

DEAE Beads 6FF

Product Number: DB001

Shipping and Storage

Store at 4-30°C.

Description

Ionic exchange fillers are widely used for the separation and purification of downstream proteins, nucleic acids, and peptides in biopharmaceuticals and biotechnology. It mainly includes four types: strong acidic cation exchange packing, weak acidic cation exchange packing, strong alkaline anion exchange packing, and weak alkaline anion exchange packing. The ion exchange fillers are all based on highly cross-linked 6% agarose as a medium, which can tolerate high flow rates and higher chemical stability, making it suitable for laboratory and industrial large-scale purification.

DEAE Beams 6FF is a weak anion exchange filler with an ion exchange group, $-O-CH_2CH_2-N^+(C_2H_5)_2H$. The specific properties are shown in the table below.

Project	Performance
Matrix	Highly cross-linked 6% agarose microspheres
Ion exchange type	Weak anion
Ion loading capacity	Approximately 0.11-0.16mmol Cl ⁻ /mL medium
Particle size	45-165μm
Pressure flow rate	200-400 cm/h
PH stability range	2-12
Storage Buffer	20% ethanol

Column size 50×500mm, column height 15cm, linear flow rate of deionized water at 0.1MPa at 25°C.

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 and eluent used can be selected according to different ion exchange fillers. The basic principle is low salt loading and high salt elution.

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, improve protein purification efficiency, and prevent column blockage.

3. Media loading

3.1. Loading of gravity column

- 3.1.1. Take a gravity chromatography column of appropriate specifications, install the lower gasket, add an appropriate amount of pure water to rinse the column tube and gasket, and close the lower outlet.
- 3.1.2. Mix DEAE Beams 6FF evenly, use a gun tip to suck an appropriate amount of slurry and add it to the gravity column (the actual volume of the medium accounts for half of the suspension), and open the lower outlet to dry the protective liquid.
- 3.1.3. Add an appropriate amount of pure water to rinse the medium, wait for the liquid in the column to dry by gravity, and then close the outlet.
- 3.1.4. Install the upper gasket after lubrication, ensuring that there is no gap between the gasket and the packing, and keeping it level.
- 3.1.5. The loaded gravity column can be directly equilibrated by adding equilibrium solution. When not in use, protective solution can be added and stored at 4-30°C.

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3.2. Packing of medium pressure chromatography column

DEAE Beams 6FF is widely used in industrial purification, involving the filling of various medium and low pressure chromatography columns. The following introduces the method of filling chromatography columns.

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

r: Column radius cm

h: Loading height cm

Note: The volume of the suspension taken should be twice the volume of the medium, as the medium volume only accounts for half of the total suspension volume, with the other half being the protective solution.

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 1-2 cm of deionized water at the bottom of the column.

Suspend the medium 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.

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.

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 3 times the volume of deionized water to the column bed at the final column flow rate. Mark the height of the column bed.

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

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

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.

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

4. Sample purification process

4.1. Purification by incubation method

4.1.1. According to the purified sample volume, take an appropriate amount of DEAE Beams 6FF and add it to a centrifuge tube. Centrifuge at 1000 rpm for 1 minute and discard the supernatant; It can also be added to a gravity column to dry the protective liquid.

4.1.2. Add 5 times the volume of equilibrium solution to the centrifuge tube to clean the medium, centrifuge at 1000 rpm for 1 minute, and discard the supernatant; If using a gravity column, clean it directly in the gravity column and use gravity flow to dry the equilibrium solution; Repeat twice or more.

4.1.3. Add the sample, seal the centrifuge tube or gravity column tube, shake at 4°C for 2-4 hours, or incubate at 37°C for 30 min-2 hours.

4.1.4. After incubation, centrifuge at 1000 rpm for 1 minute, discard the supernatant or filter the collection medium, and retain the supernatant as a flow-through for electrophoresis identification.

4.1.5. Clean the medium with 5 times the volume of cleaning solution, centrifuge at 1000 rpm for 1 minute or filter through a gravity column tube to remove the supernatant (be careful not to suck up the medium), repeat 3-5 times, and it is recommended to replace the centrifuge tube in between.

4.1.6. Add 3-5 times the column volume of eluent for elution, incubate at room temperature for 10-15 minutes, centrifuge at 1000 rpm for 1 minute, or collect the eluent using a gravity column tube. Repeat 2-3 times.

4.2. Purification by gravity column method

4.2.1. Equilibrate the packed DEAE Beams 6FF gravity column with 5 times the column volume equilibration solution, keeping the packing in the same buffer system as the target protein, and repeat 2-3 times.

4.2.2. Add the sample to a balanced gravity column and retain it for at least 2 minutes to ensure sufficient contact between the sample and the medium. Collect the effluent and repeat the loading process to increase binding efficiency.

4.2.3. Wash impurities with 10-15 times the volume of the column, remove non-specific adsorbed impurities, and collect the impurities.

4.2.4. Using 5-10 times the column volume of eluent for elution, collecting in sections, and collecting one tube for each column volume for separate detection, can ensure that all bound target proteins are eluted and obtain high-purity and high concentration proteins.

4.3. Purification by medium pressure chromatography column method

4.3.1. After loading DEAE Beams 6FF, various conventional medium and low pressure chromatography systems can be used.

4.3.2. Fill the pump pipeline with deionized water. Remove the upper stopper, connect the chromatography column to the chromatography system, open the lower outlet, connect the pre loaded column to the chromatography system, and tighten it.

4.3.3. Wash the storage buffer with 3-5 times the volume of deionized water.

4.3.4. Use an equilibrium liquid chromatography column with at least 5 times the volume of the column bed.

4.3.5. Use a pump or sample ring to load the sample. Note: The increase in viscosity of the sample can cause significant back pressure on the chromatography column even when the sample volume is small. The sample size should not exceed the binding capacity of the column. A large sample volume may also cause significant back pressure, making the injector more difficult to use.

4.3.6. Rinse the column with detergent until the UV absorption reaches a stable baseline (usually at least 10-15 column volumes).

4.3.7. Use a one-step or linear gradient elution method with eluent. In a one-step elution process, typically 5 times the column volume of elution solution is sufficient. Gradient elution can use a small gradient, such as 20 times column volume or more, to separate proteins with different binding strengths.

5. SDS-PAGE detection

Use SDS-PAGE to detect the purification effect on the samples obtained from purified products (including effluent components, wash impurities components, and wash elution components) and the original samples.

Packing cleaning and preservation

1. Regular cleaning

The ion exchange packing can be washed with 1M NaCl or even higher ion strength solution or high pH solution after each use, and then equilibrated with at least 5 times the column volume of equilibration solution until the ion strength or pH value is stable.

2. CIP (Cleaning In Place) cleaning

Ion exchange fillers can be reused without regeneration, but with the increase of non-specific binding proteins and protein aggregation, the flow rate and binding load often decrease. In this case, the fillers can be cleaned according to the following method.

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

Wash with 2 times the column volume of 1M NaOH solution, then immediately wash with 5 times the column volume of PBS, pH 7.4.

Remove non-specific adsorption substances caused by hydrophobic adsorption

Wash with 3-4 times the column volume of 70% ethanol or 3-4 times the column volume of 1% Triton™X-100, then immediately wash with 5 times the column volume of PBS at pH 7.4.

Remove some ion bond binding substances

Wash with 2-4 times the column volume of 2M NaCl, then immediately wash with 5 times the column volume of PBS at pH 7.4.

3. Packing preservation

3.1. Unused fillers are stored in covered containers, with the lid tightened and stored at 4-30°C.

3.2. Wash the used packing with pure water for 5 times the column volume first, then rinse with 20% ethanol for more than 2 times the column volume, and then store the packing at 4-30°C. It is recommended to rinse with 20% ethanol for more than 2 times the column volume every 1-2 months to replace the old protective solution.

Problem and Solution

Problem	Root cause analysis	Recommendations
The column's back pressure is too high	The packing is blocked	Perform packing cleaning and preservation for packing materials The cracking solution contains tiny solid particles. It is recommended to use a filter membrane (0.22 or 0.45µm) or centrifuge to remove them before column loading.
The elution sample is relatively mixed	Repeated use of fillers	Clean or replace fillers according to Packing Cleaning and Preservation
	Insufficient cleaning of miscellaneous items	Increase the volume of cleaning solution to ensure sufficient balance of fillers/cleaning impurities
	The charged performance of the sample is similar	Optimize elution conditions