BESTCHROM
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Diamond MIX-A Mixed-mode strong anion exchange resin

Instruction for use

Contents

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 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. However, when doing ion exchange chromatography, it is required that the salt content in the sample should not be too high. The mixed-mode ion exchange resin introduces a benzene ring on the ligand, which makes this type of resin have a certain tolerance to salt, eliminating the desalting step of high salt samples, and expanding the application range of ion exchange.

IEX resin is composed of three parts: (1)Cross-linked agarose matrix, that has the characteristics of porousness, hydrophobicity and good chemical stability, Diamond base matrix is a high-rigidity agarose base frame, which is made by chemical restructuring and modification of the traditional Bestarose 6FF base frame, with better mechanical properties.(2)The functional group fixed on the base matrix is a mixed-mode charged group, usually composed of a charged part and a hydrophobic part.(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.

Diamond MIX-A is mixed-mode strong anion exchange resin formed by coupling phenyl glycidyl ether functional groups and 2,3-epoxypropyltrimethylammonium chloride functional groups to high-rigidity agarose microspheres through two steps of crosslinking.

2.Technical characteristics

Appearance	White slurry
Matrix	High rigidity agarose
Functional group	Compound strong anion group
Average particle size $^{+}$	~75μm
Ionic capacity	90~120μmol Cl^-/mL packed resin
Max. pressure	0.5 MPa
Pressure flow velocity^{++}	≥1200cm/h（0.5MPa BXK 100/500 H=20 cm 20℃）
Chemical stability	Stable in common aqueous buffers: 1M NaOH+++,1M HAC+++,10mM NaOH, 6M GuHCl,8M Urea, 70% ethanol , 30% isopropyl alcohol, 20% ethanol, 2% benzyl alcohol Avoid contact with oxidizing agents, cationic detergents.
pH stability	2~14(CIP),3~12(working)

Operating temperature	Operating temperature:2~40℃, do not freeze
Storage++++	2~30℃, 20% ethanol or 2% benzyl alcohol
Recommended flow velocity	90-500cm/h

+The average particle size is the cumulative resin particle size of the filler volume distribution.

++The flow velocity is linear flow velocity of BXK100/500 column, column bed height 20cm, 20℃ 0.5MPa pressure.

+++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 chromatography

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×1.12(Compression factor=1.12)

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

  Required resin slurry[1] volume = Suspended 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 of packing solution (20% ethanol + 0.4M 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 resin surface according to the compression ratio of 1.12, 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
Detection	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

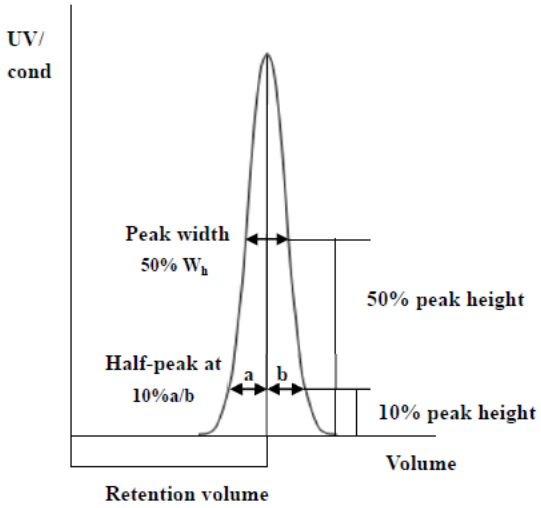
  (The units of V_R and W_h should be the same)

  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 .

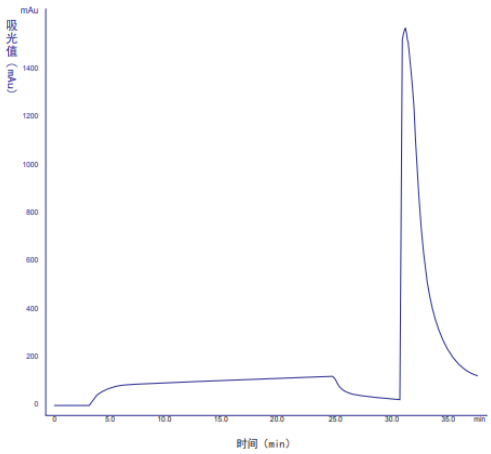
3.3 Chromatographic method

- Buffer selection: Buffer salts whose buffer groups do not act on the resin should be selected. The pH of the equilibrium buffer is usually 1 pH unit higher than the isoelectric point of the target, and should be adopted to facilitate the combination of substances. Meanwhile, the stability of samples in the buffer solution should be considered. Elution buffer needs to be determined according to the actual situation, if the main principle of binding is ion exchange, Elution buffers are usually made by adding a high concentration of salt (e.g.1M NaCl) or low pH elution to equilibration buffer.If hydrophobicity plays a role in binding, a low salt and pH buffer should be selected as the elution buffer.
- Flow velocity: According the column bed height to use the flow velocity(usually 90~500cm/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 0.45μm microporous membrane 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 Diamond MIX-A.

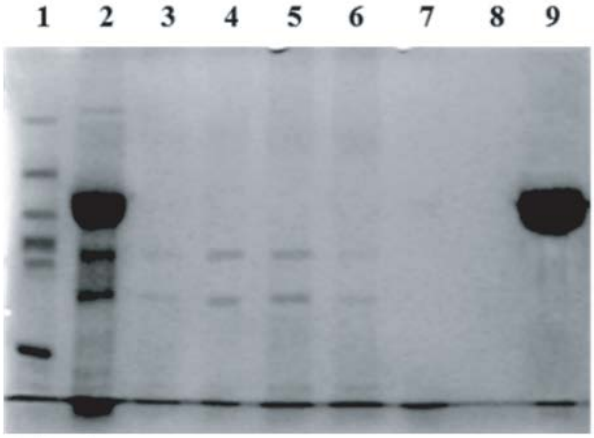
- Cleaning: Wash the column with equilibration buffer until the UV absorption value is close to baseline.
- Elution: Linear gradient or step-gradient can be used to increase the elution strength in the elution buffer, eluting substances with different binding strength from the chromatography column, collecting different components and detecting the location of the target.
- Regeneration: Flush the column with a high concentration of salt (eg: 2M NaCl)
- Re-equilibration: After cleaning with equilibration buffer, the second sample can be loaded. Repeat the process if necessary.

4. Application

A recombinant pichia pastoris fermentation broth was purified by Diamond MIX-A



Column:Ezload 50/20 Diamond MIX-A
Equilibration buffer :20mM PB,0.1M NaCl,pH7.0
Elution buffer :20mM PB,0.5M NaCl,pH7.0
Sample: 300mL of a recombinant pichia pastoris fermentation broth



Lane 1:Marker
Lane 2:fermentation liquor
Lane 3-6: The flow in lane
Lane 7-8: Flow through
Lane 9:Elution

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 2~3CV of 1M NaOH first, then clean immediately with 5~10CV of purified water.
- Removal of lipoproteins and lipids: Clean with 70% ethanol or 30% isopropanol by volume of

5~10CV first, then clean 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 or 2% benzyl alcohol preservation solution does not have sterilization and depyrogenation, it is recommended that Diamond MIX-A can be treated with 1M NaOH for 0.5-1h to reduce the risk of microbial contamination before and during use.

7. Storage

Diamond MIX-A is supplied in 20% ethanol or 2% benzyl alcohol. It should be stored in 20% ethanol 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.

8. Disposal and recycling

Diamond MIX-A is very difficult to degrade in nature, incineration is recommended to protect the environment.

9. Order information

Product	Cat. No.	Pack size
Diamond MIX-A	AI0101	25mL
	AI0102	100mL
	AI306311	500mL
	AI0103	1L
	AI0104	5L
	AI0105	10L
	AI306315	20L
	AI306316	40L

Prepacked columns	Cat. No.	Pack size
EzFast Diamond MIX-A	EI01021	1×1mL
	EI316351	5×1mL
	EI316303	1×5mL
	EI316353	5×5mL
EzScreen Diamond MIX-A	EI01025	1×4.9mL
	EI01035	5×4.9mL
EzLoad 16/10 Diamond MIX-A	EI049	1 pcs
EzLoad 26/10 Diamond MIX-A	EI050	1 pcs