TK-Col IMAC FF

TK-Col 16/10 IMAC FF

TK-Col 26/10 IMAC FF

Affinity Chromatography Prepacked Columns

Product Manual

Content

1. Product Introduction

TA-IMAC FF is a metal chelating affinity chromatography medium that can be widely used for the separation and purification of proteins and peptides. The principle of TA-IMAC FF is to utilize the interaction of histidine, cysteine and tryptophan side chains of proteins with various transition metal ions, such as Cu^{2+}, Zn^{2+}, Ni^{2+}, Co^{2+}, Fe^{3+}, so as to achieve the purpose of separation and purification.

TA-IMAC FF is made of crosslinked agarose and ammonia triacetic acid (NTA), which can chelate the tetravalent level of metal ions, making the chelated metal ions more stable, tolerating a certain concentration of reducing agent, possessing the advantages of good physical and chemical stability, good specificity, and fast flow rate.

TK-Col IMAC FF preloaded columns are ready-to-use affinity chromatography columns filled with TA-IMAC FF medium in TK-EC 1ml ,TK-EC 4.9 ml ,TK-EC 5ml ,TK-EC 20ml chromatography vacutainer columns. TK-Col 16/10 IMAC FF preloaded columns are ready-to-use affinity columns filled with TA-IMAC FF media in TK-EC 16/20 chromatography vacutainers; TK-Col 26/10 IMAC FF preloaded columns are ready-to-use affinity columns filled with TA-IMAC FF media in TK-EC 26/20 chromatography vacutainers. 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-IMAC FF technical parameters

Appearance	White slurry, layered on placement
Base frame	Highly cross-linked 6% agarose
Particle Size Distribution Range	45-165μm
Metal chelating capacity	Ni^{2+}/Zn^{2+}: 15μmol/mL media, Cu^{2+}: 25μmol/mL media
Dynamic Binding Load	Ni^{2+}: ~40mg His-tagged protein/mL media
Chemical stability (in the absence of chelated metal ions)	40°C 1 week: 10mM HCl, 0.1M NaOH, 8M urea, 6M guanidine hydrochloride; 40°C 12h: 1M NaOH, 70% acetic acid;
Storage	2~30°C, 20% ethanol or 2% benzyl alcohol
Maximum Pressure Resistance	0.3MPa
pH stability	3-12 (working); 2-14 (CIP, when no chelated metal ions)
Recommended Flow Rate	<150cm/h

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 IMAC FF	TA-IMAC FF	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 IMAC FF		19.1-21.1	16×100（±5）	<5			
TK-Col 26/10 IMAC FF		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

- **Chelates metal ions:**
  - Rinsing 2 column volumes with purified water.
  - Over the chromatography column with 0.5 column volume of 0.2 M metal ion solution;
  - Remove unbound metal ions with 5 column volumes of purified water;
  - Washing the chromatography column with 5 column volumes of elution buffer;
  - Equilibrate the chromatography column with equilibration buffer and set aside.

The general metal ion environment is neutral (pH 7-8). Zinc ions are selected for pH ≤ 5.5 to avoid solubility of high pH salts, and iron ions are selected for pH ≈ 3 to avoid formation of insolubles

- Buffer selection: the buffer applicable to the His tag purification process is preferred to be phosphate buffer 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	—	**Arginine**	—

(cholate, SDS)			
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.

Use 2-5 column volumes of solution solution (50 mM PB, 0.5 M NaCl, 0.1-0.2 M EDTA, pH 7.0) to remove metal ions; Fe3+ tends to form insoluble matter in neutral solution, so overnight removal of metal ions with 50 mM EDTA is recommended. Remove residual EDTA by passing 2-3 column volumes of 0.5 M NaCl through the column;

Permeate the chromatography column with 0.5 column volume of 0.2M 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 5: 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

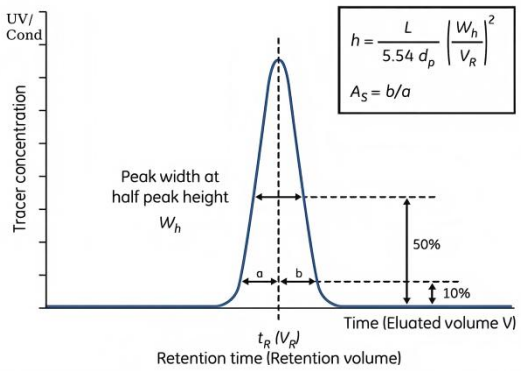
N=theoretical plate number

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 metal 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 sterilization and de-pyrogenic effect, it is recommended that TA-IMAC FF media can be treated with 70% ethanol for more than 12h before 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-IMAC FF media are sold with 20% ethanol or 2% benzyl alcohol as a preservation solution.After use, TA-IMAC FF is stored in 20% ethanol at 2-30°C in an airtight container. It is recommended that the preservative be replaced with fresh preservative every 3 months to prevent evaporation of ethanol and microbial growth.

6. Destruction and recycling

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

7. Ordering Information

Table 6 Article number and packaging

Product	Item No.	Norm
TK-Col IMAC FF	Y6221	1×1ml
	Y6222	5×1ml
	Y6223	1×5ml
	Y6224	5×5ml
	Y622403	1×4.9ml
	Y622404	1×20ml
TK-Col 16/10 IMAC FF	Y6225	1pac.
TK-Col 26/10 IMAC FF	Y6226	1pac.