GE Healthcare

Instructions 56-1190-99 AE **Gel Filtration media**

Superdex 30 prep grade
Superdex 75 prep grade
Superdex 200 prep grade

Superdex™ 30, 75 and 200 prep grade are preparative gel filtration media with a unique composite matrix of dextran and agarose. This matrix combines the excellent gel filtration properties of dextran with the physical and chemical stability of highly cross-linked agarose. Some characteristics of Superdex gel filtration media are listed in Appendix C, Table 1.

With their high physical and chemical stability, very high batch-to-batch reproducibility, and Regulatory Support File back-up, Superdex 30 prep grade, Superdex 75 prep grade and Superdex 200 prep grade are ideal for all stages of an industrial scale operation - from research and process development through scale-up and into pruduction.

The instructions that follow are based upon packing Superdex prep grade media in an XK 16, 26 and 50 columns.

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

Superdex™

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1. Material needed

Superdex prep grade
Column XK 16/100 column, XK 26/100 column or
XK 50/100 column
Packing Equipment Packing reservoir XK16/70 column, XK26/70 column (note: an extra column is needed for packing)
Packing Connector XK 16 or Packing Connector XK 26 (note: an extra column is needed for packing)
Packing reservoir RK 50 reservoir
Pump ÄKTAdesign pumps: Pump P-901 or Pump P-903

Note: Superdex prep grade is optimally packed at constant pressure. Consequently a pump with pressure gauge is recommended e.g. Pump P-901 or Pump P-903.

2. Preparing the gel

1. Equilibrate all material to room temperature.

2. Suspend the medium by shaking and pour into a graduated glass filter or a graduated laboratory beaker. Avoid using a spatula or glass rod, which may cause breakage of the beads. See in table 1 for the medium slurry volume that is needed for different columns. Add distilled water to give a final slurry concentration of according to table 1 settled gel.

Table 1: Packing instructions Superdex Prep Grade

Column	Slurry concentration %	Slurry volume (ml)	Compression %	Packed bed height (ml)	Flow rate rate step 1 (ml/min)	Time for packing step 1 (min)
XK 16/100	52 +– 2	430	15	93–94	3 +–0.5	90
XK 26/100	60 +– 2	1000	10	93–94	4 +–0.5	90
XK 50/100	65–70	3000	10	94–95	10 +–2	120

Column	Pressure step 2 (MPa)	Flow rate step 2 (ml/min)	Time for packing step 2 min)	Maximum flow rate of packed column (ml/min)	Maximum pressure of packed column (MPa)
XK 16/100	0.45 +–0.05		30	3	0.35
XK 26/100	0.35 +–0.05			6	0.35
XK 50/100		20 +5 (not below 20)	30	16	0.3

3. Assembling the column

Details of the column parts and packing equipment can be found in the instructions supplied. Before packing ensure that all parts, particularly the nets, net fasteners and glass tube, are clean and intact.

1. Attach the packing reservoir and the packing connector (only XK 16/100 column and XK26/100 column) to the column.
2. Inject 20% ethanol into the column bottom end piece with a syringe. Ensure that there are no air bubbles trapped under the net. Close the tubing with a stopper.
3. Flush the column and reservoir with distilled water, leaving a few ml at the bottom. Mount the column vertically on a laboratory stand.

4. Packing the column

These instructions are for packing Superdex prep grade media in an XK16/100, XK26/100 or XK50/100 column.

1. Pour the medium 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. Immediately fill the remainder of the column and reservoir with distilled water. Mount the lid on the packing reservoir and connect it to the pump.
2. Open the column outlet and start step 1 of the packing by pumping distilled water through the column according to the flow rate and time in table 1. Start step 2 by choosing a flow rate or a pressure according to recommended values in table 1.

If it is a pressure value it means that the flow rate had to be adjusted in the beginning several times to keep the pressure. when the medium bed has been stabilized run at that flow rate in 30 minutes. Switch off and disconnect the pump. Close the column outlet.

3. Take the column from the stand and remove the packing reservoir over a sink. Remount the column vertically and fill to the top with distilled water.
4. Wet the column adaptor by submerging the plunger end in 20% ethanol, and drawing through with a syringe. Ensure that all bubbles have been removed. Insert the adaptor into the top of the column, taking care not trap air under the net.
5. With the adaptor outlet open, push the adaptor into the column and down onto the medium bed, allowing the distilled water to displace any air remaining in the tubing.
6. Lock the adaptor in position, connect it to the pump, open the column outlet and continue packing at maximum flow rate for 20 minutes.
7. Mark the position of the bed surface on the column. Stop the pump, close the column outlet and reposition the adaptor to approximately 3 mm below the marked position.

The column is now ready for equilibration.

If required, the quality of packing can be checked using the testing procedure described in Appendix A.

5. Equilibration

Equilibrate the column with 2 column volumes of running buffer at a flow rate of 60 cm/h. A larger volume may be required if detergent solutions are used.

6. 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.

7. Samples

The sample volume should be in the range of 0.1–1% for analytical purposes and < 5 % for preparative purposes of the total bed volume. To ensure long column life, samples should be centrifuged or filtered (0.45 µm) before use.

8. Flow rates

Maximum flow rates see table 1. Recommended flow rate is 10–60 cm/hour. The lower the flow rate, the better the resolution. In a cold room at 4 °C, aqueous buffers approximately double in viscosity compared to room temperature, and flow rates should be lowered accordingly.

9. Re-equilibration

Re-equilibrate the column between samples is normally not needed. If proteins are retarded in the medium it is indications of separation mechanisms other than pure gel filtration. To avoid retardation of proteins at least 0.15 M NaCl is added to the buffer solution.

10. Cleaning-in-place (CIP)

To remove precipitated material, wash the column in the reversed flow direction with 1–2 column volumes of 0.5 M NaOH at a flow rate at 20 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 and check the pH of the eluted buffer to check that the column has been fully re-equilibrated before loading the next sample.

Remove strongly non-specifically bound proteins, lipoproteins ans lipids by washing the column in the reversed flow direction with 1–2 column volumes of 70% ethanol or 30% isopropanol at 10 cm/h Apply organic solvents in increasing concentration gradients to avoid air bubble formation when using high concentrations. Alternatively, wash the column with 1–2 column volumes of 0.1–0.5% detergent in a basic or acidic solution. After treatment with detergent always remove residual detergent by washing with five bed volumes of 70% ethanol. After washing, always re-equilibrate the column before re-use and check the pH of the eluted buffer.

11. Sanitization

Sanitization reduces microbial contamination of the medium bed to a minimum. To sanitize, wash with 0.5–1.0 M NaOH at room temperature for a contact time of 30–60 minutes. Re-equilibrate the column with sterile buffer before use.

12. Storage

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

Appendix A

Converting from linear flow (cm/hour) to volumetric flow rates (ml/min) and vice versa

It is convenient when comparing results for columns of different sizes to express flow as linear flow (cm/hour). However, flow is usually measured in volumetric flow rate (ml/min).

To convert between linear flow and volumetric flow rate use one of the formulae below.

From linear flow (cm/hour) to volumetric flow rate (ml/min)

where
Y = linear flow in cm/h
d = column inner diameter in cm

Example:
What is the volumetric flow rate in an XK 16/70 column (i.d. 1.6 cm) when the linear flow is 150 cm/hour?
Y = linear flow = 150 cm/h
d = inner diameter of the column = 1.6 cm

From volumetric flow rate (ml/min) to linear flow (cm/hour)

where
Z = volumetric flow rate in ml/min
d = column inner diameter in cm

Example:
What is the linear flow in an HR 5/5 column (i.d. 0.5 cm) when the volumetric flow rate is 1 ml/min?
Z = Volumetric flow rate = 1 ml/min
d = column inner diameter = 0.5 cm

Volumetric flow rate = 150 x p x 1.6 x 1.6

60 x 4 ml/min = 5.03 ml/min

Volumetric flow rate (ml/min) = Linear flow (cm/h)

60 x column cross sectional area (cm^2)

Y x p x d2

60 4

=

From ml/min to using a syringe
1 ml/min = approximately 30 drops/min on a HiTrap 1 ml column
5 ml/min = approximately 120 drops/min on a HiTrap 5 ml column

Appendix B

Testing the packed column

To check the efficiency of the column packing, determine the theoretical plate number and peak symmetry. If the column is packed according to the instructions described above typical values should be:

Efficiency: N >13,000 theoretical plates per meter
Peak symmetry: A_s = 0.70-1.30

Solutions required

Distilled water
Sample: acetone 2 % in distilled water

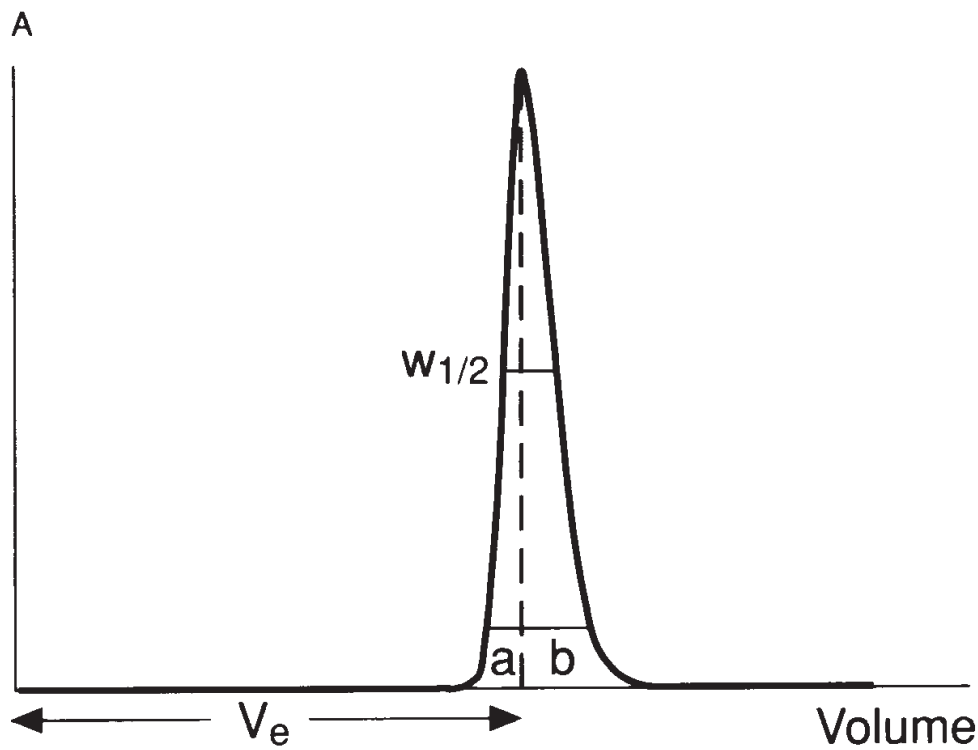
1. Establish a linear flow rate of 60 cm/hour, see appendix A through the packed column with distilled water or buffer solution.
2. Inject 0.1 % of column volume acetone (100 µl per cm^2 cross-sectional area) onto the column at a linear flow rate of 60 cm/hour see appendix A. Record the absorbance at 280 nm from time of injection until the acetone peak has been detected and the monitor signal has returned to baseline.
3. Calculate the column efficiency (plate number)
   Referring to the figure below, calculate the column efficiency (N) as follows:

$$N = 5.54 (V_e/W_{1/2})^2 \times (1000/L)$$

Referring to the figure below, calculate the symmetry factor (A_s) by the formula:

$A_s = b/a$

V_e = Elution volume in ml
$W_{½}$ = Peak width at half peak height in ml
L = Medium bed height (mm)
a = First half peak widht at 10% peak height
b = Second half peak widht at 10% peak height



13. Troubleshooting

If peak symmetry is too low, increase the first (step 1 packing) flow rate.

If the peak symmetry is too high decrease the first (step 1 packing) flow rate or increase the compressing (step 2) pressure/ flow rate.

For more detailed handling when packing the column, see Column Packing -The Movie, code no 18-1165-33.

Prepacked columns with media can be ordered from product catalogue; Biodirectory or from custom Products. Contact the local sales office for an offer.

Appendix C

Table 1. Gel characteristics

Fractionation range (globular proteins)- M_r	< 10,000 (Superdex 30 prep grade) 3,000–70,000 (Superdex 75 prep grade) 10,000–600,000 (Superdex 200 prep grade)
Bead structure	Composite of dextran and agarose
Bead size	24–44 µm (mean 34 µm)
Maximum operating pressure	0.3 MPa (3 bar 42 psi)
Maximum linear flow rate*	90 cm/hour
Recommended volumetric flow rate*	0.4–0.8 ml/min (XK 16/70)
Recommended linear flow rate*	30–60 cm/hour
Chemical stability	All commonly used buffers, 0.2 MNaOH, 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)**	3–12
pH stability (short term)**	1–14
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 medium is stable over a long period of time without adverse effects on its subsequent separation 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.

14. Ordering Information

Product	Pack size	Code No.
Superdex 30 prep grade	25 ml	17-0905-10
Superdex 30 prep grade	150 ml	17-0905-01
Superdex 75 prep grade	25 ml	17-1044-10
Superdex 75 prep grade	150 ml	17-1044-01
Superdex 200 prep grade	25 ml	17-1043-10
Superdex 200 prep grade	150 ml	17-1043-01

Related Products

XK 16/100 column	18-8776-01
XK 26/100 column	18-8770-01
XK 50/100 column	18-8753-01
Packing connector XK 16	18-1153-44
Packing connector XK 26	18-1153-45
Packing equipment RK 50 reservoir	18-8790-01
Pump P-901	18-1114-00
Pump P-903	18-3000-00
Gel Filtration; Principles and Methods	18-1022-18

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