BESTCHROM
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Diamond Blue Affinity chromatography resin

Instruction for use

Contents

1. Introduction

Diamond Blue is a kind of affinity resin based on fine particles and high rigidity agarose. The ligand is Cibacron Blue 3GA, which is physically and chemically stable. Since the ligand is not easy to fall off, it can enjoy long service life and a wide range of applications. These mediators can not only bind to proteins through specific interactions,but also non-specific binding to proteins through electric charges.Diamond Blue has been widely used in the separation and purification of various proteins, such as dehydrogenase, kinase, transferase, serum albumin, interferon and plasma proteins.

Compared to Blue Bestarose FF, Diamond Blue has better pressure flow velocity performance and higher load capacity, helping to improve process efficiency and reduce cost.

2.Technical characteristics

Appearance	Blue slurry, can be layered
Matrix	Highly cross-linked rigid agarose
Average particle size +	75μm
Functional group	Cibacron Blue 3GA
Ligand concentration	~13μmol chromophore /mL resin
Dynamic binding capacity++	≥24mg HSA/mL packed resin
Chemical Stability	40℃ 1 week：10mM HCl,0.1M NaOH, 8M urea, 6M GuHCl 40℃ 12h：1M NaOH, 70% acetic acid
Max. pressure	0.5 MPa
Pressure flow velocity	≥1200cm/h（0.5MPa BXK 100/500 H=20 cm 20℃）
pH stability	2~13(working),2~13(CIP)
Temperature tolerance	Working temperature:2~40℃, Can't freeze.
Storage+++	2~8℃, 20% ethanol with 0.1M KH_2PO_4(pH 8.0)or 2% benzyl alcohol with 0.1M KH_2PO_4(pH 8.0)

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

++ Residence time: 4min

+++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.

- 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 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 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

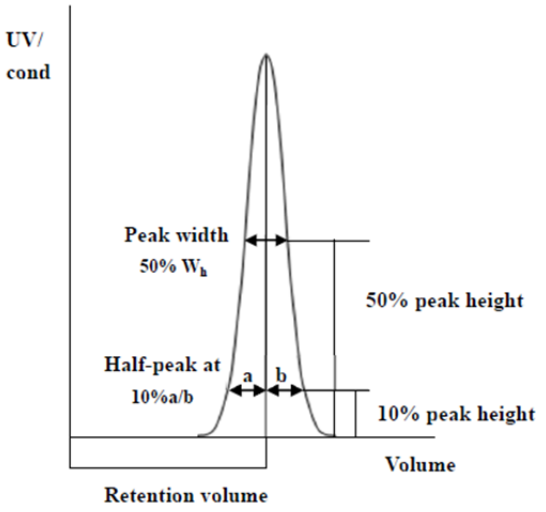
  (The units of V_R and W_h should be the same)

  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

- 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.
  - The sample concentration should not be too large. High concentration (greater than 30mg/mL) may cause fluctuations in pH and ionic strength, which affecting the 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 0.45μm filter before loading, to avoid clogging the chromatography column or reducing 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 0.1-10mmol/L of metal ions (such as Mg^{2+}、Ca^{2+}、Zn^{2+}、Mn^{2+}、Cu^{2+}、Co^{2+}、$Fe^{3+}$and Al^{3+}) to the buffer can enhance protein binding.
- Flow velocity：The flow velocity of 90 ~ 500cm/h is generally selected according to the height of the column. The larger the column height, the slower the flow velocity.
- Sample preparation: In order to prevent blocking of the column, the sample needs to be filtered by microporous membrane of 0.45μm before loading, the pH and conductivity of the sample are adjusted to be consistent with the equilibration buffer. The loading volume was determined according to the impurity content in the sample and the binding capacity of Diamond Blue.
- Equilibrium: Clean the column with a balance buffer until the UV absorption is reduced to an appropriate value.
- Sample loading: sample loading shall be carried out according to the set procedure.
- Elution: One or several of the following methods to elute the bound protein can be used:
  - Change the ionic strength of the elution buffer by increasing the concentration of salts (KCl and NaCl) in the buffer.
  - 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 3~4CV with 0.5M NaOH, then wash 3-4CV with 70% ethanol or 2M potassium thiocyanate; or use 6M GuHCl to clean 2~3CV, 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 does not have sterilization and depyrogenation,it is recommended that Diamond Blue can be treated with 70% ethanol for 12 hours to reduce the risk of microbial contamination before and during use. It can also be autoclaved at 121℃ for 15min.

6. Storage

Diamond Blue 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℃ 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 Diamond Blue is difficult to degrade in nature, incineration is recommended to protect the environment.

8. Order information

Product	Code No.	Pack size
Diamond Blue	AA310305	25mL
	AA310307	100mL
	AA310311	500mL
	AA310312	1L
	AA310313	5L
	AA310314	10L

Prepacked columns	Code No.	Pack size
EzFast Diamond Blue	EA310301	1×1mL
	EA310303	1×5mL
	EA310351	5×1mL
	EA310353	5×5mL
EzScreen Diamond Blue	EA04025	1×4.9mL
	EA04035	5×4.9mL
EzLoad 16/10 Diamond Blue	EA310304	1 pcs
EzLoad 26/10 Diamond Blue	EA310306	1 pcs