BESTCHROM
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Ni Bestarose FF Metal chelate chromatography resin

Instruction for use

Contents

1. Introduction

Ni Bestarose FF (Fast Flow) metal chelation chromatography resin is an affinity chromatography resin that pre-chelates metal ion Ni^{2+} on agarose gel with NTA as ligand. It has the advantages of large adsorption capacity, good selection type, easy regeneration, and low cost. It is widely used in the separation and purification of downstream proteins and peptides in biopharmaceutical and bioengineering, especially the efficient purification of histidine-tagged proteins.

2. Technical characteristics

Appearance	Turquoise slurry, can be layered
Matrix	Highly Cross-linked agarose, 6%
Particle size+	45~165μm
Ligand concentration	12~18μmol Ligand/mL resin
Dynamic binding capacity	~ 40mg His tag protein/ mL packed resin
Chemical stability ++	40℃ One week : 10mM HCl, 0.1M NaOH, 6M GuHCl,8M Urea. 40℃ 12h: 1M NaOH, 70% acetic acid.
pH stability+++	3~12 (working) 2~14 (CIP)
Pressure flow velocity	~600cm/h,BXK16/20,H=5cm,25℃
Max. pressure	0.3MPa
Storage++++	2~30℃, 20% ethanol or 2% benzyl alcohol
Recommend flow velocity	60~300cm/h

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

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 precipitated resin required, the resin slurry required is calculated by the follow:

  Required resin slurry[1] volume = Precipitated 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 resins in non-original concentration, 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 flow velocity can be set to 75cm/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 260cm/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
Detection	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

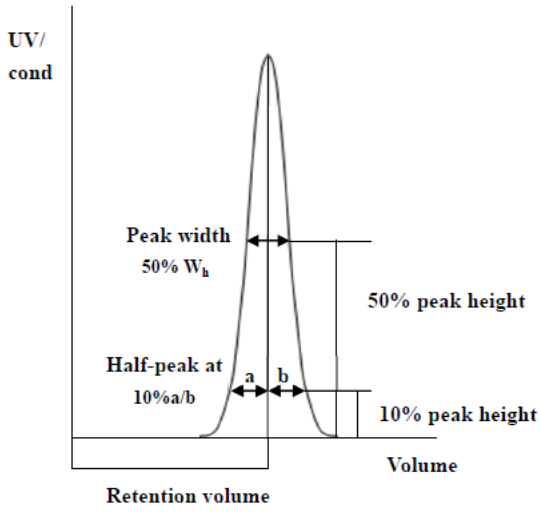
  (The units of V_R and W_h should be the same)

  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

- Buffer: Preferred phosphate buffer, pH neutral to slightly alkaline (7~8), avoid using EDTA and citrate, Tris-HCl can also be used, but should be avoided in the case of metal ions 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 (20-40mM) 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 by the substance content in the sample and the binding capacity of Ni Bestarose FF.
- Equilibration: Wash the column with equilibration buffer till the pH, conductivity and UV of flow-out reach the same as that of equilibration buffer.

  In order to reduce the impact of metal ion stripping on chromatography, it is recommended to clean with 1CV 0.5M imidazole +1M NaCl with before equilibration, then clean with 5CV of purified water, and finally equilibrate the chromatography column with equilibration buffer before loading samples.
- Loading sample: The loading volume is determined by the substance content in the sample and the binding capacity of Ni Bestarose FF.
- Cleaning: Wash the column with equilibration buffer until the UV absorption value is close to baseline.
- Elution:

➢ Competitive elution: Linear increase or gradually increase concentration of substances with affinity for metal ions, such as 0-0.5M imidazole, 0-0.5M histidine, 0-2M NH_4Cl. Gradient elution is best performed at a constant pH in equilibration buffer.

➢ The pH of the buffer can be lowered for elution. As the decrease of pH, proteins with different binding will be eluted in sequence. 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).

0.05M Chelating agents EGTA and EDTA can dissociate metal ions from the resin to achieve the purpose of elution. Ni^{2+} in the eluted product can be removed by desalting column. The resin can be used after saturated with 0.2M $NiSO_4$ again.

- 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 1-5 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).

➢ The residual EDTA was removed by 2~3CV of 0.5M NaCl passing through the column.

➢ 0.5CV 0.2M $NiSO_4$ chromatographic column.

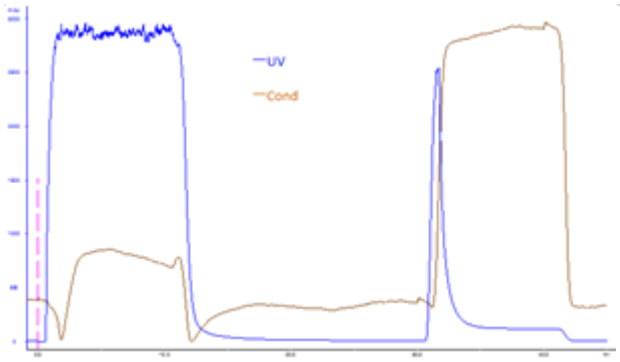
➢ Remove unbound metal ions with 5CV purified water.

➢ The chromatography column was cleaned with 5CV etric elution buffer.

➢ Equilibrate the chromatography column with a equilibration buffer and set aside.

4. Application

Application of Ni Bestarose FF in the purification of HIS-GST labeled recombinant protein



Column：EzFast 1mL
Bed height：2.5cm
Buffer solution：A: 25mM imidazole + 0.15m NaCl pH: 7.00
B: 500mM imidazole pH: 7.00
Sample：E. coli expression was recombinant with His-GST tag
Loading volume of sample：Supernatant of protein lysate 10mL

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).

The recommended CIP for different types of impurities and contaminants are as follows:

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

6. Sterilization

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

7. Storage

Ni Bestarose FF 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.

8. Disposal and recycling

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

9. Order information

Product	Cat. No.	Pack size
Ni Bestarose FF	AA0051	25mL
	AA0052	100mL
	AA0053	500mL
	AA0054	1L
	AA0055	5L
	AA0056	10L
	AA208315	20L

Prepacked columns	Cat. No.	Pack size
EzFast Ni FF	EA208301	1×1mL
	EA208303	1×5mL
	EA006	5×1mL
	EA007	5×5mL
EzScreen Ni FF	EA00525	1×4.6mL
	EA00535	5×4.6mL
EzLoad 16/10 Ni FF	EA208304	1 pcs
EzLoad 26/10 Ni FF	EA208306	1 pcs