BESTCHROM
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MegaPoly BXS Strong Cation Exchange Resin

Instruction for use

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Content

1. Introduction

Ion exchange (IEX) chromatography is a very effective method for the separation and purification of biomolecules. The method mainly relies on the interaction between positive and negative charges, and uses the charge properties and number difference of biological molecules under specific conditions to separate them. It has the characteristics of high binding capacity, good resolution, controllable condition and easy scale-up. It has been widely used in medicine, chemical industry, metallurgy, food and other fields.

MegaPoly BXS is a strong cation exchange (CEX) resin, obtained by coupling the sulfopropyl functional groups on the cross-linked Polystyrene-divinylbenzene base matrix. With an average particle size of 50 μm, the resin possesses a special pore structure, which offers large through-pores and diffusive pores. Thus, it can not only provide high flow velocity without compromising bio-molecule binding capacity and selectivity, but also ensure rapid interaction between targeted bio-molecules and functional groups, granting it merits such as high flow velocity, high binding capacity and high resolution. Accordingly, the resin is widely applied in the efficient purification of micro-bio molecules including recombinant protein, monoclonal antibodies, DNA, viruses and peptides, providing it with a promising prospect in the bio-pharmaceutical industry.

The resin has the following features:

- The cross-linked base matrix provides strong mechanical resistance and low back-pressure
- Special pore structure enhances interaction between bio-molecules and resin
- High binding capacity, high resolution and fast flow
- Physical and chemical stability
- Option for more efficient purification and separation

2. Technical characteristics

Appearance	White slurry
Matrix	Cross-linked polystyrene-divinylbenzene beads
Functional Group	Sulfopropyl
Particle size	~50μm
Dynamic binding capacity +	>100mg Human IgG /mL packed resin
Max.pressure	10MPa
Salt Tolerance	0-5M,all common salts
Chemical Stability	All common solutions: water ,1M NaOH,8M Urea,6M GuHCl,glycol,0-100% ethanol,acetonitrile,2M acetic acid,1M HCl, other common solutions.
pH stability	1~14
Operating temperature	Operating temperature:2~30℃, do not freeze.
Storage	2~30℃,20% ethanol or 0.1M NaOH
Avoid	Avoid exposure to strong oxidants, oxidizing acids, strong reducing agents, acetone or benzyl alcohol.

+10%DBC,Buffer A：50mM Sodium acetate,pH5.5,Column bed height 10cm,Flow velocity：100cm/h

3. Method of chromatography

3.1 Column packing

MegaPoly BXS has high mechanical resistance and incompressibility, can be efficiently loaded in both low pressure glass columns and high pressure stainless columns. Applicable packing methods range from traditional flow velocity method, automatic axial compression method and natural suspension method.

- Needed resin calculation: determine the required amount of MegaPoly BXS resin according to column volume.

  Required resin slurry[1] volume = Column volume ×1.06÷ Resin slurry[1] concentration. The original concentration of resin slurry[1] is shown in the follow table.

Pack size	Resin slurry [1] concentration (%)
25mL,100mL,500mL,1L,5L,10L	80
20L,40L	75

1:It refers to the original packaging resin slurry sold by Bestchrom.

Note：For resins in non-original concentration, customer can calculate the required volume

according to the actual concentration of resin slurry.

- Washing the resin: Thoroughly shake the resin and weigh certain amount of resin according to the above-mentioned calculation. Pour the resin into a funnel, pump and filtrate the resin. Wash the resin with 3mL of packing solution (20% ethanol with 0.1M NaCl). Stir well with a glass stick or stirrer to better wash shipping buffer away. Repeat the step for 3 times.
- Prepare the packing slurry: Move the washed resin from the sand core funnel into a beaker or other appropriate container, add packing solution to obtain a 50%~70% concentration 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). Take BXK16/20 for example, 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 glass tube with same diameter as the BXK column.

✧ 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. Turn on the flow velocity and degas the column hose. Connect the hose to adaptor. Lower the adapter to the clarifying solution and tighten the sealing ring after it is fully immersed in the clarifying solution. Do not bring any air bubbles in when operating.

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

- When the bed height is 10cm, the column packing pressure can be set to 2.5bar. Open the bottom plug, turn on the pump and run the setting flow velocity until the resin bed is stabilized, mark the bed height.
- Remove the packing reservoir (if any), mount the adaptor, lower the adaptor to about 0.5cm above the resin surface, set the flow velocity at 480cm/h, 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, shut top/bottom plugs, disconnect the adaptor and device, press the adaptor to about 0.3cm below the make position, complete column packing.

3.2 Evaluation of Packing

- The packing quality of chromatographic column can be confirmed by column efficiency measurement and evaluation. The tests are required after the column packing, during the column working life and when the separation and purification performance weakens. The method usually relies on the height equivalent to a theoretical plate(HETP) and the asymmetry factor(As).
- NaCl solution can be used as sample for the testing. Sample solution and mobile phase can be prepared according to the following table.

	NaCl method
Sample	0.8M NaCl aqueous solution
Sample volume	1.0%CV
Mobile phase	0.4M NaCl aqueous solution
Flow velocity	30cm/h
Detection	conductance

- Method for measuring HETP and As:

  Use UV curve or the conductance curve to calculate the height equivalent of theoretical plate (HETP), number of theoretical plates(N) and the asymmetry (As):

  HETP=L/N

  $N=5.54(V_R/W_h)^2$

  Note: V_R = retention volume

  W_h = half-peak width

  L = column height

  N = the number of theoretical plates

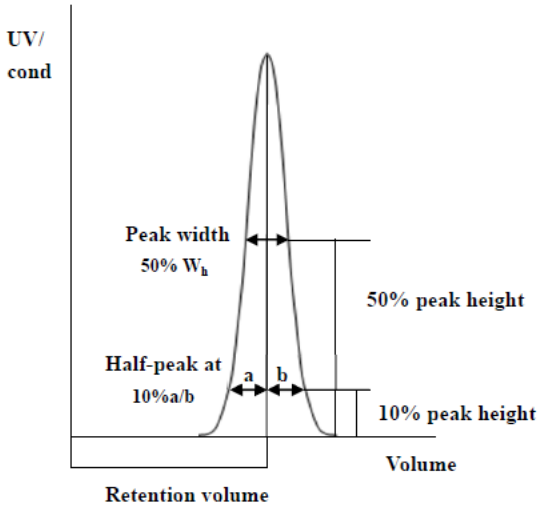
  (The units of V_R and W_h should be the same)

  As=b/a

  Note:

  a= 1st half peak width at 10% of peak height

  b= 2nd half peak width at 10% of peak height



- Evaluation the column packing

  As a guideline, if the value of HETP is less than 3 times the average particle size(d_{50}) of the resin and the As is between 0.8~1.8, the column is very efficient. The unsatisfactory results should be analyzed and the column should be repacked.

3.3 Chromatographic method

- Buffer selection: Buffer salts whose buffer groups do not act with the resin should be selected. The buffer solution with low salt (less than 5mS/cm) and low pH (usually 1-3 pH units lower than the isoelectric point of the target) should be adopted to facilitate the binding of substances. Meanwhile, the stability of samples in the buffer solution should be considered. Elution buffers are usually made by adding a buffer containing high concentration of salt (e.g. 1M NaCl) or high pH elution to equilibration buffer.

- Flow velocity: According the column bed height to set the flow velocity (usually 200cm/h),the higher the column height is, the slower the flow velocity will be.
- Sample preparation: In order to prevent blocking of the column, the sample needs to be filtered by microporous membrane of 0.45μm before loading, the pH and conductance of the sample are adjusted to be consistent with the equilibration buffer (the pH and conductance of the sample can be adjusted by dilution, ultrafiltration, and buffer change).
- Equilibration: Washing the column with equilibration buffer until the pH and conductance of the column outlet buffer are basically the same as the equilibration buffer, which usually takes 4CV.
- Loading sample: The loading volume is determined by the substance content in the sample and the binding capacity of MegaPoly BXS.
- Cleaning: Wash the column with equilibration buffer until the UV absorption value close to baseline.
- Elution: Linear gradient or step-gradient can be used to increase the elution strength in the elution buffer, eluting substances with different binding strength from the chromatography column, collecting different components and detecting the location of the target. Due to the increased salt tolerance of resin, salt and pH might need to be adjusted for elution. Make sure the elution volume and retention time are same with other resins.
- Regeneration: Flush the column with equilibration buffer containing high concentration of salt (eg: 2M NaCl).
- Re-equilibration: After washing with equilibration buffer till pH and conductance reach same, the sample can be loaded again, repeat if necessary.

4. 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:

- Wash resin with 3~5CV of 1~2M NaCl and 3~5CV of 0.5~1 M NaOH.
- Removal of strongly bond protein: Wash with 2~3CV of 2M NaCl.
- Removal of strong hydrophobic proteins and precipitating proteins: Wash with 2~3CV of 1M NaOH , then wash immediately with 5~10CV of pure water.
- Removal of lipoproteins and lipids: Wash with 5~10CV of 70% ethanol or 30% isopropanol , then wash with 5~10CV of pure water.
- The above two cleaning conditions can also be combined for cleaning, namely wash with 30% isopropanol solution containing 1M NaOH.

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.

5. Sterilization

Since the 20% ethanol or 0.1M NaOH preservation buffer does not have sterilization and depyrogenation function, it is recommended that MegaPoly BXS can be treated with 1M NaOH for more than 0.5-1h to reduce the risk of microbial contamination before and during use. It can also be autoclaved at 121℃ (add 50mM PB, pH7) for 30min.

6. Storage

MegaPoly BXS is supplied in 20% ethanol or 0.1M NaOH. It should be stored in 20% ethanol or 0.1 M NaOH, 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.

7. Disposal and recycling

MegaPoly BXS is very difficult to degrade in nature, incineration is recommended to protect the environment.

8. Order Information

Product	Cat. No.	Pack size
MegaPoly BXS	AI05701	25mL
	AI05702	100mL
	AI05703	500mL
	AI05704	1L
	AI05705	5L
	AI05706	10L
	AI05707	20L
	AI05708	40L

Prepacked columns	Cat. No.	Pack size
EzFast MegaPoly BXS	EI05721	1×1mL
	EI05731	5×1mL
	EI05723	1×5mL
	EI05733	5×5mL
EzScreen MegaPoly BXS	EI05725	1×4.9mL
	EI05735	5×4.9mL
EzLoad 16/10 MegaPoly BXS	EI05701	1 pcs
EzLoad 26/10 MegaPoly BXS	EI05711	1 pcs