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CM Bestdex C-25
CM Bestdex C-25 Dextran gel

Instruction for use

Contents

1. Introduction

Ion exchange chromatography(IEC)is a very effective method for separation and purification of biological molecules.The method mainly relies on the interaction between positive and negative charges and uses the nature and difference of the charges carried by different biomolecules under specific conditions to separate.It has the characteristics of high load capacity, good resolution, controllable conditions and easy amplification. It has been widely used in medicine, chemical industry, metallurgy, food and other fields.

The ion exchange resin is composed of three parts：(1)Cross-linked mesh base frame, which is porous, hydrophilic and chemically stable；(2)The functional group fixed on the base frame, which is a charged group, determines the nature of the ion exchange chromatography resin；(3) An ion (called an equilibrium ion) that has an opposite charge to the functional group and can be reversibly bound to the functional group.

CM Bestdex C-25 is a weak cation exchange resin formed by coupling carboxymethyl groups on cross-linked dextran microspheres. It retains the excellent hydrophilicity and pore structure of natural polysaccharide compounds, good compatibility with biologically active molecules, and very low non-specific adsorption.CM Bestdex C-25 is based on Bestdex G-25, which has a very high capacity for small molecular substances and is very suitable for the separation and purification of antibiotics, natural products and other substances. Due to the large variation of the column bed volume of the chromatography resin in different buffers, it is not suitable to be installed as a fixed bed chromatography column.

2.Technical characteristics

Appearance	White to slight yellow powdery solid
Matrix	Cross-linked dextran
Functional group	Carboxymethyl
Dry powder size	40~120μm
Swelling coefficient	7mL/g dry powder(PBS)
Ionic capacity	4.0~5.0 mmol H^+/g dry powder
Max. pressure	0.3 MPa
Working pH range	6~10
pH stability	2~13(CIP),2~12 (working)
Chemical Stability	Stable in common aqueous buffers: 6M GuHCl,8M Urea, 70% ethanol , 30% isopropyl alcohol

Temperature tolerance	Working temperature:2~40℃, Can't freeze, Can tolerate 121℃ high pressure sterilization (50mM PB pH 7)
Recommended flow velocity	60~150cm/h

3. Method of chromatographic

3.1 Resin swelling

- Pour the resin into 0.1M NaCl at 80 ~ 100℃ with 10 times the weight of dry powder and stir it a little, swell for 2~4h, or swell for 24h at room temperature. (Note: During the swelling process, do not use a magnetic stir bar to stir. (Using a magnetic stir bar will cause the resin particles to break) (1g CM Bestdex C-25 dry powder has a volume of about 7mL after swelling).
- To replace the solution, pour the gum suspension into a funnel, withdraw the liquid, and wash with approximately 3 times the volume of distilled water. (When the volume is relatively large or the conditions are not available, the upper solution can be removed after the glue is layered, and then the appropriate amount of distilled water is added to mix until the layer is removed, and the resin solution is replaced 3 times).
- If autoclaving is required, replace the resin solution with 0.1M sodium acetate solution.
- The resin after high pressure is washed with 3 times the resin volume of sterile water.

3.2 Resin use

3.2.1 Batch adsorption

If the resin is used for batch adsorption, there is no need to pack the chromatography column. The amount of resin required is calculated based on the load and the amount of the target. Put the resin prepared in step 3.1 into the solution containing the target and stir for more than 30 minutes. After the adsorption is completed, the resin is collected by precipitation to remove the supernatant or trapped by the filter, washed with the equilibrium buffer, and then eluted with the elution buffer.

3.2.2 Packing and use of chromatography columns

Note：It is best to equilibrate the resin slurry to room temperature before column packing.

- Prepare the packing slurry: Transfer the washed resin from the funnel into a beaker or other appropriate container, add distilled water to obtain a 50%~75% slurry, stir well and set aside for use.
- Take a cleaned BXK column (BXK series columns with diameters ranging from 1cm to 30cm can satisfy different scale chromatography applications). Purge the bubbles trapped at the end-piece net by draining some distilled water through the column outlet. Leave about 1cm water at the bottom of the column and close the bottom outlet. Adjust the column so that it is perpendicular to the ground.
- Slowly pour the slurry into the column at one time (use a packing reservoir if necessary). Do not bring any air bubbles into the column.

  Packing reservoir: Empty glasstube with same diameter as the BXK column.

- Fill the remainder of the column with packing solution. Connect the packing reservoir to the chromatography system, open the flow velocity, drain the bubbles in the hose, close the flow velocity, and tighten the top cover of the packing reservoir.

  Note: This operation is only applicable to BXK 50 and below chromatographic columns.

✧ After pouring, stir well again with stirrer, and then wash the resin particles on the inner wall of the column from top to bottom with the packing solution, and let the resin settle naturally until there is about 1cm of clarifying solution on the suspension. Mount the adapter and connect the adapter to the chromatography system or peristaltic pump. Lower the adapter to descend to contact with the clarifying solution and tighten the sealing ring after it is fully immersed in the clarifying solution. With the outlet of the top piece is opened, slowly move the adapter down until all bubbles are drained.

Note: This operation is only applicable to BXK 100 and above columns. Flushing the inner wall reduces the resin particles sticking between the seal ring and the column wall, avoiding the risk of leakage.

- Set the flow velocity (The packing flow velocity must be at least 1.5times of the normal flow velocity, do not exceed the maximum flow velocity). Open the bottom plug of the column and start the peristaltic pump or chromatography system according to the flow velocity set above. The pressure in the column is set to be less than the maximum pressure of the chromatography column. If pressure exceeds 0.3MPa, during column packing, the flow velocity needs to be reduced appropriately.
- After the resin surface is stable, keep it for more than 30 minutes, mark the position of the resin surface, and then stop the pump.
- Remove the packing reservoir (if any), mount the adaptor, lower the adaptor to about 0.5cm above the resin surface, and continue to press the column using the above flow velocity until the bed is completely consolidated, mark the consolidated bed height.
- Stop the pump, open top plug, close the bottom plug, loosen the O-ring seal slightly, press the adaptor to about 0.3cm below the marked position, tighten the O-ring seal, close adaptor stop plug, and complete the column packing.

3.3 Chromatographic method

- Buffer solution: To facilitate the binding with sample and impurities, please choose buffer solution with low salinity (lower than 5mS/cm) and low pH value. By choosing the buffer with appropriate pH and salinity, sample can either bind with resin or simply flow through. For example, a very commonly used method is to bind with targeted molecules while let impurities flow through. Alternatively, this method can also work by binding with impurities while let targeted molecules flow through. Generally speaking, it is important to choose buffer solution that is efficient and easy to bind with targeted molecules while taking account of the stability of the above-mentioned molecules in the buffer.
- Flow rate: According the column bed high to use the flow velocity 60~150cm/h, the higher

column bed high and lower flow velocity.

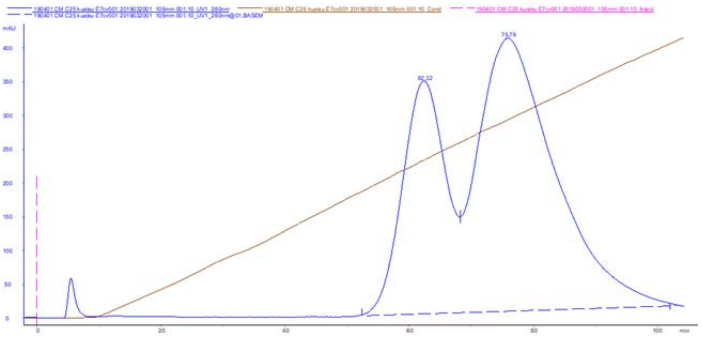
- Sample preparation: In order to prevent blocking of the column, the sample needs to be filtered by microporous membrane of 0.45μm before loading.
- Flow-through mode：collecting flow through peaks.
- Equilibration: Washing the column with equilibration buffer until the pH and conductivity of the column outlet buffer are basically the same as the equilibration buffer, which usually needs 3-5CV.
- Sampling: The loading volume is determined according to the substance content in the sample and the binding load of CM Bestdex C-25.
- Rinse with equilibration buffer until the UV absorption value drops to an appropriate value.
- Elution：
  - Salt concentration elution: it can be eluted by gradient elution.
  - PH elution: The charged state of the target protein is changed by changing the pH of the eluent, and the cationic filler is elution by increasing the pH of the eluent.

Note: It is necessary to pay attention to the change of the column bed volume with the buffer during the use of the resin. If the pressure is too large due to the expansion of the cylinder, the cylinder should be properly lifted to prevent damage to the chromatography column.

- Regeneration: Depending on the nature of the sample, filler regeneration is usually done with a high ionic strength buffer (e.g. 1~2M NaCl) and/or a reduction/increase in pH, followed by rebalancing in a binding buffer. In some processes, such as denatured proteins or lipids, which are not easily elute during regeneration, these can be removed by in-place cleaning.
- Rebalancing: After rinsing with equilibration buffer, and wait for the pH and conductivity to be basically the same as the balance buffer,the second sample can be loaded and repeated.

4. Application

Using CM Bestadex C-25 to separate lysozyme and quinine sulfate



Equilibrium buffer: 40mM PB, pH6.0

Elution buffer: 40mM PB+0.6M NaCl, pH6.0

Column：BXK16/20

Sample: lysozyme and quinine sulfate mixture

5. Cleaning-in-place(CIP)

With the increasing use of chromatography resin, the accumulation of contaminants on the chromatography column is also increasing. Cleaning-in-place can prevent the accumulation of contaminants and maintain a stable working state. Determine the frequency of CIP according to the degree of contamination of the resin (if the contamination is serious, CIP should be carried out after each use to ensure repeatability of the results).

The recommended CIP for different types of impurities and contaminants are as follows:

- 2~3CV of 2M NaCl was used to wash out the proteins with relatively tight binding.
- Removal of strong hydrophobic proteins and precipitating proteins: Clean with 1M NaOH of 2~3CV first, then rinse immediately with 5~10CV pure water.
- Removal of lipoproteins and lipids: Clean with 70% ethanol or 30% isopropanol by volume of 5~10CV first, then rinse with pure water by volume of 5~10CV.
- The above two cleaning conditions can also be combined for cleaning, namely 30% isopropanol solution containing 1M NaOH.

Note: Because CM Bestdex C-25 has a large volume change in organic reagent, it is recommended to clean and reinstall the column in container after column removal.

Note: 70% ethanol or 30% isopropanol should be degassed before use. In the CIP process, the flow velocity can be chosen as 30~60cm/h. Reverse flushing can be used when the blockage is serious.

6. Sterilization

CM Bestdex C-25 can be autoclaved at 121℃ (50mM PB, pH7) for 30min.

7. Storage

CM Bestdex C-25 is available in dry powder form. It is should be stored in 20% ethanol and sealed at 2-30℃ after use, in order to prevent ethanol volatilization and microbial growth, it is recommended to replace the storage solution every 3 months.

8. Disposal and Recycling

CM Bestdex C-25 is very difficult to degrade in nature, incineration is recommended to protect the environment.

9. Order information

Product	Code No.	Pack size
CM Bestdex C-25	AI0261	25g
	AI0262	100g
	AI0263	500g
	AI0264	1kg
	AI0265	5kg
	AI0267	10kg
	AI0268	20kg
	AI0266	25kg