BESTCHROM
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Diamond CD-S
High resolution strong cation exchange resin
Instruction for use

Content

1. Introduction

Ion exchange (IEX) chromatography 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 number difference of biological molecules under specific conditions to separate them. It has the characteristics of high load, good resolution, easy to control and easy scale-up. It has been widely used in medicine, chemical industry, metallurgy, food and other fields.

Diamond CD-S is a strong cation exchange resin, obtained by coupling sulfopropyl functional groups on the high rigidity agarose beads in small size. The small bead size provides resin with high binding capacity and high resolution, making it ideal for the intermediate stage and polishing of purification process.

2. Technical parameters

Appearance	White to slight yellow suspension
Matrix	High rigidity agarose
Functional group	Sulfopropyl
Average particle size+	~60μm
Dynamic binding capacity++	＞100 mg IgG /mL resin
Max pressure	0.5MPa
Chemical stability	Common solutions: 1M NaOH+++, 6M GuHCl, 30% isopropanol, 70% ethanol Avoid exposure to strong oxidants and cation exchange detergents
pH stability	3~14 (CIP), 4~12 (operation)
Operating temperature	2~40°C, do not freeze
Storage	2~30°C, 20% ethanol
Recommended flow rate	100~300cm/h

+Average particle size is the mean value of resin volume distribution accumulation.

++10%DBC,Buffer A:50mM Sodium acetate,pH5.5,10cm column , flow rate：100cm/h.

+++1M NaOH only used for washing.

3. Method of chromatography

3.1 Column packing

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

- According the column volume to calculate the amount of Diamond CD-S required. Resin volume=column volume×1.15(Compression factor=1.15)

  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 listed 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 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 (100% of precipitated resin volume) to get 45%~55% packing 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 column for instance, 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 plug. 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 packing reservoir with packing solution. Connect the packing reservoir to the chromatography system, open the flow rate, drain the bubbles in the hose, close the flow rate, and tighten the top cap 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 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 rate can be set to 300cm/h. Open the bottom plug, start the pump and run the setting flow rate until the bed is stabilized. Mark the bed height.
- Remove the packing reservoir (if any), lower the adapter to about 0.5cm above the resin surface, and continue to press the column using the above flow rate until the bed is completely consolidated, marking the consolidated bed height.
- Stop the pump, open the adaptor upper plug, shut the column bottom plug, slightly loose the O-ring seal, press resin according to 1.15 concentration ratio, tighten the O-ring seal, close plug, complete column packing.

3.2 Evaluation of Column efficiency

- Test the column efficiency to check the quality of packing. The tests are required after the column packing, during the column working life and when the separation and purification performance is deteriorated. The method of the expressing the efficiency of a packed column is in terms of the height equivalent to a theoretical plate (HETP), theoretical plate number (N) and the asymmetry factor (As).
- Acetone or NaCl can be used as sample for the testing. Sample solution and eluent buffer 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 rate	30cm/h	30cm/h
Monitor	UV280 nm	Conductivity

- Method for measuring HETP and As:

  According to the UV curve or the conductivity curve to calculate the Height equivalent to a theoretical plate (HETP), number of theoretical plates (N) andthe 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 bed height

N = the number of theoretical plates

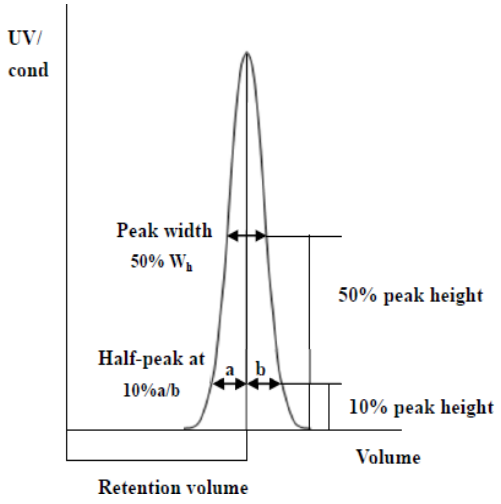
(The units of VR and W_h should be the same)

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, it 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 react with resin should be selected. The equilibration buffer should be low salt and low pH buffer (usually 1 pH unit lower than the isoelectric point of the target substance) to facilitate the binding of target substances. Meanwhile, the stability of target substance in the buffer should be considered. Elution buffers are usually obtained by adding a high concentration of salt (e.g. 1M NaCl) buffer or high pH buffer to equilibration buffer.
- Flow rate: According to the column bed height to use the flow rate of 60-300 cm/h, the higher the column bed height is, the slower the flow rate is.
- Sample preparation: In order to prevent blocking of the column, the sample needs to be filtered by microporous membrane 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 buffer change with Bestdex G-25).
- Equilibration: Washing the column with equilibration buffer until the pH and conductivity of the column outlet buffer reach the same as that of equilibration buffer, which will usually take 3-5CV.
- Loading sample: Determine the loading volume according to the sample components and binding capacity of Diamond CD-S.
- Cleaning: clean with the equilibration buffer till UV adsorption is close to baseline.
- Elution: Linear gradient or step-gradient can be used to increase the elution strength of 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 till pH and conductivity 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:

- 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 distilled water.
- Removal of lipoproteins and lipids: Wash with 5~10CV of 70% ethanol or 30% isopropanol, then wash with 5~10CV of distilled 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 rate can be set at 30~60cm/h. Reverse cleaning can be used when the blockage is serious.

5. Sterilization

Since the 20% ethanol shipping buffer does not have sterilization and depyrogenation function, it is recommended to treat Diamond CD-S with 1M NaOH for more than 0.5-1h to reduce the risk of microbial contamination before and during use.

6. Storage

Diamond CD-S is preserved in 20% ethanol or 2% benzyl alcohol. The used Diamond CD-S should be sealed in 20% ethanol at 2-30% to prevent the ethanol evaporation and microbial growth. It is recommended to replace storage solution regularly.

7. Disposal and recycling

It is difficult to degrade Diamond CD-S in nature, incineration is recommended to protect the environment.

8. Order information

Product	Cat. No.	Pack size
Diamond CD-S	AI05501	25mL
	AI05502	100mL
	AI05503	500mL
	AI05504	1L
	AI05505	5L
	AI05506	10L
	AI05507	20L

Pre-packed column	Cat. No.	Pack size
EzFast Diamond CD-S	EI05521	1×1mL
	EI05531	5×1mL
	EI05523	1×5mL
	EI05533	5×5mL
EzScreen Diamond CD-S	EI05525	1×4.6mL
	EI05535	5×4.6mL
EzSelect Diamond CD-S	EI05526	8×600µL
EzLoad 16/10 Diamond CD-S	EI05501	1pcs
EzLoad 26/10 Diamond CD-S	EI05511	1pcs