TK-Col Ni HP（NTA）

TK-Col 16/10 Ni HP（NTA）

TK-Col 26/10 Ni HP（NTA）

Affinity Chromatography Prepacked Columns

Product Manual

Content

1. Product Introduction

TA-Ni HP (High Performance) metal chelating chromatography medium is a kind of affinity chromatography medium made by pre-chelating metal ion Ni2+ on high resolution agarose gel with NTA as the ligand, which has the advantages of high resolution, high adsorption capacity, good selectivity, easy regeneration, low cost, etc. It is widely used in the downstream protein and peptide separation and purification of bio-pharmaceuticals and bio-engineering, especially the high efficiency purification of histidine-tagged proteins. It is widely used for the purification of proteins and peptides downstream of biopharmaceutical and bioengineering, especially for the efficient purification of histidine-tagged proteins

TK-Col Ni HP pre-packed columns are ready-to-use affinity chromatography columns packed with TA-Ni HP medium in TK-EC 1ml, TK-EC 4.9 ml, TK-EC 5ml, TK-EC 20ml columns; TK-Col 16/10 Ni HP pre-packed columns are ready-to-use affinity chromatography columns packed with TA-Ni HP medium in TK-EC 16/20 columns; TK-Col 16/10 Ni HP pre-packed columns are ready-to-use affinity chromatography columns packed with TA-Ni HP medium in TK-EC 16/20 columns. TK-Col 16/10 Ni HP preloaded columns are ready-to-use affinity columns filled with TA-Ni HP media in TK-EC 26/20 columns. This series of columns eliminates the hassle of loading the columns by the customer and the risk of poor column performance. This type of preloaded columns is widely used in laboratory process development, small amount preparation of samples, and is suitable for the separation and purification of biomolecules such as recombinant proteins with His tags. It has the following features:

- Ready-to-use
- Volume stabilization of the column bed
- Good physical and chemical resistance

2. Technical parameters

Table 1 TA-Ni HP (NTA) Technical Parameters

Appearance	Blue-green slurry, layered on placement
Base Frame	Highly cross-linked 6% agarose
Average particle size	34μm (24~44μm)
Matrix Density	~ 15μmol/mL media
Dynamic Binding Load	~ 40mg His-tagged protein/mL media
Chemical stability (when metal ions are removed)	40℃ 1 week: 10mM HCL, 0.1M NaOH, 8M urea, 6M guanidine hydrochloride; 40℃ 12h: 1M NaOH, 70% acetic acid;
Maximum Pressure Resistance	0.3MPa
pH stability	3-12 (working); 2-14 (CIP, when removing metal ions)
Recommended Flow Rate	<150cm/h
Storage	2~30℃, 20% ethanol or 2% benzyl alcohol

Table 2: Technical parameters for each pre-assembled column (see end page for item number)

Product name	Prepacked resin	Prepacked column volume ml	Inner diameter× Column bed height mm×mm	Recommended flow rate + ml/min	Storage	Pressure resistance	Sieve plate aperture (μm)
TK-Col Ni HP（NTA）	**TA-Ni HP(NTA)**	1	7×25	<1	2-8°C, 20% ethanol or 2% benzyl alcohol (for international shipments).	0.3MPa（3bar）	10
		4.9	8×100	<3			
		5	16×25	<5			
		20	16×100	<5			
TK-Col 16/10 Ni HP（NTA）		19.1-21.1	16×100（±5）	<5			
TK-Col 26/10 Ni HP（NTA）		50.4-55.7	26×100（±5）	<13			

3. Methods of use

- *TK-Col 16&26 series chromatography columns are made of glass and should be handled gently to prevent breaking or affecting the column efficiency.*
- *To avoid clogging the column, all samples and buffers need to be filtered through 0.45um membrane.*
- *In order to get a good separation effect, avoid too much temperature difference between the buffer and the column.*
- *Keep the column out of direct sunlight.*
- *Chromatography columns can be used in a chromatography cooler, but the flow rate needs to be reduced appropriately.*

3.1 Connecting the column to the chromatography system

- Open the package and take out the column
- Check whether the column is intact, and whether the column has been dried out during transportation, if any of the above situations occurs, please contact Chutian Microsphere sales representative in time.
- Fix the column next to the chromatography system and pay attention to the flow direction of the column.
- Start the chromatography system, make sure the air bubbles in the chromatography system are drained, and set the alarm pressure of chromatography system to 0.3MPa, then adjust and keep the flow rate running at 0.2ml/min.
- After the chromatography system is purged of air bubbles, open the upper and lower plugs of the chromatography column and connect the chromatography column under low flow rate operation.

3.2 Pretreatment of chromatography columns

- Rinse, the chromatography column is stored in 20% ethanol or 2% benzyl alcohol (for international transportation) during transportation, first rinse off the storage solution with 2 column volumes of distilled water.
- Sterilization, for sample safety, it is recommended to rinse 2 column volumes with 0.5M NaOH before rinsing 2 column volumes with distilled water for the first use.

3.3 Equilibration of Chromatographic Columns

- Buffer selection: buffers: buffers applicable to the His tag purification process are preferred to be phosphate buffers with a neutral pH range (between 7-8), avoiding the application of EDTA and citrate, etc.Tables 3 and 4 Effect of commonly added reagents on proteins.
- The equilibration buffer needs to contain a low concentration of imidazole (20-40 mM), which reduces the nonspecific binding of host proteins to the medium, and the same concentration of imidazole should be added to the sample.
- The buffer must contain 0.15 to 0.5 M NaCl to eliminate ion exchange.
- Use the recommended flow rate to flush the chromatographic column with equilibration buffer. The pH and conductivity of the buffer to be exported are the same as those of the buffer before entering the column that means the column is well equilibrated, which generally requires 2~5 column volumes.

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	**—**	—

3.4 Flow rate

- According to the type of chromatographic column, generally choose the flow rate within the recommended flow rate range, for the height of the column in the 10~15cm high case can choose <150cm/h flow rate, the higher the column height the slower the flow rate. (See Table 2)

3.5 Sampling

- Sample and sample volume: The pH and conductivity of the sample need to be adjusted to be consistent with the binding buffer, and in order to prevent the sample from clogging the column, the sample needs to be filtered with a 0.45 μm microporous filtration membrane before sampling, and the volume of the sample is determined according to the impurity content in the sample and the binding loading of the medium.

3.6 Rinse

- Rinse with equilibration buffer until the UV absorption value drops to the appropriate value.

3.7 Eluent

- Competitive elution: substances with affinity for metal ions can be added linearly or in one step, e.g. 0-0.5 M imidazole and 0-2 M NH4Cl. Gradient elution is best performed at a constant pH of the equilibration buffer.
- The pH of the buffer can be lowered by for elution. When the pH of the buffer is lowered below 4, the metal ions will dissociate from the medium and thus achieve elution. (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.) The 0.05M chelating agents EGTA and EDTA can dissociate the metal ions from the medium to achieve the purpose of elution, and Ni2+ in the eluted product can be removed by desalting column. The medium can be used after saturated with 0.1M NiSO4 again.

3.8 Regeneration and rebalancing

Regeneration: Impurity residues and shedding of metal ions will affect the chromatographic performance and loading of the column. Rechelation of metal ions is recommended after every 1-5 cycles, depending on production needs.

Nickel removal with 2-5 column volumes of nickel removal buffer (50 mM PB, 0.5 M NaCl, 0.1-0.2 M EDTA, pH 7.0);

Remove residual EDTA by passing the column with 2-3 column volumes of 0.5 M NaCl;

The chromatography column was passed with 0.5 column volume of 0.2 M NiSO4;

Remove unbound metal ions with 5 column volumes of purified water;

Wash the column with 5x elution buffer;

Equilibrate the column with equilibration buffer and set aside.

3.9 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 4: 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

3.10 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

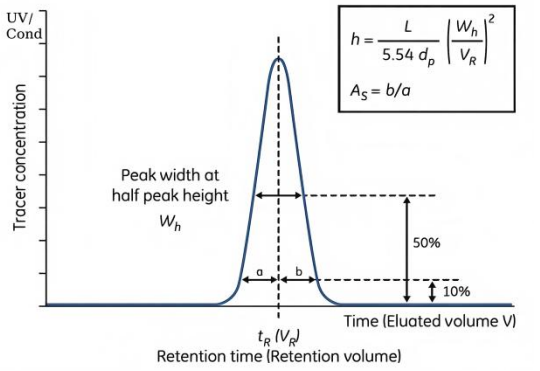
N=theoretical plate number

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



3.11 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.

4. 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).

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

5. 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-Ni HP(NTA) media can be treated with 70% ethanol for more than 12h prior to and during use, or the media after nickel removal can be treated with 1M NaOH for 0.5~1h to reduce the risk of microbial contamination.

TA-Ni HP(NTA) is sold with 20% ethanol or 2% benzyl alcohol as preservation solution. After use, TA-Ni HP(NTA) is stored in 20% ethanol at 2~30°C in an airtight container. It is recommended that the preservation solution be replaced with fresh preservation solution every 3 months in order to prevent ethanol evaporation and microbial growth.

6. Destruction and recycling

Since the packing material in the TK-Col Ni HP(NTA) series of pre-packed columns is difficult to degrade in nature, incineration is recommended for environmental protection.

7. Ordering Information

Table 5 Article number and packaging

Product	Item No.	Norm
TK-Col Ni HP（NTA）	Y6213	1×1ml
	Y6214	5×1ml
	Y6215	1×5ml
	Y6216	5×5ml
	Y621603	1×4.9ml
	Y621604	1×20ml
TK-Col 16/10 Ni HP（NTA）	Y6219	1pac.
TK-Col 26/10 Ni HP（NTA）	Y6220	1pac.