GE Healthcare
Life Sciences

Affinity purification using ÄKTA™ start

Training cue card

This protocol will help you understand the practical principles of affinity chromatography by taking you step-by-step through the purification of a Histidine-tagged fusion protein.

Requirements

- ÄKTA start system
- Frac30 fraction collector
- 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, 200 mM imidazole, pH 7.5 (Prepare at least 200 ml of buffer)
- Sample: Histidine-tagged fusion protein 1 mg/ml in 25 mM sodium phosphate buffer (Prepare 10 ml of sample)
- Column: HisTrap™ FF 1 ml
- Fraction tubes: 1.5 ml microcentrifuge tubes
- 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

1. Place the bottles containing Buffer A and Buffer B in the buffer tray on top of the instrument



Fig 1. ÄKTA start instrument with Frac30 fraction collector.

2. Immerse both buffer inlets (A and B) in the corresponding bottle.
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 Figure 1.

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

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

5. Prime the entire flow path (buffer tubing to fractionation tubing) with Buffer A to ensure the tubing is filled with Buffer A before starting the chromatography run. Perform ***Washout fractionation tubing***:
   a. Place the fractionation tubing in the waste bottle
   b. From ÄKTA start instrument home screen (Fig 2), tap ***Method Run***

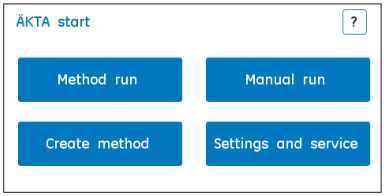


Fig 2. ÄKTA start display: Screenshot of the main menu.

c. Tap ***Prepare system*** from Method run screen.
d. Select ***Washout fractionation tubing*** (Fig 3).
e. Set the run parameters. Tap ***Run*** to initiate the method.

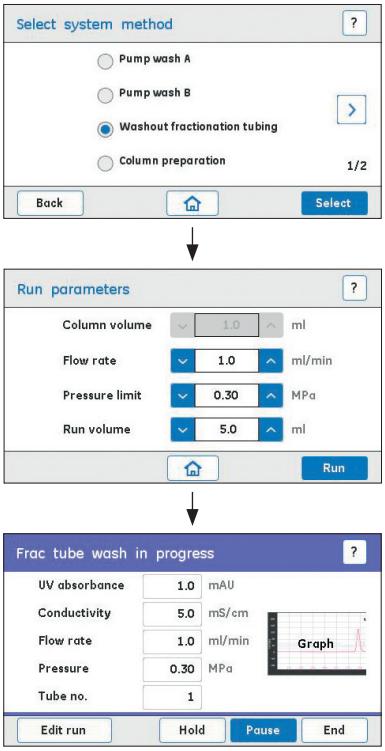


Fig 3. ÄKTA start display: Screenshots of the ***Prepare system methods***, ***Select parameters*** and ***Fractionation wash*** run screens.

6. Prime the sample flow path (sample inlet tubing to Wash valve) with Buffer A to ensure the sample tubing is filled with buffer before starting the chromatography run.
   a. Ensure that a 1 mm internal diameter ETFE tubing is connected to the port I (Sample) of the Sample valve.
   b. Place the sample tubing in the Buffer A bottle.
   c. From ÄKTA start instrument display home screen (Fig 4), tap ***Manual run***.
   d. Enter Flow rate 5 ml/min.

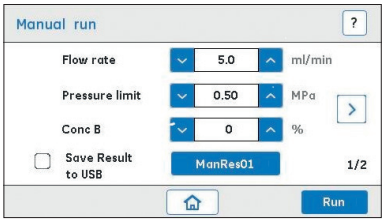


Fig 4. ÄKTA start display: Screenshot of the ***Manual run*** screen (1/2).

   e. Uncheck ***save Result to USB***
   f. Tap the forward arrow to go to screen (2/2)
   g. Set ***Sample valve*** to Sample (Fig 5).

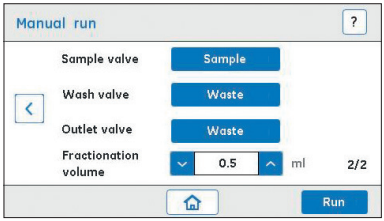


Fig 5. ÄKTA start display: Screenshot of the ***Manual run*** screen (2/2).

   h. Tap ***Run*** to initiate the manual run.
   i. After 2 minutes run, tap ***End*** to end the run.
   j. Tap ***Exit*** to go to the home screen

7. Prepare Frac30 fraction collector (Fig 6).
   a. Fill the inner row of holders with 1.5 ml microcentrifuge tubes.
   b. Move the dispenser arm to the dispensing position.
   c. Insert the fractionation tubing into the tubing holder.

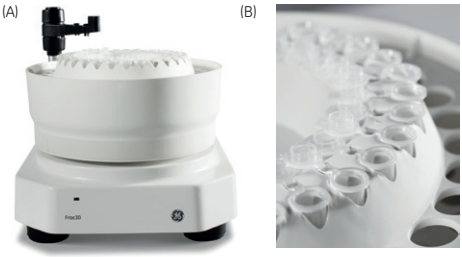


Fig 6. A) Frac30 fraction collector. B) Fraction collector showing placement of the microcentrifuge tubes.

Connecting the column

Connect the HisTrap FF 1 ml column to the system (Fig 7). To avoid introducing air into the column, connect the column "drop to drop".

8. Attach a column clamp to the column holder rail on the instrument.
9. Remove the column stoppers and mount the column on the union connector.
10. Fix the column to the column clamp.

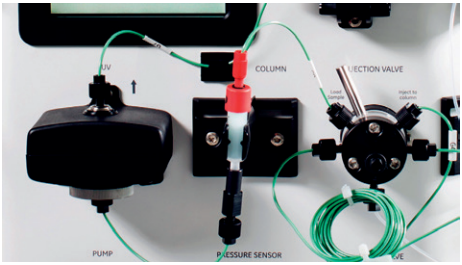


Fig 7. Image showing the column position.

11. Remove the G5 tubing from the union connector (Manual injection valve to the top/inlet of the column).
12. 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.
13. Connect the G6 tubing (column outlet to UV) to the bottom of the column holder/union connector.

Loading sample

14. Immerse the sample inlet tubing in the sample container.

Note:

- When the sample is applied via the Pump, the Injection valve has to be manually set to position **Load** (as illustrated in Fig 8)
- Ensure that the volume of sample is sufficient to prevent air entering the tubing.
- Make sure that there are no trapped air bubbles in the tubing.



Fig 8. Image showing Manual injection valve in **LOAD** position.

Starting the run

15. Insert a USB memory stick into the USB port of the instrument.

Note: The result files will be saved in the GE folder which is automatically created by the instrument once the USB memory stick is plugged in.

16. Tap ***Method run*** from ÄKTA start instrument display home screen.
17. Tap **Templates** (Fig 9) from the Method run screen.

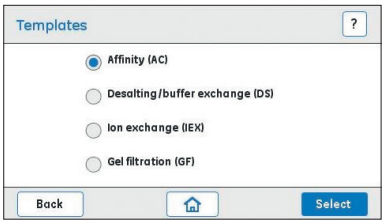


Fig 9. ÄKTA start display: Screenshot of the ***Templates*** screen.

18. Select ***Affinity***, and tap ***Select***.
19. The following run parameter screen (1/3) appears (Fig 10).

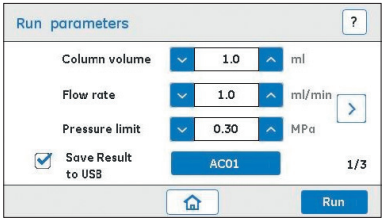


Fig 10. ÄKTA start display: Screenshot of ***Run parameters*** screen (1/3).

20. Provide a result file name (e.g. AC01). Only the two digits of the result file name can be modified.
21. Tap the forward arrow to go to screen (2/3) (Fig 11).

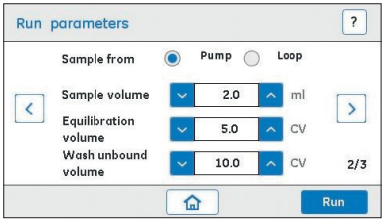


Fig 11. ÄKTA start display: Screenshot of ***Run parameters*** screen (2/3).

22. Select sample from ***Pump***.
23. Enter ***Sample volume*** = 2 ml.
24. Enter ***Wash out unbound volume*** = 10 CV. Tap the forward arrow to go to screen (3/3) (Fig 12).

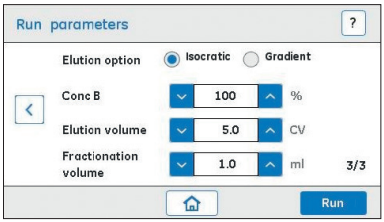


Fig 12. ÄKTA start display: Screenshot of ***Run parameters*** screen (3/3).

25. Enter ***Fractionation volume*** = 1 ml.
26. Tap ***Run*** to start the run.

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 13).

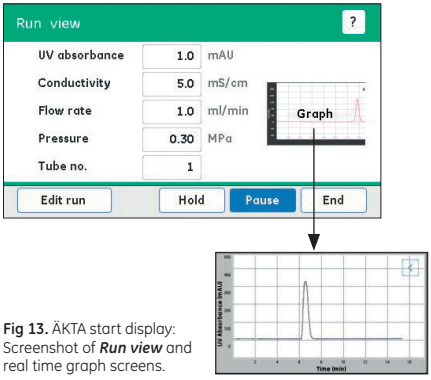


Fig 13. ÄKTA start display: Screenshot of ***Run view*** and real time graph screens.

27. After the completion of the run tap ***Exit***.
28. Remove the USB memory stick from the ÄKTA start instrument.

Typical result

29. Insert the USB memory stick into a computer to open the chromatography result file (AC01) in any image viewing software (Microsoft® picture manager/ paint etc.). A representative chromatogram for the chromatography run is shown in Figure 14.

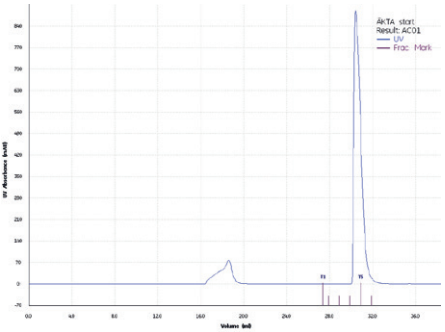


Fig 14. Chromatogram (.bmp image) of affinity purification on ÄKTA start.

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 the 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 HisTrap FF instructions.

Check your knowledge

Q1: Why do you need to prime the sample flowpath with Buffer A?

a. To have a UV and conductivity signal baseline before starting the purification.
b. To make sure the flowpath is filled with the correct buffer and there are no air bubbles in the tubing.
c. To neutralize the sample before sample loading.

Q2: Which predefined system methods are there on the ÄKTA start display?

(More than one answer possible)

a. Pump wash A
b. Sample wash
c. Pump wash B
d. Column CIP
e. Wash out fractionation tubing
f. Column preparation

Q3: How is the sample eluted from the column?

a. Gradient elution
b. Isocratic elution
c. A and B

Q4: What position must the Injection valve have in order to apply the sample via the sample pump?

a. Inject
b. Load
c. All of the above

Q5: Which run parameters can be changed at the start of the purification run?

a. Column volume, sample volume, equilibration volume
b. Flow rate, pressure limit, Conc B buffer
c. Sample application method, Elution option
d. Wash unbound volume, Elution volume, Fractionation volume
e. Result name
f. All of the above

Q6: 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

Answers

1. B
2. A, C, E, and F
3. B
4. B
5. F
6. E

Ordering information

Product	Quantity	Code number
HisTrap FF	5 × 1 ml	17-5319-01
Column Clamp	1	28-9563-19
Union 1/16" female-1/16" female	1	11-0003-39

Reference information

Document	Code number
ÄKTA start System cue card	29-0240-42
ÄKTA start Maintenance cue card	29-0240-43
ÄKTA start Operating instructions	29-0270-57
HisTrap FF instructions	11-0008-88

Related literature

Product	Code number
Application notes	
Purification of N-terminal histidine-tagged protein using ÄKTA start	29-0642-77
Purification of GST-tagged protein using ÄKTA start	29-0642-98
Purification of antibodies using ÄKTA start and HiTrap™ Protein G HP column	29-0643-02
Depletion of albumin from serum samples using ÄKTA start	29-0642-95
Training cue cards	
Gel filtration using ÄKTA start	29-1120-91
Desalting using ÄKTA start	29-1094-91
Anion exchange purification using ÄKTA start	29-1107-59

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