BESTCHROM
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Diamond Layer 700 BA Mixed-mode Chromatography resin

Instruction for use

Contents

1. Introduction

Diamond Layer 700 BA is novel mixed-mode purification chromatography resin designed for intermediate purification and polishing purification of viruses and other biomolecules.

The resin has a two-layer structure, nucleic microspheres and a thin shell. The nucleic microspheres are prepared by crosslinking the benzylamine ligands to high rigidity agarose, and have anionic and hydrophobic effects. The thin shell is a layer of agarose structure without ligand, which has a molecular sieve function. In the purification process, large molecules such as viruses cannot enter the inside of the microsphere through the shell, so they flow through directly. Most impurities can enter the inside of the microsphere and bind to the resin through ion and hydrophobic interaction. These characteristics make Diamond Layer 700 BA resin an excellent choice for virus purification resin in vaccine production. The size exclusion of outer layer is 700KD. Biomolecules smaller than the corresponding molecular weight could enter the microsphere and bond with ligand groups, while biomolecules bigger than the corresponding molecular weight would be blocked in the microsphere outflow and pass through the chromatography column.

The main characteristics of Diamond Layer 700 BA resin include:

- The double-layer structure of the microspheres enables the resin to effectively adsorb impurities while the target protein flows through to achieve the purification effect.
- Due to the flow through of the target, the impurities are bond in hydrophobic and ionic mode, allowing relatively wide operating conditions.
- Compared with the conventional molecular sieve resin, the high sample loading volume and high flow velocity improve the production efficiency.

2.Technical characteristics

Matrix	Highly cross-linked rigidity agarose
Functional group	benzylamine
Average particle size^{+}	85μm
Appearance++	White to slight yellow slurry
Dynamic binding capacity	~13mg ovalbumin/mL packed resin
Average molecular weight exclusion	Mr≈700 000
Pressure flow velocity	≥500cm/h(H=20cm，pressure＜2bar)
Chemical Stability	Stable in common aqueous buffers: 1M NaOH+++,1M HAC+++,70% ethanol , 20% ethanol , 30% isopropyl alcohol, 6M GuHCl, 0.5% twain 80, 1M NaOH+30% isopropyl alcohol
pH stability	3~13(working), 2~14(CIP)

Storage++++	2~30℃, 20% ethanol or 2% benzyl alcohol
Avoid	Avoid contact with oxidizing agents, citrate buffer solution.

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

++Due to ligand properties, the color gradually changes from white to light yellow with the increase of storage time, and the color change has no effect on product performance.

+++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×1.15(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 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.

- Replace solution: Suspend the resin by shaking and pour the slurry into a funnel, remove the liquid, wash with about 5CV of packing solution (20% ethanol with 0.4M NaCl), (When resin volume is too large or other conditions are not convenient，please replace solution in the following manner. Settle resin till it is stratified, remove the supernatant, add packing solution, stir the mixture well, aspirate the supernatant after it is stratified. Repeat the operation for 5 times.
- 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 sirrer, 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 300cm/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 1.15, 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).
- NaCl solution can be used as sample for the testing. Sample solution and mobile phase can be prepared according to the following table.

	NaCl method
Sample	0.8M NaCl(Dissolved in water)
Sample volume	1.0%CV
Mobile phase	0.4M NaCl Aqueous solution
Flow velocity	30cm/h
Detection	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

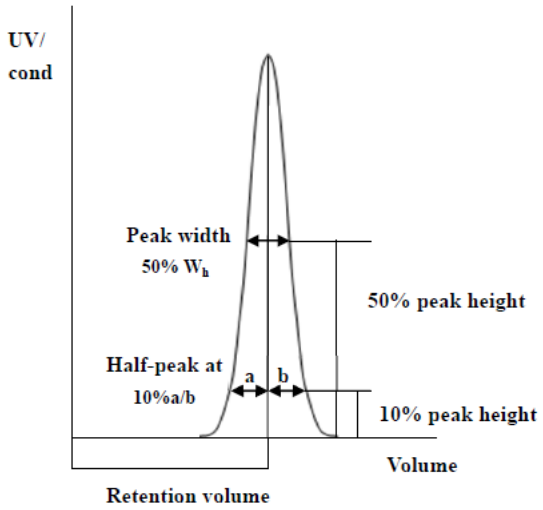
(The units of V_R and W_h should be the same)

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 .

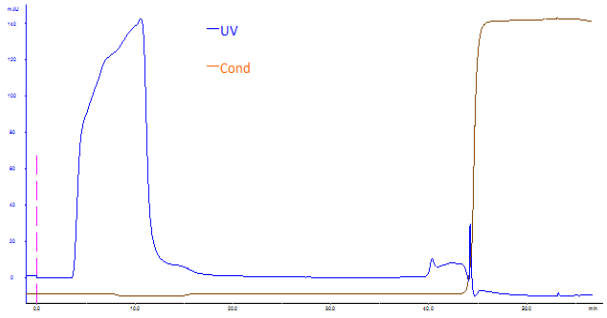
3.3 Chromatographic method

- Buffer selection: choose a buffer salt whose buffer group does not interact with the resin. The loading pH is recommended to use 7~9 to bind the impurity protein and nucleic acid in the sample, and the buffer material is Tris, PB, etc., and citrate buffer is not recommended. The resin is an anionic and hydrophobic composite type, so the loading salt concentration can be from 0 to 1M. The specific loading pH and salt concentration need to be optimized.
- Flow velocity: According the column bed height to set the flow velocity (usually 90~500cm/h), the higher column bed height is, the lower flow velocity will be.
- Sample: In some cases, high DNA and RNA content will affect the performance of the resin. Therefore, it is recommended that before using Diamond Layer 700 BA for sample purification, the DNA/RNA content can be reduced by operations such as ion exchange, alternatively cut nucleic acid into small molecules by broad-spectrum nuclease. Broad-spectrum nuclease can degrade DNA or RNA so that it can enter the microsphere and bind to the resin, meanwhile the broad-spectrum nuclease can also enter the microsphere and bind to the resin.
- Equilibrium: 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: Since the working principle of Diamond Layer 700 BA resin is to trap impurities in the micropores core of the resin, and let the target material flow through , in order to determine the maximum sample volume when maintaining the target purity, it is recommended that during process development process, get final loading amount by detecting and analyzing flow-through under various loading volume. Thus, to improve the recovery rate and purity of the target substance.

- Cleaning: wash the column with equilibration buffer to elute the impurities remaining in the resin until the UV absorption is close to the baseline.
- Regeneration: The impurities bound to the resin need to be eluted with strong elution conditions. Therefore, the elution conditions after each sample loading are the same as the CIP conditions. Please refer to the "5. CIP " section.
- Rebalance: After rinsing with equilibration buffer, sample can be loaded again, repeat the process if necessary.

4. Application

Isolation of lentiviruses using Diamond Layer 700 BA



Column：EzScreen4.4mL
Buffer A：PBS　pH7.5
Buffer B：1M NaOH+1M NaCl
Sample：virus
Flow velocity：0.6mL/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 cleaning conditions for Diamond Layer 700 BA is as follows:

- Use 30% isopropanol or 27% n-propanol solution containing 1M NaOH to reverse wash approximately 5CV.The specific concentration and processing time need to be optimized according to the actual situation of the sample.

  Note：The choice of cleaning solution needs to consider whether the column material is tolerable, so as not to damage the column.

6. Sterilization

Since the 20% ethanol or 2% benzyl alcohol preservation solution does not have sterilization and depyrogenation, it is recommended that Diamond Layer 700 BA can be treated with 1M NaOH for 0.5-1h to reduce the risk of microbial contamination before and during use.

7. Storage

Diamond Layer 700 BA 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 Layer 700 BA is very difficult to degrade in nature, incineration is recommended to protect the environment.

9. Order information

Product	Cat. No.	Pack size
Diamond Layer 700 BA	AI04901	25mL
	AI04902	100mL
	AI04903	500mL
	AI04904	1L
	AI04905	5L
	AI04906	10L
	AI04907	20L

Prepacked columns	Cat. No.	Pack size
EzFast Diamond Layer 700 BA	EI04921	1×1mL
	EI04931	5×1mL
	EI04923	1×5mL
	EI04933	5×5mL
EzScreen Diamond Layer 700 BA	EI04925	1×4.9mL
	EI04935	5×4.9mL
EzLoad 16/10 Diamond Layer 700 BA	EI04901	1 pcs
EzLoad 26/10 Diamond Layer 700 BA	EI04911	1 pcs