BESTCHROM
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Benzamidine Bestarose 4FF Affinity chromatography resin

Instruction for use

Contents

1. Introduction

Benzamidine Bestarose 4FF is an affinity chromatography resin made by coupling p-amino benzamidine to Bestarose 4FF agarose gel. It is commonly used for the separation and purification of serine proteases or the removal of serine proteases from biological samples. The benzamidines are broad-spectrum inhibitors of serine proteases (such as trypsin, thrombin, urokinase, kallikrein, prokinin releasing enzyme, etc) and can be used as ligands for purifying such substances.

2.Technical characteristics

Appearance	White slurry, can be layered
Matrix	Cross-linked agarose, 4%
Particle size+	45~165μm
Functional group	P-aminobenzidine
Ligand concentration	≥12μmol Ligand/mL resin
Dynamic binding capacity	≥35mg Trypsin/mL packed resin
Max. pressure	0.3 MPa
Pressure flow velocity	＞150cm/h（0.1MPa BXK50/30 H=25cm 20℃）
Chemical stability	Stable in common aqueous buffers: 6M GuHCl,8M Urea, PH =1, 2, 3, 4 hydrochloric acid solution, 0.025M borax solution with pH=8, 9, 10, 11
pH stability	2~8(working)、1~9(CIP)
Storage++	2~8℃， 20% ethanol with 50mM NaAc(pH4.0) or 2% benzyl alcohol with 50mM NaAc(pH 4.0)
Recommended flow velocity	30-300cm/h

+Particle size is normally distributed, and particles within this range account for more than 95% 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.

- 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: 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). Take BXK16/20 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 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 column packing pressure can be set to 0.1MPa. Open the bottom plug, turn on the pump and run the setting flow velocity until the resin 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 packing pressure 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

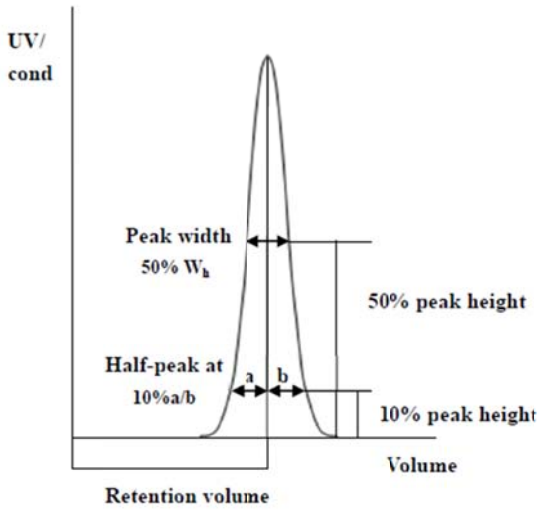
  (The units of V_R and W_h should be the same)

  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

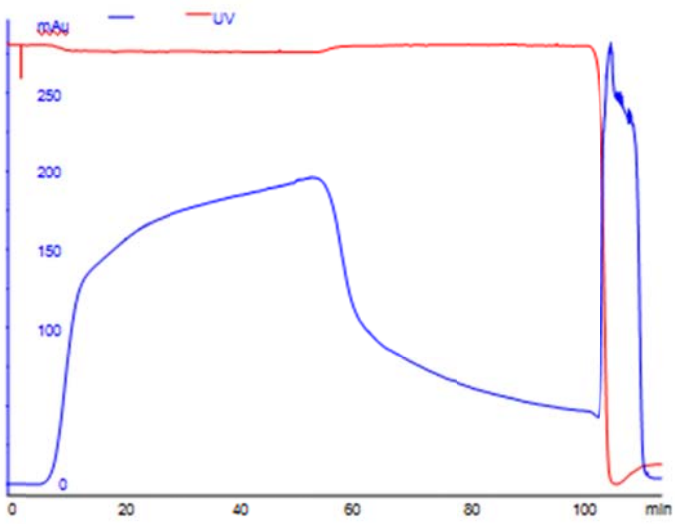
  As a guideline, if the value of HETP is less than 3 times the average particle size(d_{50}) of the resin and the As is between 0.8~1.8, the column is very efficient. The unsatisfactory results should be analyzed and the column should be repacked .

3.3 Chromatographic method

- Sample
  - ➢ To avoid clogging the chromatography column, the sample solution needs to be centrifuged or filtered with a 0.45μm filter before loading.
  - ➢ The viscosity of the sample needs to be appropriate. High viscosity samples will cause uneven flow velocity during the chromatography process and affect the binding efficiency.
- Binding buffer: Neutral buffer is generally used, such as 50mM Tris, 0.5M NaCl, pH 7.4.
- Flow velocity: It is recommended to use 30 ~ 300cm / h flow velocity.
- Sample preparation: Adjust the pH and conductivity of the sample to be consistent with the equilibrium buffer, and determine the sample volume according to the impurity content and flow velocity in the sample.
- Sampling: sample the prepared samples according to the set conditions.
- Rinse: Rinse with binding buffer until the UV absorption value drops to an appropriate value.
- Elution method 1: Usually lower the pH for elution, such as: 50mM glycine, pH3.0.
  The collected elution was immediately neutralized with 1M Tris, pH9, and 60 ~ 200μl of 1M Tris was needed for 1mL elution.
- Elution method 2：Add p-aminobenzidine for competitive elution, such as 50mM Tris, 0.5M NaCl, 20mM p-aminobenzidine, pH7.4.
- Regeneration：2CV of high pH buffer (0.1MTris-HCl, 0.5M NaCl, pH8.5) and low pH buffer (0.1M sodium acetate, 0.5M NaCl, pH3) were washed 3 times alternately； 10CV combined with buffer equilibrate the chromatography column.

4. Application

Application of Benzamidine Bestarose 4FF in purification of trypsin



Column：EzFast 5 mL
Sample：Chymotrypsin mixture
Balance fluid：50mM PB、500mM NaCl，pH 7.0
Elution buffer：0.1M Gly，pH 2.7
Note：Immediately after collecting the eluate, 60-200 μl of 1M Tris-HCl, pH 9.0 was added to each mL of eluate

5. 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).

- Precipitation or denaturation cleaning:
  Wash with 2CV of 6M guanidine hydrochloride and then with 5CV of equilibration buffer.
- Cleaning of hydrophobic binding substances:
  Wash with 2CV of 70% ethanol, then wash with 5CV of equilibration buffer.

6. Sterilization

Since the 20% ethanol with 50mM NaAc(pH4.0) or 2% benzyl alcohol with 50mM NaAc(pH 4.0) preservation solution does not have sterilization and depyrogenation,it is recommended that Benzamidine Bestarose 4FF resin can be treated with 20% ethanol containing 0.1M acetic acid for more than 12 hours before and during use to reduce the risk of microbial contamination.

7. Storage

Benzamidine Bestarose 4FF is supplied in 20% ethanol with 50mM NaAc(pH4.0) or 2% benzyl alcohol with 50mM NaAc(pH 4.0). It should be stored in 20% ethanol solution with 50mM NaAc(pH 4.0) 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.

8. Disposal and Recycling

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

9. Order information

Product	Code No.	Pack size
Benzamidine Bestarose 4FF	AA0291	25mL
	AA0292	100mL
	AA111311	500mL
	AA0294	1L
	AA0295	5L
	AA111314	10L

Prepacked columns	Code No.	Pack size
EzFast Benzamidine 4FF	EA111301	1×1mL
	EA111303	1×5mL
	EA111351	5×1mL
	EA111353	5×5mL
EzScreen Benzamidine 4FF	EA02925	1×4.9mL
	EA02935	5×4.9mL
EzLoad 16/10 Benzamidine 4FF	EA111304	1 pcs
EzLoad 26/10 Benzamidine 4FF	EA111306	1 pcs