GE Healthcare

Instructions 56-1190-96 AC **Gel Filtration**

Sephadex G-25 Medium

Sephadex™ G-25 Medium is an economic gel filtration media based on cross-linked dextran. The hydrophilic matrix minimizes nonspecific adsorption and gives high recoveries during desalting and buffer exchange of proteins and nucleic acids. Characteristics of Sephadex G-25 Medium are listed in Appendix B, Table 1.

The instructions that follow are based upon packing Sephadex G-25 in the recommended XK 16/40 column. Flow rates are given in specific volumetric values, with reference to the linear flow rate. To modify these instructions for a column with different dimensions, refer to Appendix A.

Detailed information on the technique of gel filtration can be found in the handbook: Gel Filtration; Principles and Methods", from GE Healthcare.

Sephadex

Table of contents

Material needed

Sephadex G-25 Medium

Column XK 16/40

Packing Reservoir RK 16/26, Valve (LV-3, LV-4 or Valve INV-907 if an ÄKTA system is available) or Packing connector XK16 and second Column XK 16/40 Pump (ÄKTAdesign pump P-901)

Graduated cylinder or beaker, large beaker

Glass rod, 5 ml syringe

Small spoon or plastic spatula

Buffer

1. Preparing the media suspension

Sephadex is supplied as a dry powder and must be swollen before use. During swelling excessive stirring should be avoided as it may break the beads. Do not use magnetic stirrers.

1. Swell the medium in excess buffer, at room temperature for 3 hours, or in a water bath at 90 °C for 1 hour. The eluent buffer should not contain agents which significantly increase the viscosity. The column may be equilibrated with viscous buffers at reduced flow rates after packing is completed.

2. Prepare a media slurry in a ratio of 75% settled gel to 25% buffer and degas under vacuum, if the gel was swollen at room temperature.
3. Allow all material to equilibrate to room temperature.

2. Assembling the column

1. Details of the column parts can be found in the instructions supplied with the column. Before packing ensure that all parts, particularly the nets, net fasteners and glass tube, are clean and intact.
2. Attach the packing reservoir firmly to the column. Or attach the packing connector to the column and then the second column that serves as a packing reservoir.
3. Connect the column end piece to a syringe and submerge it in buffer. Fill using the syringe, ensuring that there are no air bubbles trapped under the net. Close the tubing with a stopper and attach the end piece to the column.
4. Flush the column with buffer, leaving a few ml at the bottom. Mount the column vertically on a laboratory stand.

3. Packing the column

These instructions are for packing Sephadex G-25 in the recommended XK 16/40 column. Flow rates are given in specific volumetric values, with reference to the linear flow rate. To modify these instructions for a column with different dimensions, refer to Appendix A.

1. Resuspend and pour the media slurry into the column in one continuous motion. Pouring down a glass rod held against the wall of the column helps prevent the introduction of air bubbles. Fill the reservoir to the top with buffer. Screw on the reservoir top tightly, and connect it to the pump or injection valve on the ÄKTA system. Open the column outlet.
2. Open the bottom outlet of the column and pack at 10 ml/min (300 cm/h) until the gel bed has reached a constant height.
3. Stop the pump, close the column outlet and remove the column from the stand. Unscrew and remove the packing reservoir over a sink, or unscrew the packing column and then the packing connector.
4. Re-mount the column on the stand and carefully fill with buffer to form a meniscus at the top of the column.
5. Wet the adaptor by drawing buffer through it using a syringe. Insert the adaptor at an angle into the column, ensuring that no air is trapped under the net. Adjust the adaptor O-ring to give a sliding seal on the column wall.
6. Make all tubing connections at this stage. There must be a bubble-free eluent connection between the column and the pump and the column and the injection valve system.
7. Slide the adaptor slowly down the column so that any air in the tubing's is displaced by eluent. Valves on the inlet side of the column should be turned in all directions during this procedure to ensure that air is removed.

8. Lock the adaptor in position on the gel surface. Open the column outlet and continue packing until the gel bed is stable. Re-position the adaptor on the gel surface as necessary.

For desalting and buffer exchange, the column is now ready for use.

4. Equilibration

Equilibrate the column with 160 ml of running buffer. A larger volume may be required if detergent solutions are used.

5. Buffers

Buffer composition does not directly influence the resolution which can be obtained in gel filtration chromatography and buffers can be chosen to match the requirements of the sample. However, an ionic strength equivalent to 0.15 M NaCl or greater is recommended to avoid ionic interactions with the gel matrix.

To ensure long column life, all buffers should be centrifuged or filtered (0.45 µm) before use.

6. Samples

The sample volume can be up to 20 ml (25% of the total bed volume) for desalting and buffer exchange. To ensure long column life, samples should be centrifuged or filtered (0.45 µm) before use.

7. Elution

The recommended flow rate range for an XK 16/40 column packed with Sephadex G-25 Medium is 5 ml/min (150 cm/hour) Gel filtration is a non-interactive technique, and all sample substances should elute in a volume equivalent to the volume of the column. Re-equilibration is not needed between runs with the same eluent.

8. Cleaning-in-place (CIP)

In some applications, substances such as denatured proteins or lipids do not elute in the regeneration procedure. These can be removed by the cleaning procedure described below. The need for column cleaning may be indicated by:

- Increased back-pressure
- Colour changed at the top of the column
- Reduced resolution
- A space between the upper adaptor and the gel surface

To remove precipitated material, wash the column in the reversed flow direction with 40–80 ml of 0.2 M NaOH or a solution of a non-ionic detergent at a flow rate at 0.6 ml/min (18 cm/hour). The total contact time with the cleaning solution should be 1–2 hours. After washing, always re-equilibrate the column before re-use.

The cleaning procedures given above can also be performed on a Buchner funnel.

9. Sanitization

Sanitization reduces microbial contamination of the gel bed to a minimum. To sanitize, wash with 0.2 M NaOH at room temperature for a contact time of 30–60 minutes. For the XK 16/40 column, set the flow rate at approximately 0.6 ml/min (18 cm/hour). Re-equilibrate the column with sterile buffer before use.

10. Storage

Dry Sephadex should be stored at +4 °C to +30 °C.
Packed columns and used gel should be stored in 20% ethanol at +4 °C.

Appendix A

Converting to columns of different dimensions

Flow rates

Flow rates quoted in this instruction are for an XK 16/40 column. To convert flow rates for columns of different dimensions:

1. Divide the volumetric flow rates (ml/min) quoted by a factor of 2 (the cross-sectional area in cm^2 of the XK 16/40) to give the linear flow rate in cm/min.
2. Maintain the same linear flow rate and calculate the new volumetric flow rate according to the cross-sectional area of the specific column to be used.

$$\text{Linear flow rate} = \frac{\text{Volumetric flow rate}}{\text{Column cross-sectional area}}$$

Volumes

Volumes (buffers, gradients, etc.) quoted in this instruction are for an XK 16/40 column that has a bed volume of 100 ml (bed height × cross-sectional area). To convert volumes for columns of different dimensions, increase or decrease in proportion to the new column bed volume.

$$\text{New volume} = \text{Old volume} \times \frac{\text{New bed volume}}{\text{Old bed volume}}$$

Appendix B; Tables

Table 1. Media characteristics.

Fractionation range (globular proteins)- M_r	1000–5000
Fractionation range (dextrans)- M_r	100–5000
Bead structure	Cross-linked dextran
Bead size (Dry)	50–150 µm
Bead size (Wet)	86–258 µm
Maximum operating pressure	Obeys Darcys Law
Recommended volumetric flow rate*	5 ml/min (XK 16/40)
Recommended linear flow rate*	150 cm/hour
Maximum linear flow rate*	300 cm/hour
Chemical stability	All commonly used buffers, 0.2 M NaOH, 0.2 M HCl, 1 M acetic acid, 8 M urea, 6 M guanidine HCl, 1% SDS, 24% ethanol, 30% propanol, 30% acetonitrile
pH stability (long term)**	2–13
pH stability (short term)**	2–13
Autoclavable	at 121 °C, pH 7 for 30 min.

* At room temperature in aqueous buffer.

** pH stability, long term refers to the pH interval where the gel is stable over a long period of time without adverse effects on its subsequent chromatographic performance.
pH stability, short term refers to the pH interval for regeneration, cleaning-in-place and sanitization procedures. All ranges given are estimates based on our knowledge and experience.

11. Ordering Information

Description	Pack size	Code No.
Sephadex G-25 Medium	25 g	17-0033-10

Related Products

Column XK 16/40	18-8774-01
Packing connector X K 16	18-1153-44
Packing reservoir RK 16/26	18-8793-01
Valve LV-3	19-0016-01
Valve LV-4	19-0017-01
Pump P-901	18-1114-00
ÄKTAexplorer 100	18-1112-41
ÄKTApurifier 100	18-1404-00
Gel Filtration; Principles and Methods	18-1022-18
Column Packing – The Movie (instructional CD)	18-1165-33

www.gehealthcare.com/protein-purification
www.gehealthcare.com

GE Healthcare Bio-Sciences AB
Björkgatan 30
SE-751 84 Uppsala
Sweden

GE Healthcare Europe GmbH
Munzinger Strasse 5
D-79111 Freiburg
Germany

GE Healthcare UK Ltd.
Amersham Place
Little Chalfont
Buckinghamshire, HP7 9NA
UK

GE Healthcare Bio-Sciences Corp.
800 Centennial Avenue
P.O. Box 1327
Piscataway, NJ 08855-1327
USA

GE Healthcare Bio-Sciences KK
Sanken Bldg.
3-25-1, Hyakunincho
Shinjuku-ku, Tokyo 169-0073
Japan

imagination at work

56-1190-96 AC 02/2006

Elanders Östervåla 2006