BESTCHROM
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IMAC Bestarose HP Metal chelate chromatography resin

Instruction for use

Contents

1. Introduction

IMAC Bestarose HP (High Performance) is a high-resolution metal chelating affinity chromatography resin, combined with a variety of transition metal ions such as Cu^{2+}, Zn^{2+}, Ni^{2+}, Co^{2+}, Fe^{3+}, etc, which can be used to separate and purify proteins and peptides. The principle is to use the interaction between the side chains of histidine, cysteine and tryptophan of the protein and metal ions, so as to achieve the purpose of separation and purification.

IMAC Bestarose HP is formed by cross-linking agarose and nitrilotriacetic acid (NTA). It can chelate the four-valent position of metal ions, make the chelated metal ions more stable, as well as tolerate higher reducing agents. It has good chemical stability, good specificity and fast flow velocity.

2.Technical characteristics

Appearance	White slurry, can be layered
Matrix	Highly cross-linked agarose, 6%
Particle size +	24~44μm(the average particle size is 34μm)
Chelating ability	~15μmol Ni^{2+}/mL resin
Dynamic binding capacity	~ 40mg His tag protein/ mL packed resin(chelating nickel ion)
Chemical stability ++	1 week at 40℃: 10mM HCl, 0.1M NaOH, 8M urea, 6M GuHCl; 40℃ 12h: 1M NaOH, 70% acetic acid;
Max. pressure	0.3MPa
pH stability+++	3~12(working) 2~14(CIP)
Storage++++	2~30℃, 20% ethanol or 2% benzyl alcohol
Recommended flow velocity	＜150cm/h

+Particle size is normally distributed, and particles within this range account for more than 75% of the total.

++ Stability when removing metal ions

+++ CIP refers to pH stability when metal ions are removed

++++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(Due to the resin particles are fine, need to choose the aperture. 10um screen). 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 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):

  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

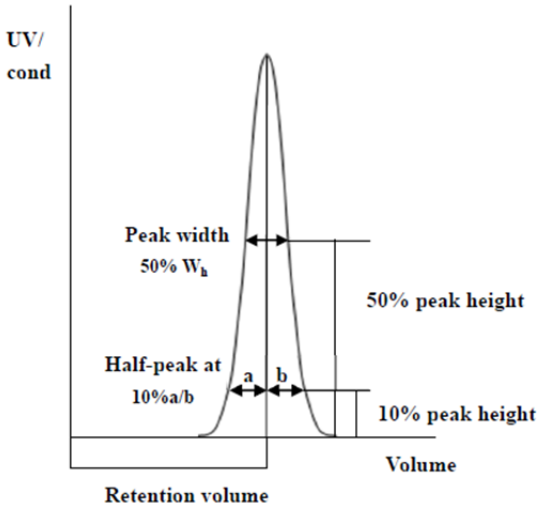
  (The units of V_R and W_h should be the same)

  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

- Chelate metal ions：
  - Rinse 2CV with purified water，
  - Pass the chromatography column with 0.5CV of 0.2 M metal ion solution
  - Use 5CV of purified water to remove unbound metal ions
  - Wash the chromatography column with 5CV of elution buffer
  - Equilibrate the chromatographic column with equilibration buffer and wait for use.

  The general metal ion environment is neutral (pH7-8). Zinc ion selects pH≤5.5 to avoid the solubility of high pH salt; iron ion selects pH≈3 to avoid the formation of insoluble matter.
- Buffer: Preferred phosphate buffer, pH range in neutral (7~8), avoid the use of EDTA and citrate, Tris-HCl can also be used, but should be avoided in the case of metal and protein affinity is very weak. Common additive reagents and concentrations that do not and affect metal chelation chromatography are listed in Table 1 and Table 2 respectively.

  In order to reduce the non-specific binding of the host protein to the resin, low concentrations of imidazole (20-40 mM) are usually added to the equilibration buffer and the sample.

  NaCl of 0.15 ~ 0.5M must be added to the buffer solution to eliminate ion exchange.

Table 1 Additives that do not affect protein binding to immobilized metal ion affinity resin

Additives	Concentration	Additives	Concentration
Phosphate、Tris、Borate、HEPES	20-100mmol/L	Non-ionic detergent	2%
NaCl	2mol/L	Triton X-100	2%
KCl	1mol/L	Tween-20	2%
Guanidine hydrochloride	6mol/L	Octyl glucoside	2%
Urea	8mol/L	Dodecyl maltoside	2%
glycerin	50%	$C_{12}E_8$,$C_{10}E_6$	2%
Isopropanol	60%	PMSF (Protease inhibitor)	1mmol/L
Ethanol	30%	Pepsin inhibitor (Pepsin inhibitor)	1μmol/L
Amphoteric detergent (CHAPS)	1%	Leupeptin (Protease inhibitor)	0.5μg/mL
1% Benzamidine (Protease inhibitor)	1mmol/L	/	/

Table 2 Additives that may disrupt protein binding to the immobilized metal ion affinity resin

Additives	Concentration	Additives	Concentration
2-mercaptoethanol	20mmol/L	Histidine	Can be used instead of imidazole
Strong reducing agents (DTT and DTE)	0.1mmol/L	Glycine	—
Chelating agents (EDTA and EGTA)	0.1mmol/L，take Ni^{2+} from the resin	Glutamine	—
Ionic detergent (cholate, SDS)	—	Arginine	—
Sodium azide	3mmol/L	Ammonium chloride	—
Citrate	Can tolerate low concentrations	—	—

- 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 is determined according to the substance content in the sample and the binding load of IMAC Bestarose HP.
- Equilibrium: The pH, conductivity and UV of the chromatographic column are the same as that of the equilibrium liquid phase when the equilibrium buffer is cleaned to the effluent.

  In order to reduce the impact of metal ion shedding on chromatography, it is recommended to clean 0.5M imidazole containing 1M NaCl with 1CV before balancing, then clean 5CV with purified water, and finally balance the chromatography column with balancing buffer before loading samples.
- Sampling: Sample the prepared samples according to the set conditions.
- Elution:
  - Competitive elution: linear increase or one-step increase of substances with affinity for metal ions, such as 0-0.5M imidazole, 0-2M NH_4Cl, 0-0.5M histidine. Gradient elution is best performed at a constant pH in equilibration buffer.
  - The pH of the buffer can be lowered for elution. When the pH of the buffer is lower than 4, metal ions will dissociate with the resin to achieve the purpose of elution. (If the target protein is sensitive to low pH, it is recommended to add 1/10 volume of 1M Tris-HCl to the eluted collection solution, pH 9.0 for neutralization).

    EGTA or EDTA solution with a chelating agent of 0.05M can dissociate metal ions from the resin to achieve elution. This method can also be used for elution of denatured or precipitated proteins. This method is not recommended. The metal ions in the eluted products can be removed by a desalting column.
- Regeneration: Impurity residue and shedding of metal ions will affect the column's

chromatographic performance and loading capacity. It is recommended that metal ions be re-chelated after every one to five cycles according to production needs.

- Nickel was removed with 2~5CV of buffer solution (50mM PB, 0.5M NaCl, 0.1-0.2M EDTA, pH 7.0); Fe^{3+} is easy to form insoluble matter in neutral solution, so it is recommended to use 50mM EDTA overnight to remove metal ions.
- The residual EDTA was removed by 2~3CV of 0.5M NaCl passing through the column.
- 0.5CV of 0.2M metal ion solution was used to cross the chromatography column.
- Remove unbound metal ions with 5CV purified water;
- The chromatography column was cleaned with 5CV tric elution buffer;
- Balance the chromatography column with a balancing buffer and set aside.

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).

- First remove nickel ions;
- To remove the protein adsorbed by ion exchange: wash the column with 2M NaCl solution of 2-3 times the bed volume, and then wash the column with distilled water of 3 times the bed volume;
- Precipitated or denatured material: can be removed with 1M NaOH for 0.5-1h;
- Hydrophobic binding substance: 2CV 70% ethanol or 30% isopropanol to wash the column, immediately with at least 5CV of filter-sterilized equilibration buffer, reverse washing.

5. Sterilization

Since the 20% ethanol or 2% benzyl alcohol preservation solution does not have sterilization and depyrogenation, it is recommended that IMAC Bestarose HP can be treated with 70% ethanol for more than 12h or the resin after nickel removal can be treated with 1M NaOH for 0.5-1h to reduce the risk of microbial contamination before and during use.

6. Storage

IMAC Bestarose HP is supplied in 20% ethanol or 2% benzyl alcohol. It should be stored in 20% ethanol and sealed at 2-30℃ 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

IMAC Bestarose HP is very difficult to degrade in nature, incineration is recommended to protect the environment.

8. Order information

Product	Code No.	Pack size
IMAC Bestarose HP	AA207105	25mL
	AA207107	100mL
	AA207111	500mL
	AA207112	1L
	AA207113	5L
	AA207114	10L
	AA207115	20L

Prepacked columns	Code No.	Pack size
EzFast IMAC HP	EA01021	1×1mL
	EA01031	5×1mL
	EA01023	1×5mL
	EA01033	5×5mL
EzScreen IMAC HP	EA01025	1×4.9mL
	EA01035	5×4.9mL
EzLoad 16/10 IMAC HP	EA01001	1 pcs
EzLoad 26/10 IMAC HP	EA01011	1 pcs