Smartarose and Smartarose CL

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1. Product Description

Smartarose is a spherical agarose gel filtration matrix. Smartarose 4B and Smartarose 6B have 4% and 6% agarose, respectively. Smartarose CL gels are derivatives of Smartarose 4B and Smartarose 6B with better chemical and physical properties and faster flow rates. Smartarose CL gels are resistant to organic solvents and therefore suitable for separation of substances in organic solvents. Smartarose and Smartarose CL have a wide range of separation and are suitable for characterization or separation of samples with different molecular .

Table 1. Characteristics of **Smartarose**

Smartarose	4B	6B	CL-4B	CL-6B
% agarose	4	6	4	6
Optimum 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 (μm)	45-165	45-165	45-165	45-165
Recommended Linear velocity * (cm/h)	11	14	26	30
pH stability **	4-9	4-9	3-13	3-13
Chemical compatibilities	Tolerance to common gel chromatography solutions, such as 8M urea and 6M guanidine hydrochloride.		Tolerance to common gel chromatography solutions, such as 8M urea and 6M guanidine hydrochloride. It is also tolerant to organic solvents such as ethanol, DMF, THE, acetone, DMS, chloroform, dichloromethane, dichloroethane, pyridine, triethyl phosphate and acetonitrile.	
Physical properties	Changes in volume due to changes in pH or ionic strength are negligible.			
Sterilization	Chemical process		Autoclavability 20 min at 120°C in phosphate buffer, pH7.0	

* $\text{Linear velocity} = \frac{\text{Volume flow rate } (cm^3/h)}{\text{Column cross-sectional area } (cm^2)}$

** The pH range was estimated based on experiments and experience.Note: Long-Term pH stability refers to the pH range in which the gel's tomographic properties do not change over a long period of time.Short-term pH stability refers to the stable pH range during gel regeneration and cleaning.

2. Packing Column

2.1 Packing Preparation

The column packing method has two steps. The second step should be carried out under constant pressure. See Table 2 for column pressure. The resolution of gel chromatography increased with the increase of column bed height. Accordingly, column bed height should be above 60 centimeters.

Table 2. Recommended column velocity and pressure

	Step 1 flow velocity(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
Smartarose CL-4B	30	0.025, 0.25, 3.6
Smartarose CL-6B	30	0.045, 0.45, 6.4

2.2 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.

2.3 Packing Smartarose and Smartarose CL

1) Resuspend the beads stored in its container by shaking (avoid stirring the medium). Pouring the slurry down a glass rod held against the column wall will minimize the introduction of air bubbles.
If using a packing reservoir, immediately fill the remainder of the column and reservoir with packing buffer. Mount the adapter or lid of the packing reservoir and connect the column to a pump. Avoid trapping air bubbles under the adapter or in the inlet tubing.
2) Open the bottom outlet of the column and set the pump to run at the desired flow velocity. Ideally, the medium is packed at a constant pressure. If the packing equipment does not include a pressure gauge, use a packing flow velocity listed in Table 2. If the recommended pressure or flow velocity can not be obtained, use the maximum flow velocity the pump can deliver. This should also give a reasonable well-packed bed. Do not exceed 75% of the packing flow velocity in subsequent chromatographic procedures.
3) When the bed has stabilized, close the bottom outlet and stop the pump.
If using a packing reservoir, disconnect the reservoir and fit the adapter to the column. If using the column, carefully place the top filter on top of the bed before fitting the adapter.
4) With the adapter inlet disconnected, push the adapter down, approximately 2 mm into the bed, allowing the packing solution to flush the adapter inlet.
5) Connect the pump, open the bottom outlet and continue packing. The bed will be further compressed at this point and a space will be formed between the bed surface and the adapter.
6) Close the bottom outlet. Disconnect the column inlet and lower the adapter approximately 2 mm into the bed. Connect the pump. The column is now ready to use.

2.4 Performance testing

In order to check the quality of chromatography column, column efficiency test should be carried out to determine the theoretical plate number and peak asymmetry factor.
Elution Buffer: Deionized water
Sample: 2% (v/v) acetone in deionized water
As shown in FIG. 1, the number of theoretical trays is calculated using the following formula: $N/m = 5.54 (V_R/W_h)^2 \times 1000/L$
The formula for calculating the peak asymmetry factor(A_S) is as follows:
$A_S = b/a$

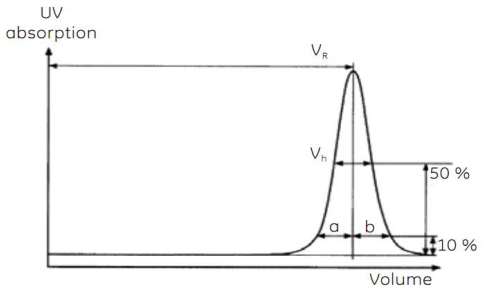

FIG.1. An example of column efficiency detection method

If the operation is carried out in accordance with the instructions, the test values of the column are shown in the following table:

Gel	Number of theoretical plates	Peak asymmetry factor
Smartarose 4B, 6B, CL-4B, CL-6B	> 3000	0.8-1.5

3. Operate Procedure

3.1 Sample preparation

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

3.2 Equilibration

Eluent should be used to balance at least two column volumes before loading, or until baseline is stable.Solutions containing detergents may need to be balanced longer.

3.3 Sample Purification

The above sample volume is recommended to be 2-5% of column volume.
Sample loading can be done through sample tubing or sample loading ring.

3.4 Regenerating the medium

Regeneration usually involves flushing 2 column volume with water and then flushing 2-3 column volume with buffer .For different samples, a complete in-place cleaning (CIP) is recommended after approximately 5 cycles.

4. Cleaning And Preservation

4.1 Cleaning-in-place (CIP)

Cleaning-in-place (CIP) is a cleaning procedure that removes contaminants such as lipids, precipitates, or denatured proteins that may remain in the packed column after regeneration. Regular CIP also prevents the build-up of these contaminants in the media bed and helps to maintain the capacity, flow properties and general performance of the media.
When the following situations occur, the media needs to be cleaned:
- Increased back-pressure
- Colour changed at the top of the column
- Reduced resolution
- A space between the upper adaptor and the gel surface

1) To remove any precipitating or denaturing material, the following method is recommended

Wash the medium with 4 column volume of 1.0-2.0 M NaOH solution at a flow rate of 40 cm/h, then immediately clean with 2-3 column volume of water.

2) Remove the strong hydrophobic binding protein, lipoprotein and lipid

Wash the column using 5-10 column volumes 30% isopropanol contacting for 15-20min. Or you can choose the 2CV acidic or alkaline solution containing detergents, for example, 0.1 M acetic acid solution contains 0.1-0.5% non-ionic detergent, contacting for 1-2 hours . Finally wash the column with 10CV distilled water.

4.2 Degerming

The elimination of bacteria can reduce microbial contamination of the gel.
The gel was cleaned with 1.0-2.0M NaOH for 30-60min. A sterile buffer of 3-5 x column volume was used to rebalance.

5. Related Products

Product	Cat. No	Size
Smartarose 4B	SEC0060	25 ml
	SEC0061	100 ml
	SEC0062	500 ml
	SEC0063	1 L
	SEC0064	10 L
Smartarose 6B	SEC0070	25 ml
	SEC0071	100 ml
	SEC0072	500 ml
	SEC0073	1 L
	SEC0074	10 L

Smartarose CL-4B	SEC0080	25 ml
	SEC0081	100 ml
	SEC0082	500 ml
	SEC0083	1 L
	SEC0084	10 L
Smartarose CL-6B	SEC0090	25 ml
	SEC0091	100 ml
	SEC0092	500 ml
	SEC0093	1 L
	SEC0094	10 L