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**Blue Bestarose HP
Affinity
chromatography resin
Instruction for use**



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1. Introduction

Blue Bestarose HP is an affinity resin based on fine particle agarose and Cibacron Blue 3GA as ligand. It has the characteristics such as high resolution, stable physical and chemical properties, firmly attached ligands (not easy to drop off), long service life, and a wide range of applications. This resin can not only bind to proteins through specific interactions, but also non-specifically bind proteins through charges and other effects. Blue Bestarose HP has been widely used in the separation and purification of various proteins, such as dehydrogenase, kinase, transferase, serum albumin, interferon and plasma protein.

2. Technical characteristics

Appearance	Blue slurry, can be layered
Average particle size ⁺	34 μ m (24~44 μ m)
Functional group	Cibacron Blue 3GA
Ligand concentration	~7.3 μ mol chromophore /mL resin
Dynamic binding capacity	>18mg HSA/mL packed resin
Max. pressure	0.3 MPa
Chemical stability	Stable in common aqueous buffers: 8M urea, 6M GuHCl, 70% ethanol
pH stability	4~12(working), 3~13(CIP)
Temperature tolerance	Working temperature: 2~40 $^{\circ}$ C, Can't freeze.
Storage ⁺⁺	2~8 $^{\circ}$ C, 20% ethanol with 0.1M KH ₂ PO ₄ (pH 8.0) or 2% benzyl alcohol with 0.1M KH ₂ PO ₄ (pH 8.0)
Recommended flow velocity	<150cm/h

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

++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 particle size of the resin, it is necessary to choose a chromatographic column with a mesh size of 10 microns or less.

- According to the column volume to calculate the amount of resin.

Resin volume = column volume \times 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 slurry¹ volume = Settlement resin volume ÷ Resin slurry¹ concentration. The original concentration of resin slurry¹ 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: Thoroughly shake the resin and weigh certain volume of resin calculated by the method mentioned above. Pour it into a funnel, drain the liquid, and wash with about 3mL distilled water/mL resin for 3 times. Use a glass stick or stirrer to stir each time when adding distilled water, which helps to wash the shipping solvent away.
- Prepare the packing slurry: Transfer the washed resin from the funnel into a beaker or other appropriate container, add distilled water to obtain a 50%~75% slurry, stir well and set aside for use.
- Take a cleaned B XK column (B XK series columns with diameters ranging from 1cm to 30cm can satisfy different scale chromatography applications). Take B XK16/20 for example, purge the bubbles trapped at the end-piece net by draining some distilled water 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 B XK 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 B XK 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):

$$\text{HETP} = L/N$$

$$N = 5.54(V_R/W_h)^2$$

Note: V_R = retention volume

W_h = half-peak width

L = column height

N = the number of theoretical plates

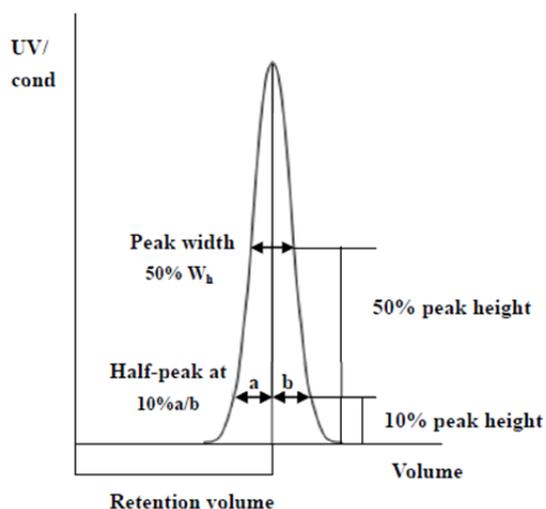
(The units of V_R and W_h should be the same)

$$\text{As} = b/a$$

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

- Sample
 - For complex protein mixed samples, the sample concentration should not be too low, the lower the binding capacity. However, for samples that specifically bind to the mediator ligand, there is no need to consider the sample concentration.
 - Sample concentration should not be too large. High concentration (greater than 30mg/mL) may cause fluctuations in pH and ionic strength, affecting binding. When the concentration is high, the sample can be diluted with binding buffer.
 - Pay attention to the viscosity of the sample. High viscosity samples will cause uneven flow velocity during chromatography.
 - The sample solution needs to be centrifuged or filtered with a 0.45 μ m filter before loading, in order to avoid clogging the chromatography column or reduce the resolution efficiency and service life of the chromatography column.
- Binding buffer
 - The low pH binding buffer can promote protein binding. Generally, the pH range is between 5.5-8.5.
 - Low ionic strength balance buffer can promote protein binding, the concentration of binding buffer is preferably between 5-50mmol / L.
 - The presence of metal ions will also enhance protein binding capacity. Adding 0.1-10 mmol/L of metal ions (such as Mg²⁺、Ca²⁺、Zn²⁺、Mn²⁺、Cu²⁺、Co²⁺、Fe³⁺和 Al³⁺)to the buffer can enhance protein binding.
- Flow velocity: According to the height of the column, the flow velocity of 60~150cm/h is generally selected.
- Sample preparation: In order to prevent blocking of the column, the sample needs to be filtered by microporous membrane of 0.45 μ m before loading, the pH and conductivity of the sample are adjusted to be consistent with the equilibration buffer. The loading volume is determined according to the substance content in the sample and the binding load of Blue Bestarose HP.
- Equilibration: Rinse with equilibration buffer until the UV absorption value drops to an appropriate value.
- Sample loading: sample the prepared samples according to the set conditions.
- Rinse: Wash the column with equilibration buffer until the UV absorption value is reduced to an appropriate value.
- Elution: one or several of the following methods to elute the bound protein can be choosed:
 - Change the ionic strength of the elution buffer by increasing the concentration of salts (KCl and NaCl) in the buffer.
 - Change to increase the pH of the elution buffer.
 - Change the polarity of the elution buffer, such as adding 50% ethylene glycol, 10% dioxane, or other organic solvents.

- Add appropriate concentrations of specific ligands such as enzyme substrates, enzyme substrate products, cofactors, inhibitors and activators.
- Regeneration: High pH (0.1M Tris, 0.5M NaCl, pH8.5) and low pH (0.1M NaAc, 0.5M NaCl, pH4.5) can be alternately cleaned to remove the strongly bound proteins and make the chromatography column regenerate.

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).

- Denatured protein: Wash 4CV with 0.1M NaOH, then wash 3-4CV with 70% ethanol or 2M potassium thiocyanate or use 6M guanidine hydrochloride to clean 2~3CV; then immediately rinse with at least 5CV of equilibration buffer.
- Strongly hydrophobic substances or lipids: Wash the column with 2-4CV of 70% ethanol or 30% isopropanol, and immediately rinse with at least 5CV of equilibration buffer.

5. Sterilization

Since the 20% ethanol with 0.1M KH_2PO_4 (pH 8.0) or 2% benzyl alcohol with 0.1M KH_2PO_4 (pH 8.0) preservation solution does not have sterilization and depyrogenation, it is recommended that Blue Bestarose HP can be treated with 70% ethanol for 12 h to reduce the risk of microbial contamination before and during use. It can also be autoclaved at 121 °C for 15min.

6. Storage

Blue Bestarose HP is supplied in 20% ethanol with 0.1M KH_2PO_4 (pH 8.0) or 2% benzyl alcohol with 0.1M KH_2PO_4 (pH 8.0). It should be stored in 20% ethanol with 0.1M KH_2PO_4 (pH 8.0) and sealed at 2-8 °C 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

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

8、Ordering Information

Product	Code No.	Pack size
Blue Bestarose HP	AA0282	25mL
	AA0283	100mL
	AA0284	500mL
	AA0285	1L
	AA0286	5L
	AA210114	10L

Prepacked columns	Code No.	Pack size
EzFast Blue HP	EA210101	1×1mL
	EA210103	1×5mL
	EA210151	5×1mL
	EA210153	5×5mL
EzScreen Blue HP	EA02825	1×4.9mL
	EA02835	5×4.9mL
EzLoad 16/10 Blue HP	EA210104	1 pcs
EzLoad 26/10 Blue HP	EA210106	1 pcs