Gel filtration using **ÄKTA** start

Instructions

This protocol will help you understand the practical principles of gel filtration by taking you step-by-step through the purification of a Bovine Serum Albumin (BSA) and cytochrome C (cyt C) mixture.

Requirements

- ÄKTA™ start system
- Frac30 fraction collector
- Computer installed with UNICORN™ start 1.0 control software
- Gel filtration buffer (GF Buffer): 25 mM sodium phosphate, pH 7.5, 150 mM NaCl (Prepare at least 500 mL of buffer)
- Sample: 5 mg/mL BSA) and 2.5 mg/mL cytochrome C in GF Buffer. (Prepare 5 mL of sample)
- Column: HiPrep™ 16/60 Sephacryl™ S-200 HR 120 mL
- 2 mL Sample loop
- Fraction tubes: 15 mL 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 bottle containing the GF Buffer in the buffer tray on top of the instrument.
2	Immerse buffer inlet A in the bottle containing the GF 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 the Figure above.*
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	Start the computer and launch UNICORN start and connect to ÄKTA start.
6	Prime the entire flow path (buffer tubing to outlet fractionation tubing) with GF Buffer to ensure the tubing is filled with GF buffer before starting the chromatography run. **a.** Place the fractionation tubing in a waste container. **b.** From UNICORN start ***System control*** module click ***Manual run***.  **c.** The ***Manual run*** dialog box will open **d.** Set the flow rate to 5 mL/min and click ***Ok*** (Figure below).

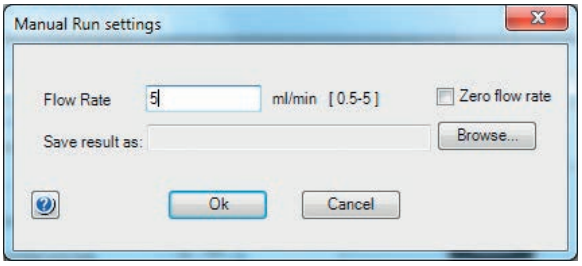

Fig 2. UNICORN start Manual run settings dialog with flow rate set to 5 mL/min.

Step	Action
	e. From the process picture, set the Wash valve to position Column and Outlet valve to position Fraction collector (Figure below). Prime the entire flow path for 2 minutes (see green highlighted part in the process picture). ***Note:*** *Ensure that there is no column in line before switching the wash valve.*

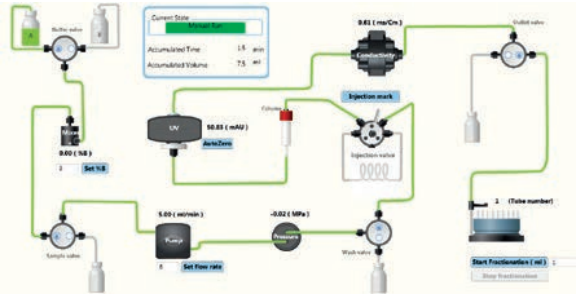

Fig 3. UNICORN start process picture illustrating priming of the flow path.

Step	Action
	f. End the manual run.
7	Prepare Frac30 fraction collector. **a.** Fill the outer row of holders with 15 mL tubes. **b.** Move the dispenser arm to the dispensing position. **c.** Insert the fractionation tubing into the tubing holder.

Connecting the column

Connect the HiPrep 16/60 Sephacryl S-200 HR column to the system (Figure below). To avoid introducing air into the column, connect the column "drop to drop".

Step	Action
1	Attach two column holders to the column holder rail on the right-hand side of the instrument.

Fig 4. Image showing the gel filtration column being attached to the ÄKTA start instrument.

Step	Action
2	Mount the column vertically on the column holders.
3	Remove the G5 and G6 tubing. Connect a 90 cm long PEEK tubing (internal diameter 0.75 mm) to the Manual injection valve (port 1). Connect a 50 cm long PEEK tubing (internal diameter 0.75 mm) to the UV monitor.
4	Start a manual run with 0.5 mL/min flow rate. Wait for the buffer to flow continuously from the 90 cm long tubing 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 from the Manual injection valve to the top part of the column.
5	Remove the column transport device and connect the 50 cm long tubing from the UV to the column outlet.
6	End the manual run. ***Note:*** *Save the transport device for use when storing the column.*

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 Figure 5. Wash the sample loop with 10 mL GF Buffer with a syringe (through port 3 of the Manual injection valve).

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

Step	Action
3	Pre-fill the loop with 2.5 mL sample. ***Note:*** • *In order to avoid sample drainage, the syringe should not be removed after filling the sample loop* • *It is recommended to overload the loop to make sure that the loop is completely filled* • *For high resolution separation in gel filtration a sample volume from 0.5% to 4% of the total column volume is recommended, depending on the type of medium used. For most applications the sample volume should not exceed 2% to achieve maximum resolution.*

Creating the method

Step	Action
1	Select ***New method*** in the UNICORN start ***Method Editor*** module.
2	Select the Predefined method for Gel Filtration.
3	Set the parameters for the method as shown in the Table below.

Step	Action
4	Save method as GF-01.

Table 1. UNICORN start method overview

Method flow	Method settings
Method settings	Column type: HiPrep 16/60 Sephacryl S 200 HR Pressure limit 0.15 MPa[1] Flow rate 0.5 mL/min Column Volume: 120 mL
Prime and equilibration	Prime Equilibration Volume: 1.2 CV[2]
Sample application	Apply sample using loop Sample Volume: 2.0 mL
Elution and fractionation	Isocratic elution: 0% B, 1.0 CV Fixed volume fractionation Fractionation Volume: 4 mL

[1] 0.15 MPa = 15 bar, 22 psi

[2] CV = Column volumes

Starting the run

Step	Action
1	Click the ***Method run*** from the UNICORN Start ***System Control*** toolbar.
2	This opens the ***Select method*** dialog box (Figure below).
3	For ***User defined***, find and select the GF-01 method in the folder pane.
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. GF-01 and then click ***Start*** to start the run.

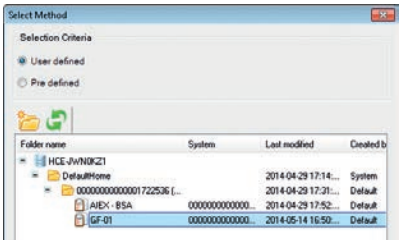

Fig 6. UNICORN start ***Select method*** dialog box.

During the run

Step	Action
1	When prompted on the screen (as depicted below in Figure below), manually turn the injection valve to ***INJECT*** position.

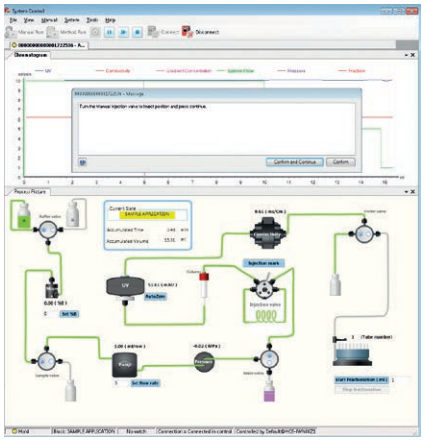

Fig 7. UNICORN start: Process picture image showing ***Sample inject*** message screen.

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 position, acknowledge the message by clicking ***Confirm and continue***. The sample is automatically injected from the loop on to the column.
3	After the required volume of sample is injected, a prompt appears on the screen (depicted in Figure below).

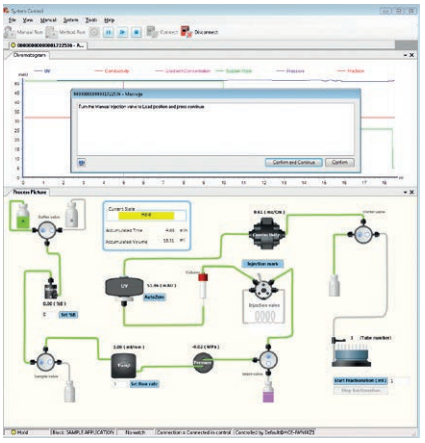

Fig 8. UNICORN start: Process picture showing ***sample inject*** message prompt following sample injection.

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 GF-01 result to open the file. A representative chromatogram for the chromatography run is shown in Figure below.

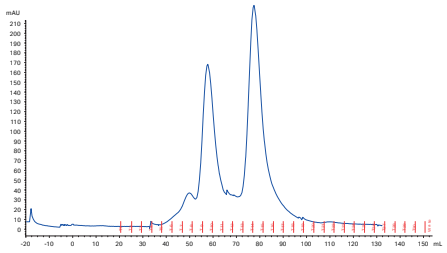

Fig 9. Representative chromatogram from UNICORN start ***Evaluation*** module. Equilibration phase is not depicted in this chromatogram.

2	From the ***Evaluation*** module a pdf report of the chromatogram can be generated, and peak integration, curve and chromatogram comparisons can 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 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.
- Run the purification at lower flow rates.
- Check the volume of sample that was loaded. Too much sample can lead to decreased resolution. If that is the case use a smaller sample volume.

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 HiPrep 16/60 Sephacryl S-200 HR instructions.

Check your knowledge

1. ***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
2. ***What is the maximum sample volume that can be applied on a gel filtration column and still achieve maximum resolution?***
   a. 5% of total column volume
   b. 2% of total column volume
   c. 0.1% of total column volume
3. ***Why should the "Sample inject" message be acknowledged as soon as the Manual injection valve has been manually turned?***
   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
4. ***Which predefined methods are there in the UNICORN start Method Editor?***
   a. Anion Exchange
   b. Desalting
   c. Gel Filtration
   d. Affinity
   e. Cation Exchange
   f. All of the above
5. ***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
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

Q1. c
Q2. b
Q3. a
Q4. f
Q5. e
Q6. a

Ordering information

Product	Quantity	Code number
HiPrep 16/60 Sephacryl S-200 HR	1 × 5 mL	17116601
Sample Loop	1 × 2 mL	18111402
Column Holder	1	28956282
PEEK tubing id 0.75 mm (1/16")	1	18111253

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
HiPrep 16/60 & 26/60 Sephacryl High Resolution instructions	28402653

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
Anion exchange purification using ÄKTA start	29110759

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