BESTCHROM
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CNBr-activated Bestarose 4B
Preactivated agarose gel

Instruction for use

Contents

1. Introduction

CNBr-activated Bestarose 4B is a pre-activated agarose gel with cyanogen bromide.Biomolecules with a primary amino group (-NH_2) can be covalently coupled to this resin for making specific affinity resin and immobilized enzymes.

The resin has the following characteristics:

- Mild reaction conditions with protein and other biological macromolecules
- Can be directly coupled to biological macromolecules without coupling spacers
- The ligand and the scaffold can form a multi-site coupling, the ligand should not fall off
- Applied to the coupling of proteins, peptides, nucleic acids, etc.

2.Technical characteristics

Appearance	White slurry，can be layered /white powder solid
Matrix	Cross-linked 4% agarose
Particle size+	45~165μm
Conjugated functional groups	Primary amino group
Amount of conjugated protein	25-60mg α-chymotrypsinogen/mL resin
pH stability++	3~11(working) 2~11(CIP)
Chemical stability+++	Stable in common aqueous buffers: 6M GuHCl,8M Urea, 70% ethanol
Max. flow velocity	≥15cm/h(BXK26/40，H=30cm, 20℃，in water)
Max.pressure	0.16bar
Storage	2 ~ 8℃, 100% acetone

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

++ No coupling ligand

+++ Data when the coupled ligand is also stable

3. Method of chromatographic

3.1 Coupling

- CNBr-activated Bestarose 4B is stored in acetone. Before coupling ligands, protective agents such as acetone must be removed.
  - Coupling solution A：1mM HCl
    Coupling solution B：0.1M $NaHCO_3$、0.5M NaCl，pH8.3**(High concentrations of salt can prevent polymer formation).**
  - Take the gel stored in acetone in a sand core funnel (dry powder can be soaked with 1mM HCl

for 10~ 15min), wash with pre-cooled coupling solution A (0 ~ 4℃), at least 30min, 1mL resin About 60mL of coupling solution A was washed.

Note: The corresponding functional groups of CNBr will hydrolyze rapidly at a higher pH.

- Ligands were replaced with coupling solution B solution, or Bestadex G-25 chromatography column in coupling solution B (ligand coupling concentration 5 ~ 10mg / mL filler).

  Description: Too high ligand concentration will have an adverse effect on affinity chromatography. Firstly, the binding rate of the adsorbed material may decrease due to steric hindrance between active sites. Secondly, the binding force between the target and the ligand is too strong to elute.

- Dilute the washed resin with coupling solution A (about 0.5mL of coupling solution A in 1mL of resin), mix with the same volume of ligand solution, mix at room temperature for 2h or stir at 4℃ overnight (cannot use magnetic stirrer).

  Note: The coupling process is usually very fast at room temperature (20-25℃), so it is important to maintain the biological activity of ligands by optimizing the coupling time.

- After the ligand coupling is completed, the gel needs to be blocked and washed.
- Block: Remove the coupling supernatant and add blocking solution (0.1M Tris-HCl, pH 8.3), 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 5 times the volume of resin.
- After washing with PBS, use or store in 20% ethanol (under the premise that the ligand is stable in 20% ethanol).

3.2 Column packing

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

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	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 according to the recommended operational flow, packing flow velocity is generally about 1.3 times of the recommended operational flow velocity.
- Open the bottom plug, start the pump and run column at set flow velocity until the bed is stability. When the pressure exceeds 0.3MPa during column packing ,mark the bed height.
- Remove the packing reservoir (if any), when slurry is fully gravity-settled, keep it for more than 3CV, 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

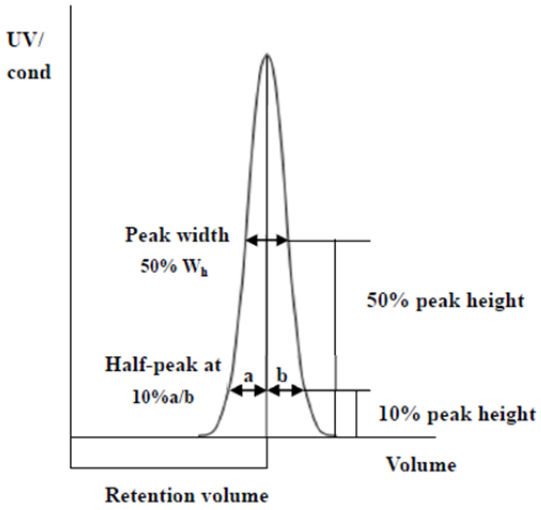
  (The units of V_R and W_h should be the same)

  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 ＜50cm / 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：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. Regeneration

The regeneration solution mainly depends on the stability of the ligand, which can only be used if the ligand is stable in the corresponding cleaning reagent. The general regeneration method is 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.
- Protein denaturant: 8M urea, 6M GuHCl.
- Organic solvent: 70% ethanol, 30% isopropyl alcohol.

5.Sterilization

CNBr-activated Bestarose 4B after coupling ligand can be treated with 70% ethanol (the ligand is stable in 70% ethanol) for more than 12～24h to reduce the risk of microbial contamination.

6. Storage

CNBr-activated Bestarose 4B with uncoupled ligand should be sealed and stored in 100% acetone at 2-8℃,the resin after coupling the ligand can be stored in 20% ethanol, in order to prevent ethanol volatilization and microbial growth, it is recommended to replace the storage solution every 3 months.

7. Disposal and Recycling

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

8. Order information

Product	Code No.	Pack size
CNBr-activated Bestarose 4B	AA017305	25mL
	AA017307	100mL
	AA0012	500mL