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SP Bestarose HP
High resolution strong
cation exchange resin
Instruction for use



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1. Introduction

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

IEC resin is composed of three parts: (1)Cross-linked agarose matrix, that has the characteristics of porous, hydrophilic and good chemical stability; (2)Functional groups fixed to the matrix, which are electrically charged groups, determine the properties of the IEC 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.

SP Bestarose HP (high performance) is a strong cation exchange resin formed by coupling sulfopropyl groups on fine-grained high-resolution agarose microspheres.

2. Technical characteristics

Appearance	White slurry, can be layered
Matrix	Cross-linked agarose, 6%
Functional group	Sulfopropyl
Particle size+	24~44μm (the average particle size is 34μm)
Ionic capacity	150~200μmol H ⁺ /mL packed resin
Dynamic binding capacity ++	~ 70 mg BSA/mL resin
Max. pressure	0.3 MPa
Chemical Stability	Stable in common aqueous buffers: 1M NaOH+++,1M HAC+++,6M GuHCl, 30%isopropyl alcohol, 70% ethanol Avoid contact with oxidizing agents, cationic detergents.
pH stability	3~14(CIP), 4~13(working)
Temperature tolerance	Working temperature:2~40°C, Can't freeze.
Storage++++	2~30°C, 20% ethanol with 0.2M NaAc or 2% benzyl alcohol with 0.2M NaAc
Recommended flow velocity	60-150cm/h

+ Average particle size is the accumulated resin particle size of packing volume distribution

++ In EzScreen 4.4mL column with column height of 10cm, 10%BSA dynamic binding load at 100mM NaHAc pH9 flow velocity of 1mL /min,

+++1M NaOH and 1M HAc only be used for cleaning.

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

3. Method of chromatographic

3.1 Column packing

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

- According the column volume to calculate the amount of resin.

Resin volume=column volume×1.15(Compression factor=1.15)

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

Required resin slurry¹ volume = Settlement resin volume ÷ Resin slurry¹ concentration. The original concentration of resin slurry¹ 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 non-original packaging, customer can calculate the required volume according to the actual concentration of resin slurry.

- Washing the resin: Thoroughly shake the resin and weigh certain volume of resin calculated by the method mentioned above. Pour it into a funnel, drain the liquid, and wash with about 3mL distilled water/mL resin for 3 times. Use a glass stick or stirrer to stir each time when adding distilled water, which helps to wash the shipping solvent away.
- 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 BXX column (BXX series columns with diameters ranging from 1cm to 30cm can satisfy different scale chromatography applications). Take BXX16/20 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 glasstube with same diameter as the BXX 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 60cm/h. Open the bottom plug, start the pump and run the setting flow velocity until the bed is stabilized, mark the bed height.
- Remove the packing reservoir (if any), Install the adaptor lower the adaptor to about 0.5cm above the resin surface, set the flow velocity at 300cm/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, 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 height equivalent to a theoretical plate (HETP) 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	Water	0.4M NaCl in water
Flow velocity	30cm/h	30cm/h
Monitor	UV280 nm	Conductivity



- Method for measuring HETP 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):

$$HETP=L/N$$

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

Note: V_R = retention volume

W_h = half-peak width

L = column height

N = the number of theoretical plates

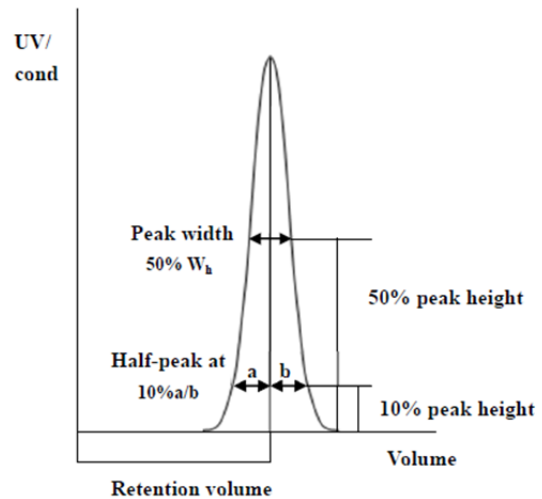
(The units of V_R and W_h should be the same)

$$As=b/a$$

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.

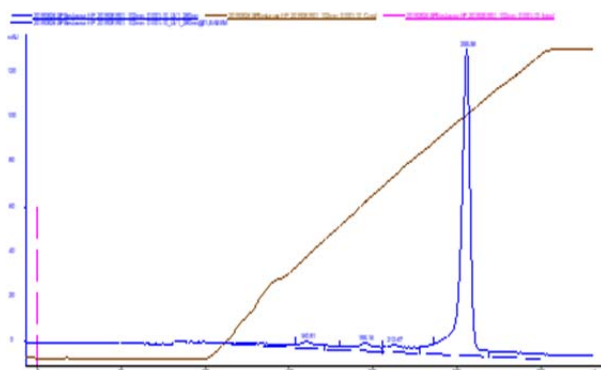
3.3 Chromatographic method

- Buffer selection: Buffer salts whose buffer groups do not act 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 elution to balance buffer.
- Flow velocity: 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 0.45 μ m 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.
- Sampling: The loading volume is determined according to the substance content in the sample and the binding load of SP Bestarose HP.
- Rinse: Wash the column with equilibration buffer until the UV absorption value is reduced to an appropriate value.

- 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 (eg: 2M NaCl)
- Rebalancing: After rinsing with equilibration buffer, the second sample can be loaded and repeated.

4. Application

SP Bestarose HP was used to isolate WGA and β -lactoglobulin



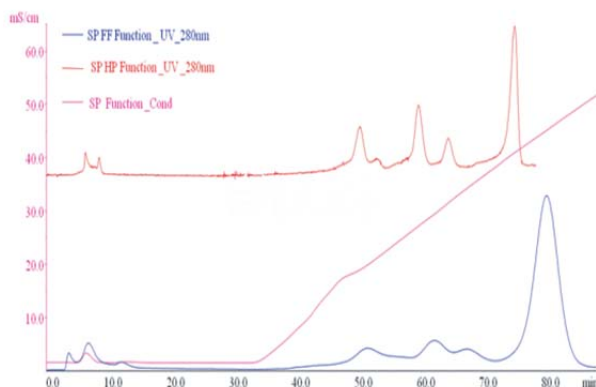
Column: EzScreen 4.2mL

Equilibration buffer: 20mM Formic acid pH 4.0

Elution buffer: 20mM Formic acid +0.75M LiCl pH 4.0

Sample: WGA and β -lactoglobulin mixture

SP Bestarose HP is better than SP Bestarose FF for the separation of the same sample.



Column: EzLoad 16/20, H=10cm

Equilibration buffer: 20mM HCOOH pH 4.0

Elution buffer: 20mM HCOOH +0.75M LiCl pH 4.0

Sample: WGA and β -lactoglobulin 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: 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

Since the 20% ethanol with 0.2M NaAc or 2% benzyl alcohol with 0.2M NaAc preservation solution does not have sterilization and depyrogenation, it is recommended that SP Bestarose HP can be treated with 1M NaOH for more than 0.5-1h to achieve the purpose of sterilization and pyrogen removal before and during use.

7. Storage

SP Bestarose HP is supplied in 20% ethanol with 0.2M NaAc or 2% benzyl alcohol with 0.2M NaAc. It should be stored in 20% ethanol with 0.2M NaAc and sealed at 2-30°C 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

SP Bestarose HP is very difficult to degrade in nature, incineration is recommended to protect the environment.

9. Order information

Product	Code No.	Pack size
SP Bestarose HP	AI0080	25mL
	AI0081	100mL
	AI0082	500mL
	AI0083	1L
	AI0084	5L
	AI0085	10L
	AI203115	20L
	AI203116	40L

Prepacked columns	Code No.	Pack size
EzFast SP HP	EI00821	1×1mL
	EI036	5×1mL
	EI203103	1×5mL
	EI037	5×5mL
EzScreen SP HP	EI00825	1×4.9mL
	EI00835	5×4.9mL
EzLoad 16/10 SP HP	EI039	1 pcs
EzLoad 26/10 SP HP	EI040	1 pcs