BESTCHROM
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Heparin Bestarose HP Affinity chromatography resin

Instruction for use

Contents

1. Introduction

Heparin Bestarose HP (High Performance) is a family-specific affinity chromatography resin made by covalently coupling heparin to fine particles with high cross-linking agarose. Widely used in the separation and purification of various biological molecules, especially various enzymes, including antithrombin III, thrombin-like, human coagulation factor IX, XI, VIII, lipoprotein lipase, collagenase, DNA polymerase. It can also be combined with human interleukin, human prostate growth factor, recombinant human vascular endothelial growth factor, cartilage growth factor, basic fibroblast growth factor, recombinant human acid fibroblast growth factor, recombinant hepatocyte growth factor, and recombinant murine heparin cofactor II. The combination of recombinant human platelet fourth factor, recombinant human endostatin, recombinant human keratinocyte growth factor and other biological macromolecules.The resin has the characteristics such as high resolution, physical and chemical stability, firmly attached ligands, long service life, and a wide range of applications.

2.Technical characteristics

Appearance	White slurry，can be layered
Matrix	Highly cross-linked 6% agarose
Particle size+	24~44μm (the average particle size is 34μm)
Functional group	Heparin
Ligand concentration	~10mg Heparin Ligand/mL resin
Dynamic binding capacity	2-5 mg bovine AT III/ mL packed resin
Chemical stability	Stable in common aqueous buffers: 8M urea, 6M GuHCl, 70% ethanol, 50mM sodium acetate(pH4), 10% glycerin, 0.1M NaOH (20℃, a week)
pH stability	5~10
Storage++	2~30℃, 20% ethanol with 50mM NaAc or 2% benzyl alcohol 50mM NaAc
Recommended flow velocity	＜150cm/h

+Particle size is normally distributed, and particles within this range account for more than 75% of the total.

++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. Due to the fine size of the resin, a chromatography column with a mesh aperture of 10

microns or less is required.

- 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 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)/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 150cm/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), Install the adaptor lower the adaptor to about 0.5cm above the resin surface, set the flow velocity at 300cm/h, 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 top plug, close the bottom plug, loosen the O-ring seal slightly, press the adaptor to about 0.3cm below the marked position, tighten the O-ring seal, close adaptor stop plug, 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

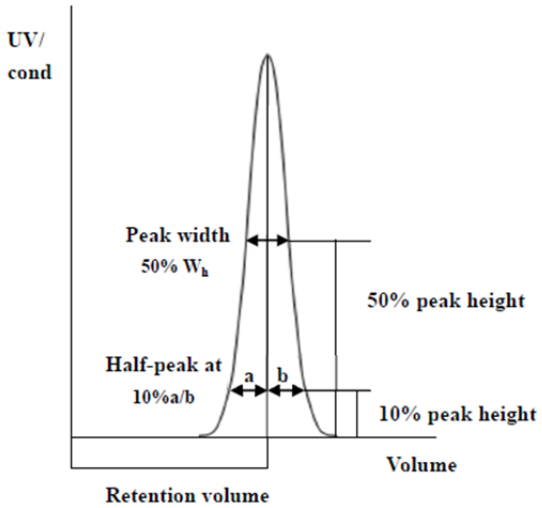
  (The units of V_R and W_h should be the same)

  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:

  Binding buffer: 20-50mM PB or Tris,pH7.4-8.0,0.15M NaCl can be added to inhibit non-specific adsorption

  Elution buffer: 20-50mM PB or Tris, 1-2M NaCl, pH 7.4-8.0,NaCl concentration needs to be adjusted appropriately according to the binding force of the target protein.
- Flow velocity: The flow velocity of 50~120cm/ h is generally selected according to the height of the column. The greater the column height, the slower the flow velocity.
- Sample preparation: In order to prevent the sample from clogging the column, the sample needs to be filtered with a 0.45 μm microporous membrane before loading, and the pH and conductivity of the sample are adjusted to be consistent with the equilibrium buffer. The loading volume is determined according to the impurity content in the sample and the combined loading of Heparin Bestarose HP.
- Rinse: Wash the column with equilibration buffer until the UV absorption at the outlet is close to the baseline.
- Elution: A linear gradient or step gradient can be used to increase the elution strength of the eluent, elute substances with different binding strengths from the chromatography column, collect different components, and detect the location of the target.
- Regeneration: Flush the column with high-concentration salts (such as 2M NaCl).
- Re-equilibration: After rinsing with equilibration buffer, the second sample can be taken 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:

- Removal of ion-bound proteins: first wash with 3~5CV of 2M NaCl, then rinse with 3~5CV of pure water.
- Removal of precipitated or denatured protein：First wash with 2CV of 0.1M NaOH, then rinse with 5~10CV of pure water, or 6M guanidine hydrochloride or 8M urea.
- Removal of hydrophobically bound proteins：Clean with 0.1 ~ 0.5% non-ionic detergent, and then rinse with 3~5CV of pure water.

  Note: The flow velocity can be selected from 30 to 60 cm/ h during CIP. Reverse cleaning is recommended.

Use immediately after washing, Rinse 3~5CV with equilibration buffer before use. If not used for a short period of time, rinse 3~5CV with 20% ethanol solution to store them.

5. Sterilization

Since the 20% ethanol with 50mM NaAc or 2% benzyl alcohol with 50mM NaAc preservation solution does not have sterilization and depyrogenation, it is recommended that Heparin Bestarose HP can be treated with 70% ethanol for 12 h to reduce the risk of microbial contamination before and during use.

6. Storage

Heparin Bestarose HP is supplied in 20% ethanol with 50mM NaAc or 2% benzyl alcohol with 50mM NaAc.It should be stored in 20% ethanol with 50mM 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

Heparin Bestarose HP is difficult to degrade in nature, incineration is recommended to protect the environment.

8. Order information

Product	Code No.	Pack size
Heparin Bestarose HP	AA0261	25mL
	AA0262	100mL
	AA0263	500mL
	AA0264	1L
	AA0265	5L
	AA212114	10L

Prepacked columns	Code No.	Pack size
EzFast Heparin HP	EA212101	1×1mL
	EA050	1×5mL
	EA049	5×1mL
	EA051	5×5mL
EzScreen Heparin HP	EA02625	1×4.9mL
	EA02635	5×4.9mL
EzLoad 16/10 Heparin HP	EA212104	1 pcs
EzLoad 26/10 Heparin HP	EA212106	1 pcs