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Smartarose 4B

Product Number: SMT4B

Shipping and Storage

Store at 4-30℃.

Description

Smartarose is a spherical agarose gel filtration matrix. The agarose content of Smartarose 4B and Smartarose 6B is 4% and 6% respectively. Smartrose CL gel is a derivative of Smartrose 4B and Smartrose 6B, with better chemical and physical properties and faster flow rate. Smartarose CL gel is resistant to organic solvents, so it is suitable for separating substances in organic solvents. Smartarose and Smartarose CL have a wide separation range and are suitable for characterizing or separating samples with different molecular weights.

Project	Performance			
Smararose	4B	6B	CL-4B	CL-6B
Agarose	4%	6%	4%	6%
Optimal separation range	70×10^3-20×10^6	10×10^3-4×10^6	70×10^3-20×10^6	10×10^3-4×10^6
Particle size	45-165μm			
Recommended linear flow rate (cm. h)	11 cm/h	14 cm/h	26 cm/h	30 cm/h
PH stability	4-9	4-9	3-13	3-13
Reagent tolerance	Resistance to common solutions for gel chromatography, such as 8 M urea, 6 M guanidine hydrochloride		It is resistant to common solutions of gel chromatography, such as 8 M urea and 6 M guanidine hydrochloride. Also tolerant to ethanol DMF、THE、 Acetone DMS、 Organic solvents such as chloroform, dichloromethane, dichloroethane, pyridine, triethyl phosphate, and acetonitrile.	
Physical properties	The volume change caused by pH or ionic strength changes can be ignored			
Sterilization	chemical method		Steam high-pressure sterilization, 120 ℃, 20 min	

Note: Linear flow rate=Volume flow rate (cm^3/h)/Column cross-sectional area (cm^2)

The pH range is estimated based on experiments and experience. Please note that long-term pH stability refers to the pH range in which the chromatographic performance of gel will not change for a long time. The short-term pH stability refers to the stable pH range during gel regeneration and cleaning.

Column installation instructions

1. Media preparation

The medium is stored in 20% ethanol. Before installing the column, it is recommended to filter out 20% ethanol and replace it with deionized water.

Mix 75% medium and 25% deionized water evenly, and degas under vacuum negative pressure. Do not use viscous reagents for column loading. After column installation, the chromatography column can be equilibrated with viscous buffer at low flow rate.

2. Media loading

The column installation method has two steps, and the second step should be carried out under constant pressure. The column pressure is shown in Table 2. The resolution of gel chromatography increases with the increase of column bed height. Therefore, it is best for the height of the column bed to be above 60 cm.

Medium	Step 1 Flow rate (cm/h)	Step 2 Pressure (MPa, bar, Psi)

Smartarose 4B	15	0.018,0.18,2.6
Smartarose 6B	30	0.025,0.25,3.6
SmartaroseCL-4B	30	0.025,0.25,3.6
Smartarose CL-6B	30	0.045,0.45,6.4

Loading of chromatography column (using a reservoir for loading)

Before installing the column, calculate the column bottom area based on the diameter of the chromatography column, and calculate the required medium volume based on the required column height. The formula is as follows: $V=1.155\Pi r^2h$

V: Required medium volume mL

1.15: Compression coefficient(Different media have different compression coefficients)

r: Column radius cm

h: Loading height cm

2.1. Rinse the bottom sieve plate and joint of the chromatography column with deionized water to ensure that there are no bubbles on the bottom sieve plate. Close the bottom outlet of the column and leave 1-2cm of deionized water at the bottom of the column.

2.2. Suspend the packing material and carefully pour the slurry continuously into the chromatography column. Pouring the slurry along the column wall with a glass rod can reduce the generation of bubbles.

2.3. If a reservoir is used, immediately fill the chromatography column and reservoir with water, place the injection distributor on the surface of the slurry, connect it to the pump, and avoid creating bubbles in the distributor or injection tube.

2.4. Open the bottom outlet of the chromatography column and turn on the pump to operate at the set flow rate. Initially, the buffer should be allowed to flow slowly through the chromatography column, and then slowly increased to the final flow rate. This can avoid hydraulic impact on the formed column bed and also prevent uneven formation of the column bed. If the recommended pressure or flow rate cannot be achieved, you can use the maximum flow rate of the pump you are using, which can also achieve a good filling effect. (Note: In subsequent chromatographic procedures, do not exceed 75% of the maximum column flow rate.) After the column bed height stabilizes, add at least twice the volume of deionized water to the column bed at the final column flow rate. Mark the height of the column bed.

2.5. Turn off the pump and close the outlet of the chromatography column.

2.6. If using a reservoir, remove the reservoir and place the distributor in the chromatography column.

2.7. Push the distributor towards the column to the marked column bed height. Allow column loading fluid to enter the distributor and lock the distributor connector.

2.8. Connect the packed chromatography column to a pump or chromatography system and begin equilibration. If necessary, the distributor can be readjusted.

3. Column effect test

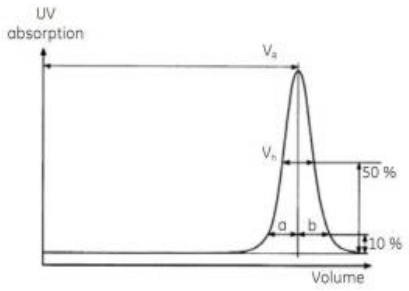
To verify the quality of the chromatography column, column efficiency testing should be conducted to determine the theoretical number of trays and peak asymmetry coefficient.

Eluent: Deionized water

Sample: 2% (v/v) acetone aqueous solution or 1 M NaCl solution

Calculate the theoretical number of trays as shown in the figure below using the following formula: N/m=5.54 (VR/Ph) 2 x 1000/L

The formula for calculating the peak asymmetry coefficient (AS) is as follows: AS=b/a (as shown in the following figure)



Purification process

1. Preparation of buffer solution

It is recommended to filter the water and buffer solution through a 0.22μm or 0.45μm filter membrane before use.

The equilibrium solution is a solution used to protect proteins after protein purification, and can be selected according to customer needs.

2. Sample preparation

It is recommended to centrifuge or filter the sample with a 0.22μm or 0.45μm filter membrane before loading to reduce impurities and prevent column blockage.

3. Sample purification

3.1. Balance

Before loading, equilibrate at least two column volumes with equilibrium solution or until the baseline is stable. Solutions containing detergents may require longer equilibration time.

3.2. Sampling

The recommended sample volume is 2-5% of the column volume. Samples can be loaded through sample tubes or sample rings. The maximum purification flow rate cannot exceed 70% of the column loading flow rate.

3.3. regeneration

Regeneration usually involves rinsing the column volume twice with water and then 2-3 times with buffer solution. If not used immediately, equilibrate with 20% ethanol and store at 4-30℃. For different samples, it is recommended to use complete in-situ cleaning (CIP) after approximately 5 cycles.

Cleaning and preservation

1. CIP (Cleaning In Place) cleaning

CIP is the removal of substances that have been tightly bound, precipitated, or denatured during previous production processes. In some cases, substances such as lipids or denatured proteins may still remain on the column after media regeneration. Specific CIP procedures need to be designed based on the known types of pollutants in the raw materials.

When the following situations occur, the medium needs to be cleaned in place:

Increased column pressure;

The color of the filler changes significantly;

Resolution reduction;

There is a gap between the upper joint and the gel surface;

If the back pressure increases, check the suspension of valves, pipelines, etc. before cleaning the medium.

1.1. Remove non-specific binding proteins and lipoproteins

Wash one column volume with 0.5 M NaOH, equilibrate with deionized water or buffer to neutrality, and flow rate 40 cm/h.

1.2. To remove some sediment or denatured substances, it is recommended to use the following method

Clean the medium with a 1.0-2.0 M NaOH solution of 4 column volumes at a flow rate of 40 cm/h, and immediately wash with 2-3 column volumes of water.

1.3. Remove some strongly bound hydrophobic proteins

Remove non-specific binding proteins and lipoproteins

Wash one column volume with 0.5 M NaOH, equilibrate with deionized water or buffer to neutrality, and flow rate 40 cm/h.

2) To remove some sediment or denatured substances, it is recommended to use the following method

Clean the medium with a 1.0-2.0 M NaOH solution of 4 column volumes at a flow rate of 40 cm/h, and immediately wash with 2-3 column volumes of water.

3) Remove some strongly bound hydrophobic proteins

Wash with 70% ethanol or 30% isopropanol at 4-10 times the column volume (gradient cleaning with organic solvents to avoid foaming). Alternatively, an acid or neutral solution containing a descaling agent can be used. For example, using 1 M

Acetic acid solution contains 0.5% non-ionic detergent, with a cleaning flow rate of 40 cm/h. Subsequently, rinse 5 column volumes with 70% ethanol to remove residual detergent.

2. Save

If not used, the medium after CIP can be stored in 20% ethanol at 4-30℃.