BESTCHROM
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Diamond S
Strong cationexchange resin

Instruction for use

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1. Introduction

Diamond S is a strong cationic resin. Compared with SP Bestarose FF, it has a higher dynamic capacity at high flow velocity. The resin used for the resin is highly cross-linked rigid agarose, which can be adapted to high flow velocity operation, and its physical and chemical properties are stable. During purification and cleaning regeneration, the resin load and various properties are not affected by external buffers. The impact is more suitable for large-scale industrial production. Diamond S has the following characteristics:

- High flow velocity, large volume of processing capacity, short operation cycle, improve productivity
- High rigidity matrix, high pressure resistance

2.Technical characteristics

Appearance	White slurry, can be layered
Matrix	High-rigidity agarose containing dextran long chain
Functional group	Sulfomethyl
Average Particle size+	90μm
Max. pressure	0.5 MPa
Pressure flow velocity	≥1500cm/h(0.5MPa BXK 100/500 H=20 cm 20℃)
Ionic capacity	110~140μmol H^+/mL packed resin
Dynamic binding capacity	> 60mg β -lactoglobulin/mL packed resin > 120mg lysozyme/mL packed resin
Chemical stability	Stable in common aqueous buffers: 1M NaOH++,0.1M NaOH,1M HCl, 6M GuHCl,8M Urea,70% ethanol,20% ethanol, 0.2M NaAc + 20% ethanol ,30% isopropanol Avoid contact with oxidizing agents, cationic detergents. Avoid long time exposures (1 week, 40℃)to pH <3.
pH stability	4~12(working) 3~14(CIP)
Temperature tolerance	Working temperature:2~40℃, Can't freeze.
Storage+++	2~30℃, 20% ethanol with 0.2M NaAc or 2% benzyl alcohol with 0.2M NaAc
Recommended flow velocity	90~500cm/h

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

++1M NaOH 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×1.05(That is, the compression ratio is about 1.05. When the column bed height is higher than 30cm, the compression ratio is only about 1.02)

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

  Required resin slurry[1] volume = Settlement 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 non-original packaging, 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 (20% ethanol with 0.2M NaCl)/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 packing solution 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). Take BXK16/20 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 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.

✧ 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 the setting flow velocity until the 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, 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 the top plug of adaptor, close the bottom plug, loosen the O-ring seal slightly, press the rubber surface according to the compression ratio of 1.1, tighten the O-ring seal, close the outlet, 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

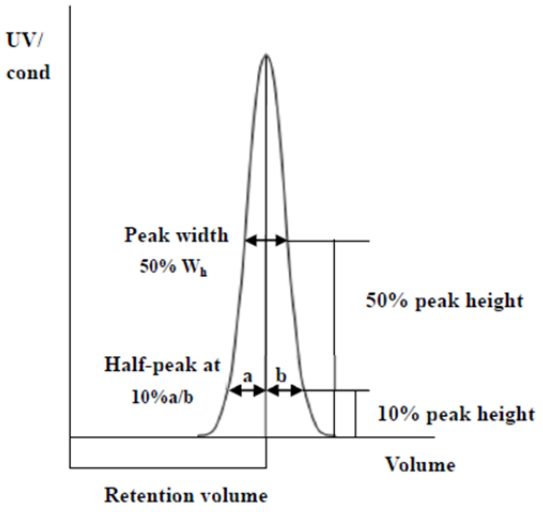
(The units of V_R and W_h should be the same)

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:The use of low-salt (less than 5mS/cm) low-pH buffer solution is conducive to the combination of samples and impurities. By choosing suitable pH and salt concentration to achieve the purpose of sample with resin or flow wear, more common method is to make the objective material with resin, impurities flow to wear, but if the process need, also can combine impurities with resin, objective material flow. The buffer is conducive to the combination of the object, and the stability of the object in the buffer should be considered.
- Flow velocity: According the column bed high to use the flow velocity 90~500cm/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, the pH and conductivity of the sample are adjusted to be consistent with the equilibration buffer ,Sample loading volume is determined according to the impurity content in the sample and the binding amount of Diamond S.
- 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 Diamond S.
- Rinse: Wash the column with equilibration buffer until the UV absorption value is reduced to an appropriate value.
- Elution:
  - Salt concentration elution: the most suitable elution condition can be determined by linear salt

concentration elution first, and then optimized into stage 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.
- 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. 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.

5. 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 Diamond S can be treated with 1M NaOH for more than 0.5-1h to reduce the risk of microbial contamination before and during use.

6. Storage

Diamond S 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℃ 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

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

8. Order information

Product	Code No.	Pack size
Diamond S	AI0181	25mL
	AI0182	100mL
	AI313311	500mL
	AI0183	1L
	AI0184	5L
	AI0185	10L
	AI313315	20L
	AI313316	40L

Prepacked columns	Code No.	Pack size
EzFast Diamond S	EI01821	1×1mL
	EI313351	5×1mL
	EI313303	1×5mL
	EI313353	5×5mL
EzScreen Diamond S	EI01825	1×4.9mL
	EI01835	5×4.9mL
EzLoad 16/10 Diamond S	EI313304	1 pcs
EzLoad 26/10 Diamond S	EI313306	1 pcs