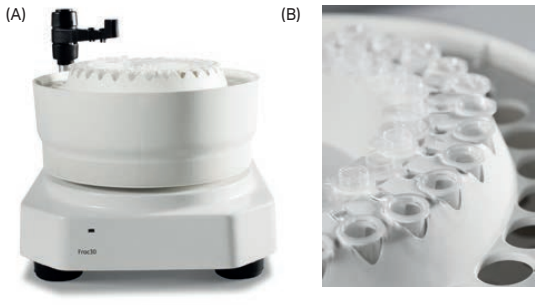


**Fig 5.** Frac30 fraction collector with collection tubes. A) Frac30 fraction collector. B) Fraction collector showing placement of the microcentrifuge tubes.

## Connecting the column

Connect the HiTrap DEAE FF 1 ml column to the system (*Fig. 6, 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. |

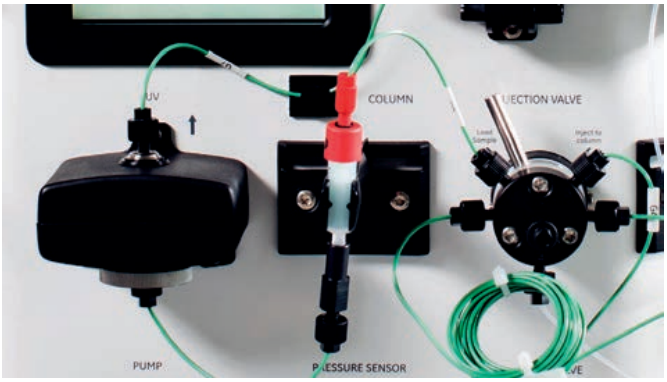


**Fig 6.** Image showing the column position.

| | |
|---|---|
| 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 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 2 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. 7, on page 3*. Wash the sample loop with 10 ml Buffer A with a syringe (through port 3 of the Manual injection valve). |


**Fig 7.** Image showing Manual injection valve in ***LOAD*** position and sample loop attached to ports 2 and 5.

| | |
|---|---|
| 3 | Pre-fill the loop with 2.5 ml sample (port 3).<br>***Note:***<br>• *In order to avoid sample drainage do not remove the syringe until the sample is loaded onto the column.*<br>• *It is recommended to overload the loop to make sure that the loop is completely filled.* |

## Creating the method

| Step | Action |
|---|---|
| 1 | Select ***New method*** in the UNICORN start ***Method Editor*** module. |
| 2 | Select the predefined method for Anion Exchange. |
| 3 | Set the parameters for the method as shown in *Table 1, on page 3*. |
| 4 | Save method as AIEX-BSA. |

**Table 1.** UNICORN start method overview

| Method flow | Method settings |
|---|---|
| Method settings | Column type: HiTrap DEAE FF, 1 mL<br>Pressure limit 0.3 MPa[1]<br>Flow rate 1 mL/min<br>Column Volume: 1 mL |
| Prime and equilibration | Prime<br>Equilibration Volume: 5 CV[2] |
| Sample application | Apply sample using loop<br>Sample Volume: 2.0 mL |
| Wash out unbound | 5 CV |
| Elution and fractionation | Gradient elution: Start % B conc at 0.0<br>Elution:<br>• Linear: 100% B, 5 CV<br>• Step: 100% B , 2 CV<br>Peak fractionation<br>• Peak fractionation settings: Level<br>• Start level: 20 mAU<br>• End level: 15 mAU<br>• Minimum peak width: 0.2 min<br>Fractionation Volume: 1.5 mL |
| Prime and equilibration | Equilibration Volume: 5 CV |

[1] 0.3 MPa = 3 bar (43.5 psi)
[2] CV = Column volumes

## Starting the run

| Step | Action |
|---|---|
| 1 | Click ***Method run*** from the UNICORN start ***System Control*** module. |
| 2 | This opens the Select method dialog box (*Fig. 8, on page 3*). |
| 3 | Find and select the AIEX-BSA method in the folder pane from ***User defined***. |
| 4 | Click the ***OK*** button to start the selected method run. This opens the ***Start protocol*** dialog. |
| 5 | Review the Variable List and change method parameters if required, and then click Next to proceed to the ***next*** page. |
| 6 | Specify a result name (e.g. AIEX-BSA) and then click ***Start*** to start the run. |

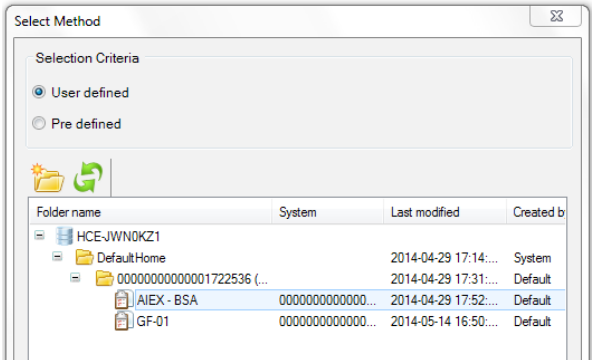


**Fig 8.** UNICORN start Select method dialog box.

## During the run

| Step | Action |
|---|---|
| 1 | When prompted on the screen (as depicted below in *Fig. 9, on page 4*), manually turn the injection valve to ***INJECT*** position. |

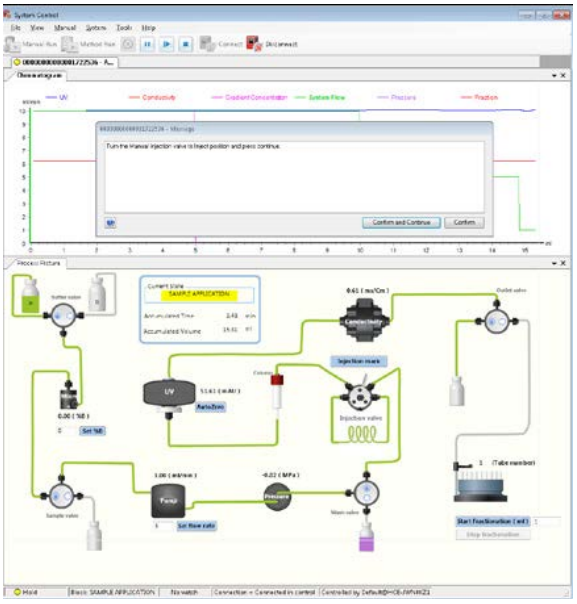

**Fig 9.** UNICORN start: Process picture image showing ***Sample inject*** message screen.

***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.*

| | |
|---|---|
| 2 | After manually switching the injection valve position, acknowledge the message by clicking ***Confirm and Continue***. The sample is automatically injected from the loop on to the column. |
| 3 | After the 2 mL of sample is injected, a prompt appears on the screen (depicted below in *Fig. 10, on page 4*). |

| Step | Action |
|---|---|

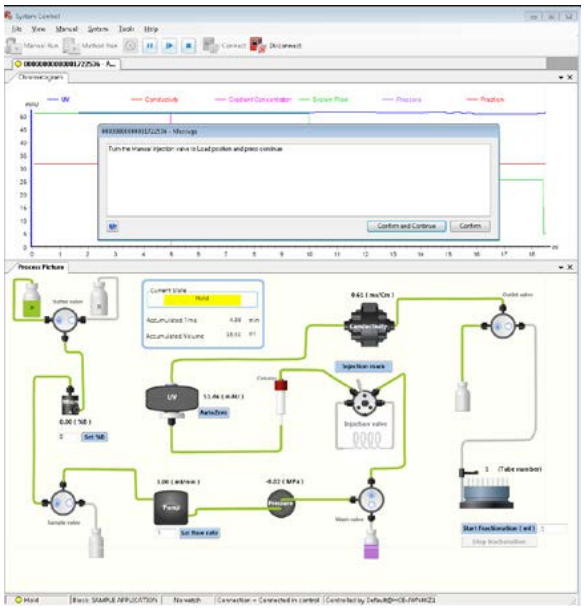

**Fig 10.** UNICORN start: Process picture image showing ***Sample inject*** message screen after injection of sample.

| | |
|---|---|
| 4 | Manually turn the injection valve to ***LOAD*** position. |
| 5 | After manually switching the injection valve position, acknowledge the message by clicking ***Confirm and continue***. |
| 6 | Upon completion of the method the run ends automatically. |

## Typical result

| Step | Action |
|---|---|
| 1 | In the ***Evaluation*** module of UNICORN start, double-click on the AIEX-BSA result to open the file. A representative chromatogram for the chromatography run is shown in *Fig. 11, on page 4*. |

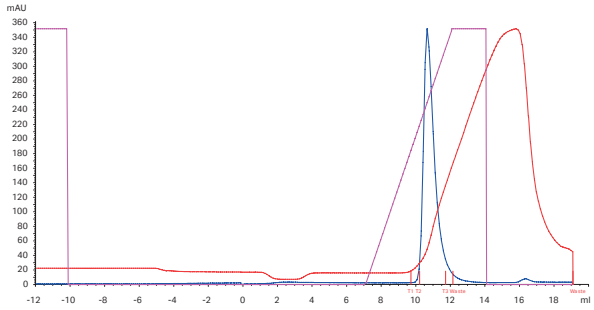


**Fig 11.** Chromatogram from UNICORNstart Evaluation module.

| | |
|---|---|
| 2 | From the ***Evaluation*** module a pdf report of the chromatogram can be generated. Peak integration, curve and chromatogram comparisons can also be performed. For details refer to the *UNICORN start user manual*. |

## 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 DEAE FF instructions*.

## Check your knowledge

1. ***Why do you need to prime the flowpath with Buffer A after priming it with Buffer B?***
   a. The order of priming the buffers does not matter.
   b. To make sure the flowpath is filled with the correct buffer before attaching the column.
   c. To have a UV and conductivity signal baseline before starting the purification.
   d. If the flowpath is primed with Buffer B before Buffer A then salt will interfere with the column function
   e. B, C, and D
2. ***Which predefined methods are there in the Method Editor?***
   a. Anion Exchange
   b. Desalting
   c. Gel Filtration
   d. Affinity
   e. Cation Exchange
   f. All of the above
3. ***Which gradient elution types can be set in the "Elution and Fractionation" phase?***
   a. Linear
   b. Linear and Step
   c. Step
   d. Isocratic
   e. All of the above
4. ***Why do you have to acknowledge the message immediately after turning the injection valve to inject?***
   a. To ensure that the injection mark coincides with the injection event
   b. To make sure the sample is loaded on the column
   c. It is not needed
5. ***Why should the column be connected to the ÄKTA start instrument using "drop-to-drop"?***
   a. To equilibrate the column faster
   b. To avoid introducing air into the column
   c. To remove the storage solution
6. ***Where can the result file be viewed after the run is completed?***
   a. In the ***Evaluation*** module
   b. On the display
   c. There is no result file

## Answers

1. e
2. f
3. e
4. a
5. b
6. a

## Ordering information

| Product | Quantity | Code number |
|---|---|---|
| HiTrap DEAE Sepharose™ FF | 5 × 1 mL | 17505501 |
| Sample Loop | 1 × 2 mL | 18111402 |
| 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 |
| UNICORN start 1.0, User manual | 29060244 |
| HiTrap DEAE FF instructions | 71501751 |

### *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*** | |
| Desalting using ÄKTA start | 29109491 |
| Affinity purification using ÄKTA start | 29115058 |
| Gel filtration using ÄKTA start | 29112091 |

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29110759 AB V:5 03/2021