TK-Col TH（Truking Hard）IEX Ion Exchange Pre-Packed Column

Product Manual

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Content

1. Product Introduction

Ion exchange chromatography (IEC) is a highly effective method for separating and purifying biomolecules. This technique primarily relies on interactions between positive and negative charges, exploiting differences in the nature and magnitude of charges carried by various biomolecules under specific conditions to achieve separation. It features high loading capacity, excellent resolution, controllable conditions, and scalability, and has been widely applied in pharmaceuticals, chemical engineering, metallurgy, food processing, and other fields.

The TH IEX Series matrix is a highly cross-linked agarose with exceptional rigidity. Compared to conventional IEX TA FF, it exhibits a higher dynamic loading capacity under high flow rate conditions, making it suitable for high-flow operations. Its physical and chemical properties remain stable throughout purification and regeneration processes, with the medium's loading capacity and performance unaffected by external buffers. This renders it particularly well-suited for large-scale industrial production.

- TH IEX possesses the following characteristics:
  - High flow rates, large volume treatment capacity, short operating cycles, improved yields
  - Highly rigid substrate, high pressure resistance
- TH IEX series consists of the following three main types of media
  - TH-Q strong anion exchange media
  - TH-SP strong cation exchange media
  - TH-S strong cation exchange media
  - TH-DEAE weak anion exchange media

TK-Col TH IEX pre-packed columns are ready-to-use ion exchange chromatography columns with TH IEX series media loaded into TK-EC16/20 or TK-EC26/20 chromatography columns, eliminating the hassle of loading the columns on your own and the risk of poor column efficiency. These pre-packed columns are widely used for laboratory process development, small amount sample preparation, and are suitable for the separation and purification of biomolecules in the fields of peptides, recombinant proteins, and antibodies. It has the following features:

- Ready-to-use
- Stable volume in the column bed
- High flow rate
- High dynamic binding load under high salt conditions
- Good physicochemical tolerance

2. Technical parameters

Table 1: Technical parameters for each type of media

Medium	TH-Q	TH-DEAE	TH-S	TH-SP
Appearance	Spherical, white paste-like substance, which separates into layers when left standing.			
Base frame	Highly rigid agarose containing long chains of dextran			
Media Type	strong anion	weak anion	strong cation	strong cation
functional group	Quaternary amine	Diethylaminoethyl	sulfonylmethyl	Sulfopropyl
Ion load	160~200μmol Cl^-/ml	290~350μmol Cl^-/ml	110~140μmol H^+/ml	170~220μmol H^+/mL
Average particle size $^+$	90μm			
Pressure	0.5 MPa			
Operating pH range	2-12	2-12	4-12	4-13
Chemical stability	Common aqueous solutions, 1 M NaOH, 6 M guanidine hydrochloride, 30% isopropyl alcohol, 70% ethanol			
pH stability	2~14 (CIP); 2~12 (working)	2~14 (CIP); 2~9 (working)	3-14 (CIP); 4-12 (working)	3~14 (CIP); 4~13 (working)
Temperature tolerance	Use temperature 4~40℃, cannot be frozen, can be autoclaved at 121℃ (add 0.1M salt) (anion: NaCl; cation: NaAc)			
load capacity	The variation between different proteins is considerable, with binding capacities ranging from several milligrams to over one hundred milligrams per millilitre of medium being within the normal range.			
Recommended Flow Rate	90~500cm/h			
Storage	2~30°C, 20% ethanol or 2% benzyl alcohol		2~30°C, 20% ethanol or 2% benzyl alcohol, 0.2M NaAc	

Table 2: Technical Specifications for Pre-Packaged Columns (Part numbers listed on the final page)

Product Name	Prepacked resin	Prepacked column volume (ml)	Inner diameter × Column bed height mm × mm	Recommended flow rate ml/min	Storage	Pressure resistant	Screen aperture (μm)
TK-Col 16/10 Hard Q	TH-Q	19.1-21.1	16×100（±5）	5.0-16.7	0.5MPa（5bar）（72.5psi）	20% Ethanol	10
TK-Col 26/10 Hard Q		50.4-55.7	26×100（±5）	13.0-44.2			
TK-Col 16/10 Hard DEAE	TH-DEAE	19.1-21.1	16×100（±5）	5.0-16.7			
TK-Col 26/10 Hard DEAE		50.4-55.7	26×100（±5）	13.0-44.2			
TK-Col 16/10 Hard S	TH-S	19.1-21.1	16×100（±5）	5.0-16.7		20% ethanol containing 0.2M NaAc	
TK-Col 26/10 Hard S		50.4-55.7	26×100（±5）	13.0-44.2			
TK-Col 16/10 Hard SP	TH-SP	19.1-21.1	16×100（±5）	5.0-16.7			
TK-Col 26/10 Hard SP		50.4-55.7	26×100（±5）	13.0-44.2			

3. Methods of use

- *To avoid clogging the column, all samples and buffers need to be filtered through a 0.45um membrane.*
- *To obtain a good separation, avoid large temperature differences between the buffer and the column.*
- *Keep the column out of direct sunlight.*
- *Layer pre-packed columns can be used in a chromatography cooler, but the flow rate needs to be reduced appropriately.*

3.1Connect the chromatography column to the chromatography system

- Open the packaging and remove the chromatography column.
- Inspect the column for damage and check if it has dried out due to air ingress during shipping. If either occurs, contact your Chutian Microsphere sales representative immediately.
- Secure the column next to the chromatography system, ensuring the flow direction is correct.
- Start the chromatography system, ensuring all air bubbles are purged. Set the system alarm pressure to 0.3 MPa, then adjust and maintain a flow rate of 0.2 mL/min.
- After purging air bubbles from the system, open both end caps of the column and connect it while maintaining the low flow rate.

3.2 Preparation of Chromatography Columns

- Rinse: Chromatography columns are stored in 20% ethanol or 2% benzyl alcohol during transport. First, flush out the storage solution with 2 column volumes of distilled water.
- Sterilization: For sample safety, it is recommended to rinse with 0.5M NaOH for 2 column volumes upon first use, followed by flushing with 2 column volumes of distilled water.

3.3 Column equilibration

- Buffer: Select buffer salts whose buffer ions do not interact with the medium's ligands. For equilibration buffers, use low-salt (less than 5 mS/cm) and high/low pH buffers (typically: cation exchange media—1 pH unit below the target's isoelectric point; anion exchange media—1 pH unit above the target's isoelectric point) to facilitate binding while considering sample stability in the buffer. Elution buffers are typically prepared by adding high-concentration salts (e.g., 1M NaCl) to the equilibration buffer.
- Flush the column with the equilibration buffer at the recommended flow rate. Equilibration is complete when the pH and conductivity of the eluting buffer match those of the buffer before entering the column, typically requiring 2–5 column volumes.

Table 3: Buffers Suitable for Anion Exchange Chromatography

pH Scope	Buffer salt	concentration（mM）	Balanced Ions	pKa(25℃)
4.3-5.3	N-Methylpiperazine	20	Cl^-	4.75
4.8-5.8	Piperazine	20	Cl^- or $HCOO^-$	5.33
5.5-6.5	L-Histidine	20	Cl^-	6.04
6.0-7.0	bis-Tris	20	Cl^-	6.48
6.2-7.2	bis-Tris propane	20	Cl^-	6.65；

8.6-9.6				9.10
7.3-8.3	Triethanolamine	20	Cl^- or CH_3COO^-	7.76
7.6-8.6	Tris	20	Cl^-	8.07
8.0-9.0	N-Methyldiethanolamine	20	Cl^-	8.52
8.0-9.0	N-Methyldiethanolamine	50	Cl^- or CH_3COO^-	8.52
8.4-9.4	Diethanolamine	20(pH8.4)	Cl^-	8.88
		50(pH8.8)		
8.4-9.4	Propane 1,3-Diamino	20	Cl^-	8.88
9.0-10.0	Ethanolamine	20	Cl^-	9.50
9.2-10.2	Piperazine	20	Cl^-	9.73
10.0-11.0	Propane 1,3-Diamino	20	Cl^-	10.55
10.6-11.6	Piperidine	20	Cl^-	11.12

Table 4: Buffers Suitable for Cation Exchange Chromatography

pH Scope	Buffer salt	concentration（mM）	Balanced Ions	pKa(25℃)
1.4-2.4	Maleic acid	20	Na^+	1.92
2.6-3.6	Methyl malonic acid	20	Na^+ or Li^+	3.07
2.6-3.6	Citric acid	20	Na^+	3.13
3.3-4.3	Lactic acid	50	Na^+	3.86
3.3-4.3	Formic acid	50	Na^+ or Li^+	3.75
3.7-4.7 5.1-6.1	Succinic acid	50	Na^+	4.21； 5.64
4.3-5.3	Acetic acid	50	Na^+ or Li^+	4.75
5.2-6.2	Methyl malonic acid	50	Na^+ or Li^+	5.76
5.6-6.6	MES	50	Na^+ or Li^+	6.27
6.7-7.7	Phosphate	50	Na^+	7.20
7.0-8.0	HEPES	50	Na^+ or Li^+	7.56
7.8-8.8	BICINE	50	Na^+	8.33

3.4 Flow velocity

- Based on the type of chromatography column, select a flow rate within the recommended range, as shown in Table 2.

3.5 Sample submission

- To prevent sample clogging of the column, samples must be filtered through a 0.45μm microporous membrane prior to injection. Adjust the sample's pH and conductivity to match the equilibration buffer (this can be achieved through dilution, ultrafiltration, or buffer replacement using TD-G25). Determine the injection volume based on the sample's substance content and the ion exchange resin's binding capacity.

3.6 Rinse

- Rinse with balanced buffer until the UV absorbance value decreases to an appropriate level.

3.7 Elution

- Elution：Linear gradient or stepwise gradient elution can be employed to increase the elution strength in the eluent, thereby eluting substances with varying binding strengths from the chromatographic column. Different components are collected, and the position of the target analyte is detected.

3.8 Rebirth

- Regeneration: rinse the chromatography column with a salt containing a high concentration (e.g. 2M NaCl).
- Re-equilibration: rinsing with equilibration buffer is sufficient for a second sample, and so on.

3.9 Column efficiency evaluation

Column efficiency determination may employ acetone or NaCl as indicators. Prepare the indicator solution and mobile phase according to the table below.

Table 5: Column Efficiency Determination Method

Method	Acetone Method for Column Efficiency Testing	NaCl Method for Column Efficiency Testing
Sample	1.0% (v/v) acetone aqueous solution	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 velocity	30 cm/h	30 cm/h
Test Data	UV 280 nm	Electrical Conductivity

3.10 Calculate column efficiency

Calculate the theoretical plate height (HETP), theoretical number of plates (N), and asymmetry factor (As) based on the UV or conductivity curve using the following formula:：

HETP=L/N

$N=5.54(VR/Wh)^2$

Where： VR= Residual Volume

Wh= Half-peak width

L= Column height

N= Theoretical plate count

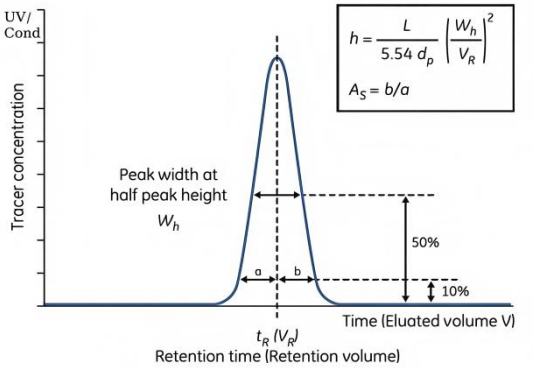
The units for VR and Wh should be consistent.

As=b/a

Where:

a = First half-width at 10% peak height

b = Second half-width at 10% peak height



4. Cleaning and regeneration

As the number of uses of the chromatography medium increases, contaminants continue to accumulate on the column. Regular in-situ cleaning prevents this buildup and maintains stable operating conditions. Determine the frequency of in-situ cleaning based on the degree of contamination of the medium (if

contamination is severe, it is recommended to perform in-situ cleaning after each use to ensure reproducibility of results).

The following cleaning conditions are recommended for different types of impurities and contaminants：

- First flush out tightly bound proteins with 2 column volumes of purified water.
- For removal of strongly hydrophobic proteins and precipitated proteins: First wash with 2–3 column volumes of 1M NaOH, then immediately flush with 5–10 column volumes of purified water.
- For removal of lipoproteins and lipid substances: First wash with 5-10 column volumes of 70% ethanol or 30% isopropanol, followed by 5-10 column volumes of purified water.
- Alternatively, combine the above two washing conditions by washing with a 30% isopropanol solution containing 1M NaOH.

Note: 70% ethanol or 30% isopropyl alcohol should undergo degassing prior to use; flow rates during in-situ cleaning may be selected between 30-60 cm/h; reverse flushing may be employed for severe blockages.

5. Sterilization and storage

TK-Col Hard IEX pre-packed columns can be treated with 1M NaOH for more than 0.5-1h to achieve sterilisation and pyrogen removal.

TK-Col Hard S/SP pre-packed columns are stored in 20% ethanol (or 10 mM NaOH) containing 0.2 M sodium acetate. To prevent ethanol volatilisation and microbial growth, it is recommended that used columns be replaced with fresh 20% ethanol once every 3 months.

TK-Col Hard Q/DEAE pre-packed columns are stored in 20% ethanol (or 10 mM NaOH). To prevent ethanol volatilisation and microbial growth, it is recommended that used columns be replaced with fresh 20% ethanol once every 3 months.

6. Destruction and recycling

Since the packing material in the TK-Col Hard IEX series of pre-packed columns is difficult to degrade in nature, incineration is recommended to protect the environment.

7. Ordering Information

Table 7: Part Numbers and Packaging

Product	Item No.	Norm
TK-Col 16/10 Hard Q	Y6096	1 stick
TK-Col 26/10 Hard Q	Y6097	1 stick
TK-Col 16/10 Hard DEAE	Y6098	1 stick
TK-Col 26/10 Hard DEAE	Y6099	1 stick
TK-Col 16/10 Hard S	Y6100	1 stick
TK-Col 26/10 Hard S	Y6101	1 stick
TK-Col 16/10 Hard SP	Y654601	1 stick
TK-Col 26/10 Hard SP	Y654602	1 stick