cytiva

Sephadex G-25 Medium

Size exclusion chromatography

Instructions for Use

Sephadex™ G-25 Medium is an economic size exclusion chromatography resin 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.

Detailed information on the technique of size exclusion chromatography can be found in the handbook *Size Exclusion Chromatography; Principles and Methods*.

Table of Contents

Read these instructions carefully before using the products.

Safety

For use and handling of the products in a safe way, refer to the Safety Data Sheets.

1 Characteristics

Sephadex G-25 Medium is a BioProcess™ size exclusion chromatography resin. BioProcess chromatography resins are developed and supported for process-scale chromatography. BioProcess resins are produced with validated methods and are tested to meet manufacturing requirements. Secure ordering and delivery routines give a reliable supply of resins for production-scale. Regulatory Support Files (RSF) are available to assist process validation and submission to regulatory authorities. BioProcess resins cover all purification steps from capture to polishing.

Table 1. Characteristics of Sephadex G-25 Medium

Matrix	Cross-linked dextran
Particle size distribution (Dry)	50 to 150 µm[1]
Swelling factor	~ 1.7
Fractionation range [M_r] globular proteins	~ 1000 to 5000
Fractionation range [M_p] dextrans	~ 100 to 5000
Recommended operating flow velocity	150 cm/h[2]
Recommended maximum operating flow velocity	300 cm/h[2]
Chemical stability	Stable to commonly used aqueous buffers
pH stability, operational[3]	2 to 13
pH stability, CIP[4]	2 to 13
Autoclavability	30 min at 121°C, pH 7

1 ≥ 90% volume share within given range

2 At room temperature in aqueous buffer

3 pH range where resin can be operated without significant change in function

4 pH range where resin can be subjected to cleaning- or sanitization-in-place without significant change in function

2 Column packing

Recommended columns

Lab-scale columns

- HiScale™ columns

  Inner diameter of 16 to 50 mm, bed volumes up to 393 mL, bed heights up to 20 cm

- XK columns

  Inner diameters of 16 to 50 mm, bed volumes up to 274 mL at bed heights of 15 cm

Production-scale columns

- AxiChrom™ columns

  Inner diameters from 50 to 200 mm, bed volumes up to 16.7 L, bed heights up to 50 cm

- AxiChrom columns

  Inner diameters from 300 to 1600 mm; bed volumes up to 1005 L, bed heights up to 50 cm

Materials needed

- Sephadex G-25 Medium
- XK column
- Packing Reservoir RK 16/26 or packing connector second XK column
- Graduated cylinder or beaker, large beaker
- Glass rod, 5 mL syringe
- Small spoon or plastic spatula
- Buffer

Preparing the resin suspension

Sephadex is supplied as a dry powder and must be swollen before use. Avoid excessive stirring during swelling as it can break the particles. Do not use magnetic stirrers.

Step	Action
1	Swell the resin in 4 to 6 mL buffer/g resin, at room temperature for 3 hours, or in a water bath at 90°C for 1 hour. The eluent buffer must not contain agents which significantly increase the viscosity. The column can be equilibrated with viscous buffers at reduced flow rates after packing is completed.
2	Prepare a resin slurry in a ratio of 75% settled resin to 25% buffer and degas under vacuum, if the resin was swollen at room temperature.
3	Allow all material to equilibrate to room temperature.

Assembling the column

Step	Action
1	Details of the column parts can be found in the instructions supplied with the column. Before packing, make sure 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.

Step	Action
3	Connect the column end piece to a syringe and submerge it in buffer. Fill using the syringe, while making sure that no air bubbles are 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. Put the column vertically on a laboratory stand.

Packing XK 16/40 columns

Flow rates are given in specific volumetric values, with reference to the flow velocity. To modify these instructions for a column with different dimensions, refer to *Appendix A, on page 12*.

Step	Action
1	Resuspend and pour the resin 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 injection valve on the ÄKTA™ system.
2	Open the column outlet and pack at 10 mL/min (300 cm/h) until the resin bed has reached a constant height..

Step	Action
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	Put the column on the stand again and carefully fill with buffer to form a meniscus at the top of the column.
5	Wet the adapter by drawing buffer through it using a syringe. Insert the adapter at an angle into the column, while making sure that no air is trapped under the net. Adjust the adaptor O-ring to give a sliding seal on the column wall.
6	Connect the top adapter to the ÄKTA system loosely so that eluent and any air can be flushed out from the tubing without entering the system.
7	Slide the adapter slowly down the column so that any air in the tubing is displaced by eluent. Start the pump at a low flow and tighten the adapter tubing to the system using drop to drop connection. Stop the pump.
8	Lock the adapter in position on the resin surface. Open the column outlet and continue packing until the resin bed is stable. Stop the pump and close the column outlet. Remove the top adapter tubing from the system. Reposition the adapter down onto the bed and further 3 mm into the bed.

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

Packing AxiChrom columns

AxiChrom columns are packed using mechanical axial compression packing. For more information regarding the AxiChrom columns and packing procedures see below documentation:

- *AxiChrom Columns (28929041)*
- *AxiChrom 50, 70 and 100 columns, operating instructions (28933108)*
- *AxiChrom 140 and 200 columns, Operating instructions (28943123)*
- *AxiChrom 300-1600 columns, Operating instructions (29065430)*

Note: *There are no specific packing instructions for Sephadex G-25 in the documents listed above.*

3 Operation

Equilibration

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

Buffers

Buffer composition does not directly influence the resolution which can be obtained in size exclusion 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 resin matrix.

To prolong column life, all buffers must be centrifuged or filtered (0.45 µm) before use.

Samples

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

Elution

The recommended flow rate for an XK 16 column packed with Sephadex G-25 Medium is 5 mL/min (150 cm/hour). Size exclusion chromatography is a noninteractive technique, and all sample substances must elute in a volume equivalent to the volume of the column. Re-equilibration is not needed between runs with the same eluent.

4 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 can be indicated by:

- Increased back pressure

- Colour changed at the top of the column
- Reduced resolution
- A space between the upper adapter and the resin surface

To remove precipitated material, wash the column in the reversed flow direction with 40 to 80 mL of 0.2 M NaOH or a solution of a nonionic detergent at a flow rate of 0.6 mL/min (18 cm/hour). The total contact time with the cleaning solution must be 1 to 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.

5 Sanitization

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

6 Storage

Dry Sephadex must be stored at 4°C to 30°C. Packed columns and used resin must be stored in 20% ethanol at 4°C to 30°C.

7 Ordering information

Description	Quantity	Product code
Sephadex G-25 Medium	100 g	17003301

Description	Quantity	Product code
	500 g	17003302
	5 kg	17003303
	40 kg[1]	17003307

[1] Pack size available upon request

Related products	
XK column 16/20	28988937
XK column 26/20	28988948
XK column 50/20	28988952
Packing connector XK 16	18115344
Packing reservoir RK 16/26	18879301
HiScale 16/20 column	28964441
HiScale 26/20 column	28964514
HiScale 50/20 column	28964445

Related literature		
Data files	Chromatography systems ÄKTApure	29021196
	Chromatography systems ÄKTA avant	28957345
	AxiChrom columns	28929041
	XK Empty Columns	28997659
	HiScale Columns	28975523
Handbook	Size Exclusion Chromatography: Principles and Methods	18102218

Appendix A

Converting to columns of different dimensions

Flow rates

To convert flow rates for columns of different dimensions:

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

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

Volumes

To convert volumes for columns of different dimensions, increase or decrease in proportions to the new column bed volume:

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

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