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Smartarose 4FF and Smartarose 6FF

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1. Product Description

Smartarose 4FF and Smartarose 6FF have excellent physical and tomographic properties based on highly cross-linked 4% and 6% agarose beads. **Smartarose 4FF and Smartarose 6FF** are widely used in industrial production because of its adaptability to various production operations and good batch repeatability. This medium can handle large volume samples, easy to operate, and withstand the biological pharmaceutical production process of temperature, pH and chemical agents and other conventional working conditions. The beads can be cleaned in position.The beads can also be sterilized if required.

Table 1. Characteristics of **Smartarose 4FF and Smartarose 6FF**

Item	Smartarose 4FF	Smartarose 6FF
Matrix	highly cross-linked 4% agarose	highly cross-linked 6% agarose
Exclusion Limit[M_r] Globular Proteins Dextrans	~3×10^7/ ~6×10^6	~4×10^6/ ~2×10^6
Particle Size (μm)	45-165	45-165
Linear velocity (cm/h)	150-250 cm/h*	200-400 cm/h*
pH stability	2-12	2-12
Chemical satbility(40°C ， 7days)	2M NaOH,70% ethanol, 30% iospropanol, 30% acetonitrile, 1% SDS, 2% asepto, 6 M guanidine hydrochloride, 8 M urea	
Autoclavability	20min at 121°C in H_2O	
Storage	20% ethanol, 4 -30 °C	

* Flow Specification：0.1MPa, SXK 50/500mm column, bed height 15 cm

2. Packing Column

2.1 Preparing the medium suspension

Smartarose 4FF and Smartarose 6FF have swollen to 20% ethanol. 20% ethanol should be removed by pumping before column loading. Prepare a media slurry in a ratio of 75% settled gel to 25% buffer and degas under vacuum. The eluent buffer should not contain agents which significantly increase the viscosity. The column may be equilibrated with viscous buffers at reduced flow rates after packing is completed.

2.2 Packing the column

1) Remove air from the column dead spaces by flushing the end-piece and adapter with packing buffer. Make sure no air has been trapped under the column net.
2) Close the column outlet leaving the net covered with packing buffer.
3) Resuspend the beads stored in its container by shaking (avoid stirring the sedimented 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.
4) Open the bottom outlet of the column and set the pump to run at the desired flow velocity. 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.
5) 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.

6) With the adapter inlet disconnected, push the adapter down, approximately 2 mm into the bed, allowing the packing solution to flush the adapter inlet.
7) 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.
8) 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 Chromatography column 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 coefficient.
Elution Buffer: 0.15M NaCl
Sample: 2% (v/v) Acetone solution or 1M NaCl
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 coefficient(A_S) is as follows:
$A_S = b/a$

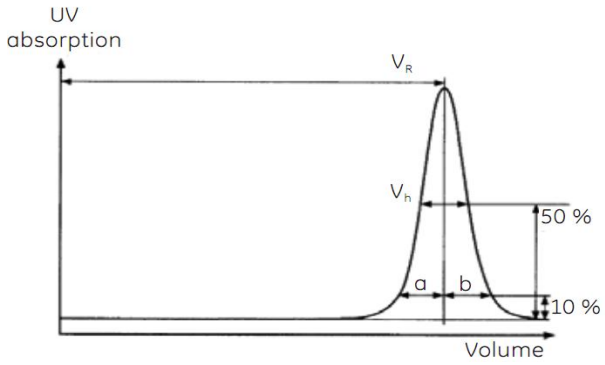

FIG.1. An example of column efficiency detection method

3. Operate Procedure

3.1 Eluent and 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 Samples

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 4FF	SEC0100	25 ml
	SEC0101	100 ml
	SEC0102	500 ml
	SEC0103	1 L
	SEC0104	10 L
Smartarose 6FF	SEC0110	25 ml
	SEC0111	100 ml
	SEC0112	500 ml
	SEC0113	1 L
	SEC0114	10 L