TA-Chelating HP

Metal Chelate Affinity Chromatography Resin

Product Manual

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Content

1. Product Introduction

TA-Chelating HP is an immobilized metal affinity chromatography medium, which is made by covalently cross-linking ligand iminodiacetic acid (IDA) into TA-HP matrix. The separation of target proteins is accomplished based on the interaction of side-chain histidine, cysteine and tryptophan with transition metal ions (Cu^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+}, etc.) immobilized on the medium.

The ligand of TA-Chelating HP media can provide three ligand sites to chelate with metal ions, and at the same time provide three ionic bonding sites to purify the target proteins with high affinity, while the same type of TA-IMAC HP media provides four ligand sites to chelate with metal ions and two ionic bonding sites to purify the target proteins, that is to say, under the same density of ligands and the same metal ions condition. That is to say, under the same ligand density and the same metal ion conditions, the affinity of TA-Chelating HP medium is stronger than that of TA-IMAC HP, so samples that cannot be adsorbed by TA-IMAC HP medium can be selected to be bound by TA-Chelating HP, but at the same time, because of the additional metal ion chelating site, TA-IMAC HP medium has a stronger strength to bind metal ions, and it can also be compatible with the reducing agents DTT and β-mercaptoethanol, therefore, it is compatible with DTT and β-mercaptoethanol. mercaptoethanol, therefore, the medium should be selected according to the protein characteristics when purifying recombinant histidine-tagged proteins.

Table 1 TA-Chelating HP technical parameters

Appearance	White slurry, layered on placement
Base frame	Highly cross-linked 6% agarose
Particle Size Distribution Range	34μm (24~44μm)
Metal chelating capacity	Cu2+: ~34μmol/mL medium
Working pH	4~8.5
Chemical stability (in the absence of chelated metal ions)	Stable in common aqueous solutions, 8M urea, 6M guanidine hydrochloride
Maximum Pressure Resistance	0.3MPa
pH Stability	3-13 (working); 2-14 (CIP, when removing metal ions)
Temperature Tolerance	Use temperature 4~40℃, can not be frozen, 121℃ autoclave (add 0.1M NaAc)
Recommended Flow Rate	60~150cm/h

2. Methods of use

2.1 Chromatography column loading

Note: It is best to equilibrate the media suspension to room temperature before loading the column.

- Calculate the amount of TA-Chelating HP needed based on the volume of the chromatography columns

  Settling volume required = column volume x 1.15 (i.e., compression ratio of approximately 1.15)

  Volume of media suspension required = volume of settling media ÷ concentration of media suspension.

Note: The concentration of Truking Microsphere's original packaging media suspension is 80%, for non-original concentration of media suspension, customers can calculate the required volume according to the actual concentration of media suspension.

- Media washing:Shake the media suspension well and measure the volume calculated by the above method, pour it into a funnel, draw off the liquid and wash it with about 3mL of purified water/mL of media, repeat the washing 3 times, each time when adding the washing liquid, you need to use a glass rod or stirring stick to stir, in order to wash off the original preservation liquid better.
- Preparation of Column Mounting Gel Suspension: Transfer the cleaned medium from the funnel to a beaker or other suitable container, add the column mounting solution until the concentration of the gel suspension is 50~75%, stir well and set aside.
- Take a cleaned TK-EC chromatography column (the diameter of TK-EC series chromatography columns ranges from 1cm to 45cm in various specifications to meet the different sizes of chromatography applications), drain the membrane air bubbles at the bottom of the column and keep about 1cm high liquid column at the bottom of the column, and adjust the column so that it is perpendicular to the ground.
- Pour the stirred gel suspension slowly into the chromatography column one at a time, taking care not to bring in air bubbles.
- Connect the upper column head to the chromatography system or peristaltic pump, drain the air bubbles under the screen of the upper column head (for the chromatography column with diameter less than 20cm, you can use the peristaltic pump or the earwash ball to suck out the air bubbles under the screen after turning the column head upwards), put the column head into the chromatography column, shake the column head so that the air bubbles can be discharged from the edge of the column head, and then screw the sealing knob tightly. (For the chromatography column with diameter >30cm, do not tighten the sealing ring too much, press down the head of the shaft to let the liquid inside the column back out through the column head to discharge the air bubbles inside the column head, and then tighten the sealing knob).
- Set the flow rate (60cm/h for TA-Chelating HP when the column height is 15-20cm), open the bottom valve/plug of the column, turn on the flow rate, and press the column with the set flow rate until the gel surface is clear and stable, and mark the position of the gel surface when it is stable.
- Remove the column loader (if any), install the upper column head, lower the column head to about 0.5cm above the gel surface, set the flow rate to 240cm/h and continue to press the column until the gel surface is clear and stable, mark the column height when the gel surface is stable.
- Stop the pump, open the valve/plug on the column head, close the valve/plug at the bottom of the column, slightly relax the sealing ring of the column head, press the column head down to about 0.3cm below the marked position, tighten the sealing ring of the column head, close the valve/plug of the column head, and the column loading is completed.

2.2 Column Effectiveness Evaluation

Column efficiency can be determined by using acetone as indicator or NaCl as indicator, and the indicator solution and mobile phase are prepared according to the following table.

Table 2: Column efficiency determination methods

Methods	Acetone Method for Column Efficacy	Column Efficacy by NaCl Method
Sample	1.0% (v/v) acetone in water	0.8M NaCl (dissolved in water)
Sample volume	1.0% column volume	1.0% column volume
Mobile phase	Water	0.4M NaCl aqueous solution
Flow rate	30 cm/h	30 cm/h
Detection Data	UV 280 nm	Conductivity

2.3 Calculating Column Effect

Theoretical plate height (HETP), theoretical number of plates (N) and asymmetry factor (As) were calculated from the UV or conductivity curves with the following equations:

HETP=L/N

$N=5.54(V_R/W_h)^2$

Where: V_R = retained volume

W_h=half peak width

L=column height

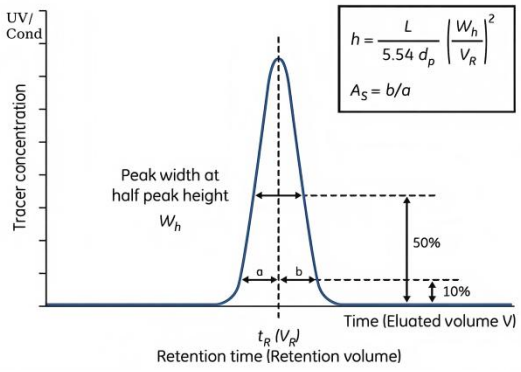
N=theoretical plate number

The units of V_R and W_h should be the same;

As=b/a

Where: a= first half peak width at 10% peak height

b= second half peak width at 10% peak height



2.4 Evaluation of results

$h=HETP/d_{50v}$

d_{50v} = median particle size volume distribution (cm)

The h-value calculated by the above formula is less than 3, and the asymmetry factor is 0.8~1.8 then it is judged to be qualified. For unsatisfactory column efficiency the reason needs to be analyzed and the column reloaded.

2.5 Chromatographic methods

- **Chelate metal ions:**

➢ Flush 5 column volumes with purified water;

➢ Rrinse 5 column volumes with equilibration buffer;

➢ Rinse 5 column volumes with purified water;

➢ Pass the column through 5 column volumes of 100 mM metal ion solution;

➢ Remove unbound metal ions with 10 column volumes of equilibration buffer;

➢ Rinse 10 column volumes with purified water;

➢ Wash the column with 10 column volumes of elution buffer;

➢ Equilibrate the column with Equilibration Buffer and set aside.

The general environment for metal ions is neutral (pH 7~8). Choose pH ≤ 5.5 for zinc ions to avoid solubility of high pH salts and pH ≈ 3 for iron ions to avoid formation of insoluble species.

- **Buffer:** preferred phosphate buffer, pH neutral to weakly alkaline (7~8), avoid EDTA and citrate, etc. Tris-HCl can also be used, but should be avoided in the case of metal ions and protein affinity is very weak.Commonly added reagents and concentrations that do not affect and affect metal chelation chromatography are listed in Tables 3 and 4, respectively.

➢ *In order to reduce the nonspecific binding of host proteins to the medium, low concentrations of imidazole (20-40 mM) are commonly added to equilibration buffers and samples.*

➢ *0.15~0.5M NaCl must be added to the buffer to eliminate ion exchange.*

Table 3 Additions that do not affect protein binding to immobilized metal ion affinity media

Additives	Common Concentration	Additives	Common Concentration
Phosphate, Borate, HEPES	20-100mmol/L	**Nonionic Stain Remover**	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%
Glycerol	50%	**C12E8 ,C10E6**	2%
Isopropyl alcohol	60%	**PMSF(Protease Inhibitor)**	1mmol/L
Ethanol	30%	**Pepsin Inhibitor(Protease Inhibitor)**	1μmol/L
Amphoteric decontaminants (CHAPS)	1%	**Leucineurin(Protease inhibitor)**	0.5μg/mL
Benzamidine 1% (protease inhibitor)	1mmol/L	/	/

Table 4 Additives that have the potential to disrupt protein binding to immobilized metal ion affinity media

Additives	Common Concentration	Additives	Common Concentration
2-Mercaptoethanol	20mmol/L	**Histidine**	Can be used to replace imidazole
Strong reducing agents (DTT and DTE)	0.1mmol/L	**Glycine**	—
Chelating agent (EDTA and EGTA)	0.1 mmol/L, competition for Ni^{2+} from the medium	**Glutamine**	—
Ionic decontaminants (cholate, SDS)	—	**Arginine**	—
Sodium azide	3mmol/L	**Ammonium chloride**	—
Citrate	Tolerates low concentrations	**—**	—

- **Flow rate:** for the height of the column in the case of 10~20cm high can choose <150cm/h flow rate, column height increases need to reduce the flow rate appropriately.
- **Sample Preparation and Sample Volume:** To prevent the sample from clogging the column, the sample needs to be filtered through a 0.45 μm microporous filter membrane and the pH and conductivity of the sample adjusted to be consistent with the equilibration buffer prior to sampling. Factors such as equilibrium solution and imidazole concentration affect the sample volume of TA-Chelating FF.
- **Equilibration:** Wash the column with Equilibration Buffer until the pH, conductivity, and UV of the effluent are the same as the equilibration solution.To minimize the effect of metal ion shedding on the chromatography, it is recommended that the column be washed with 1 column volume of 0.5M imidazole containing 1M NaCl prior to equilibration, followed by 5 column volumes of purified water, and then finally equilibrate the column with equilibration buffer before sampling.
- **Sampling:** The volume of the sample is determined according to the content of the substance in the sample and the binding capacity of TA-Chelating HP, and the sample is loaded.
- **Wash:** Wash the chromatography column with equilibration buffer until the UV absorption is close to the baseline.
- **Elution:**

➢ Competitive elution: linear or gradual increase in the concentration of substances with affinity for the metal ion, e.g., 0~2M NH4Cl, 0~0.5M imidazole, 0~0.5M histidine.Gradient elution is best performed at a constant pH of the equilibration buffer.

➢ Can lower the pH of the buffer for elution: as the pH is lowered, weakly bound and strongly bound proteins are eluted sequentially.When the pH of the buffer is lowered below 4, the metal ions dissociate from the medium and are eluted.(If the target protein is sensitive to low pH, it is recommended to add 1/10 volume of 1M Tris-HCl, pH 9.0, to the elution collection solution for neutralization).

➢ *A 0.05M solution of the chelating agent EGTA or EDTA can dissociate the metal ions from the medium for elution purposes, and this method can also be used to elute denatured or precipitated proteins.This method is generally not recommended. Ni^{2+} in the eluted product can be removed with a desalting column. The medium can be re-saturated with 0.2M NiSO4.*

- **Regeneration:** Impurity residues and metal ion shedding will affect the chromatographic performance and loading of the column.It is recommended to re-chelate the metal ions after every 1~5 cycles depending on the production needs.

➢ Nickel removal with 2~5 column volumes of nickel removal buffer (50mM PB, 0.5M NaCl, 0.1~0.2M EDTA, pH 7.0);
➢ Run with 2~3 column volumes of 0.5M NaCl to remove residual EDTA;
➢ Treate with 0.5 column volume of 0.2M NiSO4;
➢ Unbound metal ions were removed with 5 column volumes of purified water;
➢ The column was washed with 5 column volumes of elution buffer;
➢ Equilibrate the column with equilibration buffer and set aside.

3. Cleaning and regeneration

As the number of times the chromatography medium is used increases, the accumulation of contaminants on the chromatography column also increases. Regular in-situ cleaning can effectively prevent the accumulation of contaminants and maintain the stable working condition of the chromatography medium.Customers can determine the frequency of in-situ cleaning according to the degree of contamination of the media during use (if the contamination is more serious, it is recommended to carry out in-situ cleaning after each use to ensure the reproducibility of the results).

➢ Nickel ions were removed first.
➢ Removal of proteins adsorbed due to ion exchange: Wash the column with 2~3 column volumes of 2M NaCl solution, and then wash the column with 3 times the volume of purified water in the column bed.
➢ Removal of precipitated or denatured substances: Treat with 1M NaOH for 0.5~1h.
➢ Removal of hydrophobically bound substances: Wash the column with 2 column volumes of 70% ethanol or 30% isopropanol, and immediately reverse with at least 5 column volumes of sterile equilibration buffer.

4. Sterilization and storage

Since 20% ethanol or 2% benzyl alcohol preservation solution does not have sterilizing and de-pyrogenic effects, it is recommended that TA-Chelating HP media can be treated with 70% ethanol for more than 12h before and during use, or media after metal ion removal can be treated with 1M NaOH for 0.5~1h in order to reduce the risk of microbial contamination.

TA-Chelating HP media are sold with 20% ethanol or 2% benzyl alcohol as preservation solution. After use, TA-Chelating HP should be stored in 20% ethanol at 2~30°C in airtight storage. In order to prevent the volatilization of ethanol and the growth of microorganisms, it is recommended that the preservation solution be replaced with a fresh one every 3 months.

5. Destruction and recycling

Since TA-Chelating HP is difficult to degrade in nature, incineration is recommended for environmental protection.

6. Ordering Information

Table 5 Article number and packaging

Product	Item No.	Norm
TA-Chelating HP	Y5387	25mL
	Y5388	100mL
	Y5389	500mL
	Y5390	1L
	Y5391	5L
	Y5392	10L
	Y5393	20L