BESTCHROM
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BestPoly 15S Strong cation chromatography resin

Instruction for use

Contents

1. Introduction

BestPoly 15S is a strong cation exchange resin. The base frame is made of styrene and divinylbenzene. The particle size is about 15 microns, which is ideal for polishing of biomolecules.

The resin has the following characteristics:

- Uniform particle size distribution
- High resolution
- High pressure resistance, low back pressure
- Fast flow velocity
- Stable physical and chemical properties.

2. Technical characteristics

Appearance	White to slight yellow slurry, can be layered
Matrix	Styrene and divinylbenzene polymer particles
Particle size	~15μm
Functional group	Sulfomethyl
Total protein capacity	≤25mg /mL resin
Dynamic binding capacity	~80mg lysozyme/mL packed resin(20mM pH6.8, 1min residence time)
Chemical stability	Stable in common aqueous buffers: 1.0 M NaOH+, 1.0 M HCl,100% ethanol,100% isopropyl alcohol, 100% acetonitrile, 100% methanol
Max. flow velocity++	1800cm/h
Max. pressure	8MPa
pH stability	2~13(working),1~14(CIP)
Operating temperature	Operating temperature:2~40℃, do not freeze.
Storage+++	2~30℃, 20% ethanol with 0.2M NaAc or 2% benzyl alcohol with 0.2M NaAc
Recommended flow velocity	150-900cm/h

+1M NaOH only be used for cleaning.

++ In BXK100/500 column, column height 10cm, flow velocity of water at 20℃

+++2% benzyl alcohol is only used for international transport or special requirements from customer.

3. Method of chromatography

3.1 Column packing

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

Due to the fine particle size of the resin, it is necessary to choose the chromatography column with a mesh size of 5μm or less and high pressure (BXR10 columns and BHG columns can be used in lab-scale process development stage.).Resin processing should avoid friction or extrusion with hard objects, to avoid resin particles broken.

- The packing height of BestPoly 15S is generally 3-30cm.
- According the column volume to calculate the amount of resin.

  Resin volume=column volume×1.1 (Compression factor=1.1)

  According to the volume of the precipitated resin required, the resin slurry required is calculated by the follow:

  Required resin slurry[1] volume = Precipitated resin volume ÷ 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: Suspend the resin by shaking and pour into a funnel, remove the liquid, and wash with about 3mL packing solution (25% ethanol)/mL resin for 3 times. Use a glass stick or stirrer to stir each time you add the packing solution, in order to better clean the shipping buffer.
- Prepare the packing slurry: Transfer the washed resin from the funnel into a beaker or other appropriate container, add distilled water to obtain an about 25% slurry, stir well and set aside for use.
- Take a cleaned column. Take BXR10/17 for example, purge the bubbles trapped at the end-piece net by draining some packing solution 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 BXR 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.

- ✧ 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.

- When the bed height is 10cm, the flow velocity can be set to 750cm/h. Open the bottom plug, start the pump and run at the setting flow velocity until the bed is stabilized, then change pressure to constant 25bar for column packing, mark the bed height.
- Remove the packing reservoir (if any), mount adaptor, lower the adaptor to about 0.5cm above the resin surface, keep pressing the column at 25bar 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.

3.2 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 number of theoretical plates (N) and the asymmetry factor (As).
- Acetone or NaCl solution can be used as sample for the testing. Sample solution and mobile phase can be prepared according to the following table.

	Acetone method	NaCl method
Sample	1.0%(v/v)acetone in water	0.8M NaCl in water
Sample volume	1.0%CV	1.0%CV
Mobile phase	Ethanol	0.4M NaCl in water
Flow velocity	60cm/h	60cm/h
Detection	UV280 nm	Conductivity

- Method for measuring N and As:

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

$N/m=5.54(V_R/W_h)^2\times1000/L$

Note: V_R = retention volume

W_h = half-peak width

L = column height

N/m = Number of theoretical plates per meter

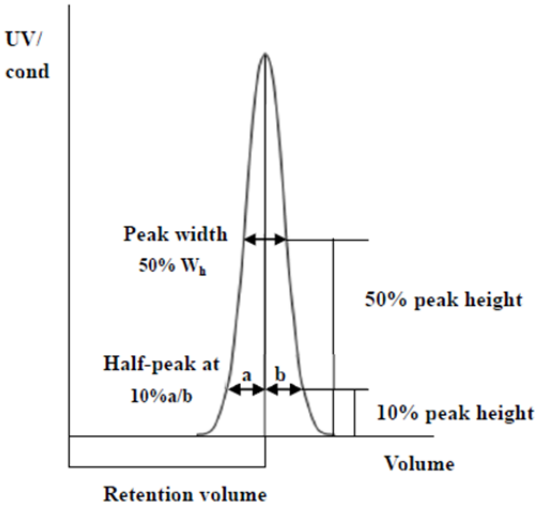
(The units of V_R and W_h should be the same)

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 N/m＞20000 and the asymmetric factor is between 0.8~1.5, the column is very efficient. The unsatisfactory results should be analyzed and the column should be repacked.

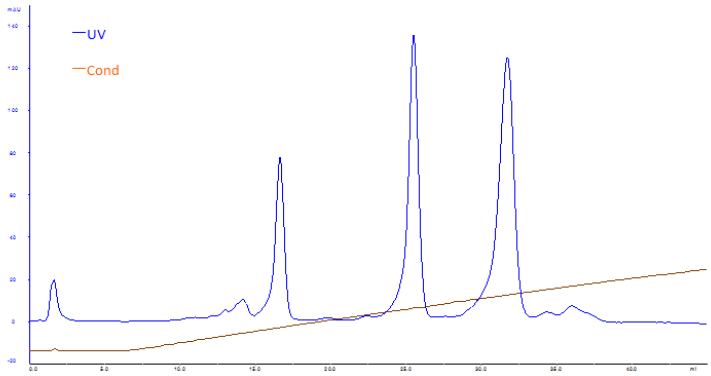
3.3 Chromatographic method

- Buffer selection: Buffer salts whose buffer groups do not act on the resin should be selected. The buffer solution with low salt (less than 5mS/cm) and low pH (usually 1 pH unit lower than the isoelectric point of the target) should be adopted to facilitate the combination of substances. Meanwhile, the stability of samples in the buffer solution should be considered. Elution buffers are usually made by adding a high concentration of salt (e.g.1M NaCl) or high pH buffer to equilibration buffer.
- Flow velocity: According the column bed height to set the flow velocity (usually 150~900cm/h), the higher column bed height is, the lower 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 conductivity of the sample are adjusted to be consistent with the equilibration buffer (the pH and conductivity of the sample can be adjusted by dilution, ultrafiltration, and desalination with Bestdex G-25).
- 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.
- Loading Sample: The loading volume is determined by the substance content in the sample and the binding capacity of BestPoly 15S.
- Cleaning: Wash the column with equilibration buffer until the UV absorption value is 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.
- Regeneration: Flush the column with a high concentration of salt (e g: 2M NaCl).

- Re-equilibration: After rinsing with equilibration buffer, sample can be loaded again, repeat the process if necessary.

4. Application

Rnase, cytochrome C and lysozyme were isolated using Bestpoly 15S



Column: BHR5/100
Buffer A：20mM PB pH6.8
Buffer B：20mM PB, 0.4M NaCl pH 6.8
Sample：RNA enzymes, 1.5 mg/mL
Cytochrome C 0.4mg/ mL
Lysozyme is 0.4 mg/mL
Flow velocity: 2 mL/min

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: 70% ethanol or 30% isopropanol should be degassed before use. Reverse flushing can be used when the blockage is serious.

6. Sterilization

Since the 20% ethanol + 0.2M NaAc or 2% benzyl alcohol + 0.2M NaAc preservation solution does not have sterilization and depyrogenation, it is recommended that BestPoly 15S can be treated with 1M NaOH for more than 0.5-1h to reduce the risk of microbial contamination before and during use.

7. Storage

BestPoly 15S is supplied in 20% ethanol + 0.2M NaAc or 2% benzyl alcohol + 0.2M NaAc. It should

be stored in 20% ethanol + 0.2M NaAc and sealed at 2-30℃ after use, in order to prevent ethanol volatilization and microbial growth, it is recommended to replace the storage solution regularly.

8. Disposal and recycling

BestPoly 15S is very difficult to degrade in nature, incineration is recommended to protect the environment.

9. Order information

Product	Cat. No.	Pack size
BestPoly 15S	AI403005	25mL
	AI403007	100mL
	AI403011	500mL
	AI403012	1L
	AI403013	5L
	AI403014	10L
	AI403015	20L
	AI403016	40L

Prepacked columns	Cat. No.	Pack size
10/15 BestPoly 15S	EI04061	1 pcs
10/10 BestPoly 15S	EI04062	1 pcs
10/20 BestPoly 15S	EI04063	1 pcs
10/25 BestPoly 15S	EI04064	1 pcs
4.6/10 BestPoly 15S	EI04065	1 pcs
4.6/20 BestPoly 15S	EI04066	1 pcs
4.6/25 BestPoly 15S	EI04067	1 pcs