BESTCHROM
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NHS-activated Bestarose 4FF Affinity chromatography resin

Instruction for use

Contents

1. Introduction

NHS-activated Bestarose 4FF is a pre-activated agarose gel, the matrix is high-flow agarose Bestarose 4FF, with spacer arms suitable for coupling small molecule ligands.

The pre-activation resin has the following characteristics:

- Easy to form amide bond with protein, strong chemical stability
- The extension arm with 10 carbon atoms reduces the steric hindrance of ligand and agarose surface
- Easy operation, avoid using highly toxic raw materials
- Fast flow velocity, suitable for large-scale applications

2.Technical characteristics

Appearance	White slurry, can be layered
Matrix	Highly cross-linked agarose, 4%
Particle size+	45~165μm
Activating group	N-hydroxysuccinimide
Ligand concentration	16~23μmol NHS /mL resin
Coupling functional group	-NH2
Max. pressure	0.3MPa
Pressure flow velocity	~150cm/h（0.1MPa BXK50/60 H=25cm 25°C）
Chemical stability++	Stable in common aqueous buffers: 6M GuHCl,8M Urea, 70% ethanol
pH stability+++	3~13(working), 2~13(CIP)
Storage	2~8℃, 100% isopropyl alcohol
Recommended flow velocity	150~300cm/h

+ The particle size is normally distributed, and the particles in this range account for more than 95% of the total

++Data when the coupled ligand is also stable

+++ No coupling ligand

3. Method of chromatographic

3.1 Coupling

- NHS-activated Bestarose 4FF is supplied in isopropanol. The following is the introduction of the preparation method of removing the isopropyl alcohol and coupling with the ligand.
  - Coupling solution A：1mM HCl

Coupling solution B：0.1M $NaHCO_3$、0.5M NaCl，pH8.3

- Take a known volume of resin stored in isopropanol in a sand core funnel, wash with pre-cooled coupling solution A (0~4℃) for at least 30 min, and 1 mL of resin with about 20 mL of coupling solution A.

  Note: NHS corresponding ligands hydrolyze rapidly at high pH.

- Ligands are dissolved in coupling solution B, or replaced in coupling solution B with Bestadex G-25 chromatography column (ligand coupling concentration 5-10 mg / mL filler).
- Dilute the washed resin with coupling solution A (about 0.5mL coupling solution A in 1mL resin) and mix it with the same volume of ligand solution, mix at room temperature for 4h or stir at 4℃ overnight (magnetic stirrer cannot be used).

  Description: The coupling process is usually very fast at room temperature, so it is important to maintain the biological activity of ligands by optimizing the coupling time.

- After the ligand coupling is completed, the packing needs to be sealed and cleaned.
- Block: Remove the coupling supernatant and add blocking solution (0.1M Tris-HCl, pH 8.5), block at room temperature for 2~4h.
- Wash: 0.1M Tris-HCl + 0.5M NaCl, pH8~9 and 0.1M acetate buffer + 0.5M NaCl, pH3~4 were washed alternately for 3~6 times, each time with liquid of 3 times gel volume.
- After washing with PBS, use or store in 20% ethanol (the ligand must be stabilized in 20% ethanol).

3.2 Evaluation of Packing

Ligand-coupled resin needs to be packed in the chromatography column before it can be used in the next purification operation.

- 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	80

  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 BXK column (BXK series columns with diameters ranging from 1cm to 30cm can satisfy different scale chromatography applications). 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 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.

  Note: This operation is only applicable to BXK 50 and below chromatographic columns.

✧ 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.

- Set the flow velocity (When the bed height is 10~20cm, the flow velocity can be up to 350cm/h).Open the bottom plug, start the pump and run the setting flow velocity until the bed is stabilized. If the pressure exceeds 0.3MPa during the packing process, the flow velocity needs to be reduced appropriately. Mark the bed height.
- After the resin surface is stable, keep it for more than 30 minutes, mark the position of the resin surface, and then stop the pump.
- 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.

3.3 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

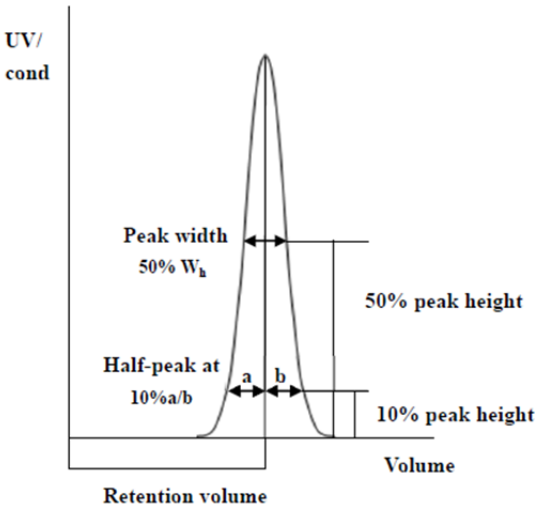
  (The units of V_R and W_h should be the same)

  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.

3.4 Use of Resin

- 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, 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 0.45μm 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 pH, salt concentration and temperature of the binding buffer mainly depend on the binding conditions of the ligand and the target.
- Flow velocity：According to the binding strength of the ligand and the target, a flow velocity of 150 ~ 300cm / h is generally selected. The weaker the binding force, the slower the flow velocity should be.
- Sample loading: Depends on the type of ligand and target.
- Rinse with binding buffer until the UV absorption value drops to an appropriate value.
- Elution: It is necessary to select an appropriate elution buffer according to the binding principle of the target substance and ligand, usually eluting in the following manner.
- Changing the pH of the elution buffer is usually based on the principle of antigen-antibody binding.
- Elution with competitive substances is usually based on the principle of enzyme and substrate binding.

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:

- 50mM Tris-HCl, pH 8-9 and 0.2M acetic acid solution, pH 3-6 alternately washed more than 3 times, each with 3CV.
- Removal of lipoproteins and lipids：First wash with 5 ~ 10CV of 70% ethanol or 30% isopropyl alcohol, then rinse with 5 ~ 10CV of pure water.
- For the removal of strongly hydrophobic proteins and precipitated proteins：First wash with 2~3CV of 6M guanidine hydrochloride, then immediately rinse with 5~10CV of pure water; or wash 2CV with 0.1M NaOH (ligand stable), then immediately use 5~10CV. Rinse with volume of pure water.

5. Sterilization

NHS-activated Bestarose 4FF after coupling ligand can be treated with 70% ethanol (the ligand is stable in 70% ethanol) for 12~24h to reduce the risk of microbial contamination before and during use. Or if the stability of the ligand permits, the column can be set for one hour in a 20% ethanol solution containing 0.1M NaOH, and then cleaned with a binding buffer of at least 5 columns.

6. Storage

NHS-activated Bestarose 4FF without coupling ligand should be sealed and stored in 100% isopropyl alcohol at 2-8℃.

7. Disposal and Recycling

NHS-activated Bestarose 4FF is very difficult to degrade in nature, incineration is recommended to protect the environment.

8. Order information

Product	Code No.	Pack size
NHS-activated Bestarose 4FF	AA0031	25mL
	AA118307	100mL
	AA0032	500mL