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**Ni Bestarose HP
Metal chelate
chromatography resin
Instruction for use**



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1. Introduction

Ni Bestarose HP(High Performance)metal chelation chromatography resin is an affinity chromatography resin made by pre-chelating metal ion Ni²⁺ on a high-resolution agarose gel with NTA as ligand. It has the advantages of high resolution, large adsorption capacity, good selectivity, 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	Cross-linked agarose, 6%
Particle size ⁺	24~44μm(the average particle size is 34μm)
Ligand concentration	~15μmol Ligand/mL resin
Dynamic binding capacity	~ 40mg His tag protein/mL packed resin
Chemical stability ⁺⁺	1 week at 40°C: 10mM HCl, 0.1M NaOH, 8M urea, 6M GuHCl; 40°C 12h: 1M NaOH, 70% acetic acid
Max. pressure	0.3MPa
pH stability ⁺⁺⁺	3~12(working) 2~14(CIP)
Storage ⁺⁺⁺⁺	2~30°C, 20% ethanol or 2% benzyl alcohol
Recommended flow velocity	<150cm/h

+Particle size is the cumulative resin particle size of packing volume distribution

++ 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 slurry¹ volume = Settlement resin volume ÷ Resin slurry¹ concentration. The

original concentration of resin slurry¹ 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 B XK column (B XK series columns with diameters ranging from 1cm to 30cm can satisfy different scale chromatography applications). Take B XK16/20 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 B XK 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 adaptor and connect the adaptor to the chromatography system or peristaltic pump. Lower the adaptor 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 adaptor down until all bubbles are drained.

Note: This operation is only applicable to B XK 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)

$$As=b/a$$

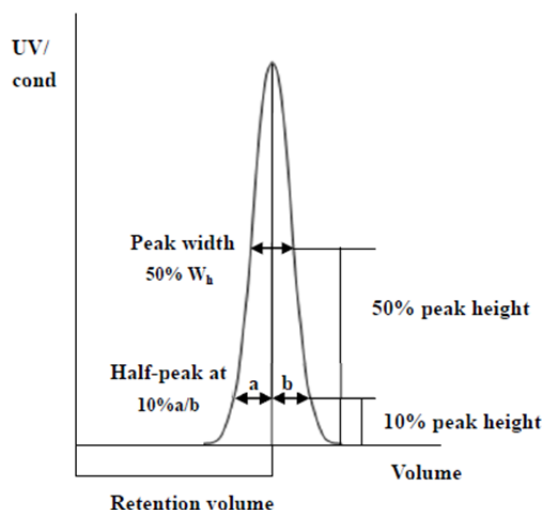
Note:

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

- Buffer: Phosphate buffer is preferred, the pH range is neutral (between 7-8), avoid using EDTA and citrate, etc. Tables 1 and 2 list common additives and concentrations that do not affect or affect metal chelation chromatography.

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 adsorption.

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 Ni Bestarose HP.
- Equilibrium: The equilibrium buffer is used to clean the chromatography column until the pH, conductivity and UV of the effluent are the same as that of the equilibrium liquid phase.

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: The loading volume is determined according to the substance content in the sample and the binding load of Ni Bestarose HP.
- Rinse: Wash the column with equilibration buffer until the UV absorption value is reduced to an appropriate value.
- Elution:
 - Competitive elution: Linear increase or one-step increase of substances with affinity for metal ions, such as 0-0.5M imidazole, 0-0.5M histidine, 0-2M NH₄Cl. 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)

Chelating agents EGTA and EDTA can dissociate metal ions from the resin to achieve the purpose of elution. Ni²⁺ in the eluted product can be removed by desalting column. The resin can be used after saturated with 0.2M NiSO₄ 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 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);
 - The residual EDTA was removed by 2~3CV of 0.5M NaCl passing through the column.
 - 0.5CV 0.2M NiSO₄ chromatographic 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).

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 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 do reverse washing with at least 5CV of filter-sterilized equilibration buffer.

5. Sterilization

Since the 20% ethanol or 2% benzyl alcohol preservation solution does not have sterilization and depyrogenation, it is recommended that Ni Bestarose HP 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.

6. Storage

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

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

8. Order information

Product	Code No.	Pack size
Ni Bestarose HP	AA0041	25mL
	AA0042	100mL
	AA0043	500mL
	AA0044	1L
	AA0045	5L
	AA0046	10L
	AA208115	20L

Prepacked columns	Code No.	Pack size
EzFast Ni HP	EA208101	1×1mL
	EA208103	1×5mL
	EA004	5×1mL
	EA005	5×5mL
EzScreen Ni HP	EA00425	1×4.9mL
	EA00435	5×4.9mL
EzLoad 16/10 Ni HP	EA208104	1 pcs
EzLoad 26/10 Ni HP	EA208106	1 pcs
EzLoad 26/20 Ni HP(Double adaptor)	EA00414	1 pcs