BESTCHROM
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Diamond Viru-S Affinity chromatography resin

Instruction for use

Contents

1. Introduction

Diamond Viru-S is an affinity chromatography resin is formed by coupling dextran sulfate to high rigidity Diamond matrix. It has a similar affinity for different virus types and is able to operate at high flow velocity under low backpressure conditions, which shortens process cycles and improves production efficiency. It is suitable for the capture stage and intermediate purification stage in the chromatography process, making large-scale production operation of biopharmaceutical possible.

Diamond Viru-S resin has the following advantages:

- Good chemical stability.
- Similar affinity for various viruses.
- High rigidity, high flow velocity, low back pressure.

2. Technical characteristics

Matrix	High rigidity agarose
Average particle size	75μm
Functional group	Dextran sulfate
Ligand concentration	70~130μmol Ligand/mL resin
Dynamic binding capacity	~60mg lysozyme/mL packed resin
pH stability	6~14 (CIP), 7~13 (working), avoid low pH operation and storage
Pressure flow velocity	≥1200cm/h (0.5MPa BXK 100/500 H=20 cm 20℃)
Chemical stability	Common aqueous solutions
Operating temperature	Operating temperature:2~30℃, do not freeze.
Storage+	2~8℃,20% ethanol or 2% benzyl alcohol

+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.

- 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 0.4M 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 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).
- 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

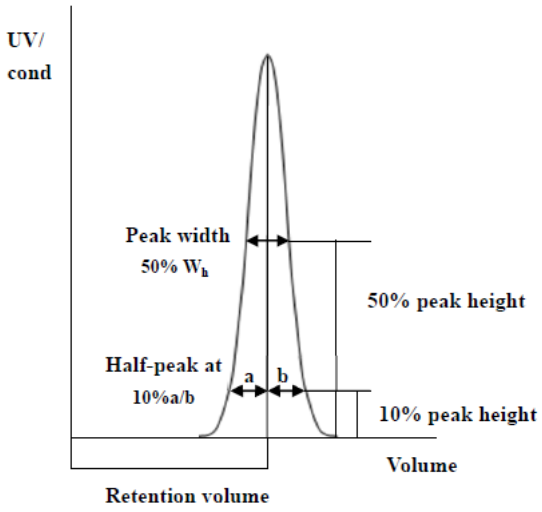
  (The units of V_R and W_h should be the same)

  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

Diamond Viru-S does not allow binding at high conductivity. When dealing with influenza viruses, it is recommended that the conductivity be less than 5mS/cm and the pH be between 6.8 and 7.8.

After column loading, it is recommended to carry out blank operation before purification operation,

including in-place cleaning (CIP).Balance the column with a 5CV equilibration buffer until the column outflow shows stable conductivity and pH.

- Buffer selection: preferred phosphate buffer, pH range from neutral 6.8 to 7.8, such as 20mM sodium phosphate pH 6.8.
- Flow velocity: According the column bed height to use the flow velocity 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: Wash 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 Viru-S.
- Cleaning: Wash the column with equilibration buffer until the UV absorption value is reduced to an appropriate value.
- Elution: A linear gradient or a step gradient can be used to increase the elution strength in the eluent, elute the virus with an elution buffer (e.g., 20mM sodium phosphate + 1.5M NaCl, pH 7.4).

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).

For each process, a specific CIP process shall be designed according to the type of pollutants. The frequency of CIP depends on the nature of the stock solution and chromatographic conditions. For Diamond Viru-S is used for the capture step, CIP is recommended after each cycle. The recommended CIP is to do reverse wash with 1M NaOH for at least 30min.

5. Sterilization

Since the 20% ethanol or 2% benzyl alcohol preservation solution does not have sterilization and depyrogenation, it is recommended that Diamond Viru-S resin can be treated with 1M NaOH for more than 0.5-1h to reduce the risk of microbial contamination before and during use. When sodium acetate or sodium phosphate buffer is used, Diamond Viru-S can be autoclaved at 121℃ for 30min to achieve the sterilization effect.

6. Storage

Diamond Viru-S is supplied in 20% ethanol or 2% benzyl alcohol. It should be stored in 20% ethanol (neutral pH) and sealed at 2-8℃ 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 Viru-S is very difficult to degrade in nature, incineration is recommended to protect the environment.

8. Order information

Product	Cat. No.	Pack size
Diamond Viru-S	AA0441	25mL
	AA0442	100mL
	AA0443	500mL
	AA0444	1L
	AA0445	5L
	AA0446	10L

Prepacked columns	Cat. No.	Pack size
EzFast Diamond Viru-S	EA04421	1×1mL
	EA04431	5×1mL
	EA04423	1×5mL
	EA04433	5×5mL
EzScreen Diamond Viru-S	EA04425	1×4.9mL
	EA04435	5×4.9mL
EzLoad 16/10 Diamond Viru-S	EA04401	1 pcs
EzLoad 26/10 Diamond Viru-S	EA04411	1 pcs