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Sepdex G25

Product Number: SP-G25

Shipping and Storage

Unopened chromatography media, please keep in the original container; The packed chromatography column should be soaked in a 20% ethanol solution first, and then the upper and lower column heads should be closed. The storage environment is 4~30 ℃.

Description

Sepdex G25 is a series of cross-linked dextran based gel filtration chromatography media, which is separated by the difference of molecular weight of different molecules. This series consists of four chromatography media with different particle sizes, arranged in descending order of particle size, with suffixes C, M, F, and SF respectively. This series of chromatography media can be well used for desalination of various biomolecules, buffer displacement, and separation and purification of small molecule substances, such as peptides.

Features

Sepdex G25 has excellent scaling up production capacity:

1. Crosslinked glucan with reliable rigidity
2. Provide multiple packaging specifications to meet the processing needs of samples with different volumes, making it easy to scale up linearly.

Chromatographic medium parameters

Parameter Table of Sepdex G25

	Sepdex G25-25C	Sepdex G25-25M	Sepdex G25-25F	Sepdex G25-25SF
Type of chromatography medium	Gel filtration			
Base frame	Cross-linked glucan			
Separation range	1kD~5kD			
Main particle size distribution*	100~300μm	50~150μm	20~80μm	20~50μm
Recommended working flow rate	300 cm/h	150 cm/h	100 cm/h	80 cm/h
Swelling coefficient	4-5 ml/g			
Voltage resistance	0.3MPa			

Note:Measurement in dry powder state, percentage distributed within the range ≥ 80%

Chemical tolerance

Chemical tolerance table of Sepdex G25

Name	Description
PH stability 1)	2-13
Tolerable solvent	Common aqueous solution, 30% isopropanol[2)], 75% ethanol[2)], 1M NaOH, 1M acetic acid, 6M guanidine hydrochloride, 8M urea

Note: 1) After being placed in an environment of 40℃ and pH 2-13 for 7 days, the physicochemical properties and functions of the chromatography medium did not show significant changes.

2)V/v, volume ratio

Column installation method

The following column loading methods are applicable to the loading of laboratory scale chromatography columns. For the loading methods of industrial chromatography columns, please consult local technical support.

1. Supplies required for column installation

1.1. Chromatographic medium: Sepdex G25

1.2. Chromatography column: Laboratory scale chromatography column and column loader

1.3. Required solution:

1.3.1. Column solution: purified water

1.3.2. Exhaust solution: purified water

1.4. Column installation tools: sand core funnel, mixing rod, measuring cylinder, etc

2. Preparation before column installation

2.1. Add the dry powder chromatography medium in a ratio of 4-6 ml purified water/g to purified water, and let it swell at room temperature for more than 4 hours.

2.2. Calculate the required volume of chromatography medium (Vm) for column installation (the volume of chromatography medium after sufficient settlement), using the following formula:

Vm=cross-sectional area of chromatography column×planned column bed height×compression ratio of chromatography medium

Note: The compression ratio of Sepdex G25 is approximately 1.15

2.3. Load the chromatography medium into a sand core funnel, clean and filter with a column loading solution that is approximately three times the volume of the chromatography medium, and replace the chromatography medium to be loaded into the column loading solution.

2.4. Prepare a gel suspension for the column chromatography medium to be loaded, with a suitable ratio of 50% -60% for Sepdex G25 chromatography medium. In order to obtain an accurate volume of the chromatography medium, the chromatography medium can be placed in a measuring cylinder and settled overnight, or the natural settling effect of the chromatography medium can be simulated by low-speed centrifugation (3000 rpm, 5 min), and then measured.

2.5. Check the standby chromatography column to ensure cleanliness and no liquid leakage.

3. Column installation

3.1. Use purified water as the bottom filter membrane (sieve) for exhaust.

3.2. After sufficient exhaust, screw a plug or close the column bottom valve at the interface at the bottom of the column, and continue to inject a small amount of purified water until it covers the bottom of the column.

3.3. Adjust the chromatography column to be vertical.

3.4. Connect the column head to the chromatography system, provide a low flow rate of 5 ml/min through the chromatography system, and exhaust the column head filter membrane (sieve) with purified water.

3.5. Mix the prepared chromatography medium suspension thoroughly with a stirring rod, and then slowly pour it into the prepared chromatography column in one go.

Note: If the volume of the suspension exceeds the volume of the empty column, a column loader or a connector should be used to connect another empty column tube for extension.

3.6. Place the deflated column head into the chromatography column, fully adhere to the liquid level of the suspension, and eliminate all bubbles. Then tighten the column head sealing ring.

3.7. Start the system pump, adjust the flow rate to 300 cm/h, and use liquid flow to compress the column bed. During this period, the pressure should not exceed 0.3 MPa. If overpressure occurs, it is necessary to reduce the flow rate.

Conversion table for flow velocity of chromatography columns with different specifications

	10mm	16mm	26mm	50mm
60 cm/h	0.8 ml/min	2.0 ml/min	5.3 ml/min	19.6 ml/min
100 cm/h	1.3 ml/min	3.3 ml/min	8.8 ml/min	32.7 ml/min
150 cm/h	2.0 ml/min	5.0 ml/min	13.3 ml/min	49.1 ml/min
200 cm/h	2.6 ml/min	6.7 ml/min	17.7 ml/min	65.4 ml/min
300 cm/h	3.9 ml/min	10.0 ml/min	26.5 ml/min	98.1 ml/min
600 cm/h	7.9 ml/min	20.1 ml/min	53.1 ml/min	196.3 ml/min

3.8. After the column bed stabilizes (the adhesive surface no longer descends), mark the position of the adhesive surface at this time. Stop the pump and press the column head down to 3-5mm below the marked position.

3.9. Continue rinsing the equilibrium chromatography column at a linear flow rate of 300 cm/h, and complete the column loading if the gel surface no longer drops. If the adhesive surface drops, repeat steps 8-9.

Note: It is recommended that the working flow rate does not exceed 75% of the column loading flow rate.

Column efficiency measurement

Choose one of the two testing methods shown in Table 4 for column efficiency testing. Use a mobile phase equilibrium chromatography column until the baseline is stable, load the sample into the chromatography column, continue to rinse with the mobile phase, and after the chromatographic peak returns to the baseline, end the operation. Integrate the chromatographic peak and evaluate the column loading effect.

Table 4 Two column efficiency measurement methods

	Acetone method	NaCl method
Sample	1% (v/v) acetone aqueous solution	2M NaCl aqueous solution
Sample volume	1% column volume	
Mobile phase	Water	0.2M NaCl aqueous solution
Current velocity	30 cm/h	
Detector	UV 280nm	Conductivity

The main evaluation criteria for column installation effectiveness are N/m (number of trays per meter) and As (symmetry factor), and their calculation methods are as follows:

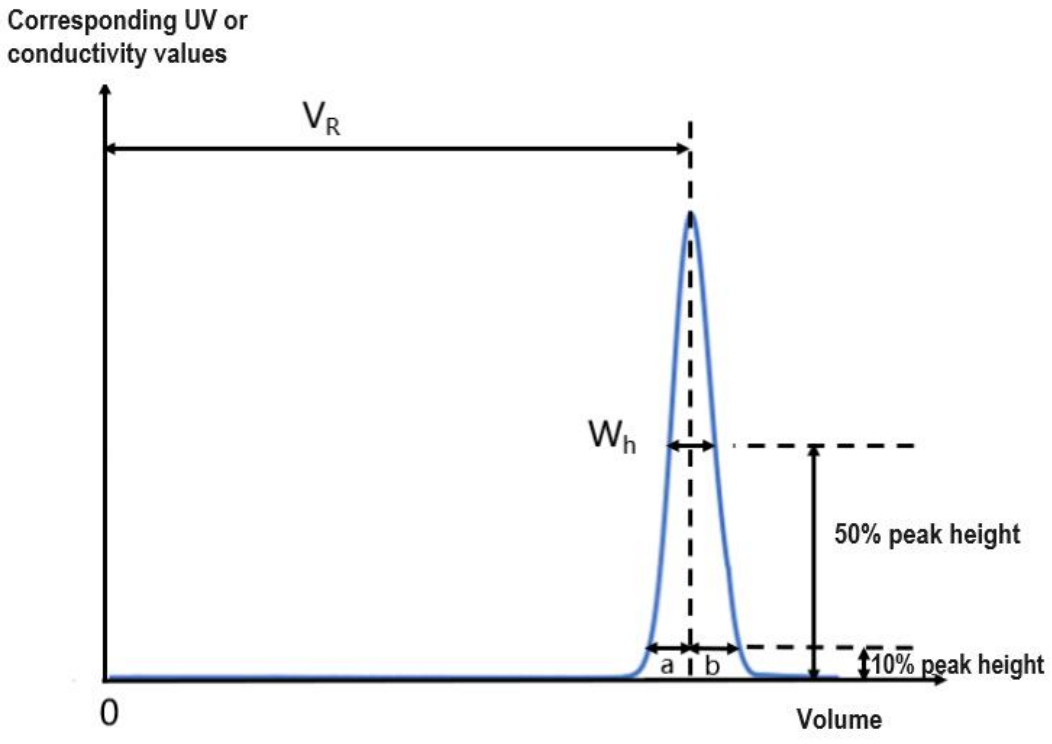
$$N/m=5.54x(\frac{V_R}{W_h})^2x\frac{1}{L}$$

$$As=b/a$$

Column efficiency qualification standard:

N/m > 2000

0.8 < As < 1.5



L=column height

VR=Reserve Volume

Wh=Half peak width

A=Left half width at 10% peak height

B=Right half peak width at 10% peak height

Protocol

1. Chromatographic conditions

1.1. Buffer selection: The stability of the sample in the buffer should be considered. If separation and purification are carried out, in order to avoid possible non-specific adsorption, it is advisable to use saline buffer instead of purified water. If buffer replacement or desalination is performed, the target buffer should be used directly.

1.2. Sample pre-treatment: To prevent the sample from blocking the column, the sample needs to be filtered with a 0.45um microporous filter membrane before loading.

2. Chromatographic steps

2.1. **Balance:** Use buffer solution to fully balance the chromatography column until the pH and conductivity are stable and basically consistent with the equilibrium buffer solution. This step usually requires 2-3 times the column volume.

2.2. **Sample loading:** Usually, the sample loading for component separation does not exceed 30% of the column volume, and the sample loading for separation and purification does not exceed 5% of the column volume. The sample concentration is not easily too high to avoid overpressure or affecting resolution.

2.3. **Elution:** Using buffer to elute and collect components with peaks at different positions, usually requiring 1-1.5 times the column volume.

2.4. **Regeneration:** Rinse the chromatography column with a high salt buffer solution (such as 1M NaCl).

2.5. **Rebalance:** Rebalance the chromatography column with buffer solution.

Cleaning and regeneration

As the number of times the chromatography medium is used increases, pollutants (such as lipids, endotoxins, proteins, etc.) continuously accumulate on the chromatography column. Regular in place cleaning is crucial for maintaining the stable working state of the chromatography column. Determine the frequency of in situ cleaning based on the degree of contamination of the chromatography medium (if contamination is severe, it is recommended to perform in situ cleaning after each use to ensure repeatability of results and extend the working life of the chromatography medium).

For different types of impurities and pollutants, it is recommended to clean them under the following conditions:

1. Removal of proteins with strong binding force: Wash with a 2M NaCl solution that is 5 times the volume of the column, or use a high salt buffer solution not lower than pH 2, such as a 1M NaAc solution
2. Removal of strongly hydrophobic proteins and precipitated proteins: First, clean with a 0.2M NaOH solution that is 5 times the volume of the column, and then clean the alkaline solution with purified water that is 5-10 times the volume of the column
3. Removal of lipoproteins and lipid substances: First, clean with 70% ethanol or 30% isopropanol, which is 5 times the volume of the column, and then rinse thoroughly with purified water, which is 5-10 times the volume of the column

Note: 70% ethanol or 30% isopropanol should be degassed before use; During the in place cleaning process, the flow rate can be selected from 30-60 cm/h; When the blockage is severe, reverse cleaning can be used.

Sterilization

To reduce microbial load, it is recommended to use a 0.2M NaOH solution to treat the chromatography medium, with a processing time of 30-60 minutes.

Destruction and recycling

Due to the difficulty in degrading Sepdex G25 series chromatography media in nature, it is recommended to use incineration treatment for discarded chromatography media in order to protect the environment.

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For chromatography media that come into contact with biologically active samples such as viruses and blood, please follow local biosafety requirements before disposal or disposal.