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

# Product Manual

# 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, extremely rigid agarose. Compared to conventional IEX TA FF, it exhibits higher dynamic loading capacity under high flow rate conditions, enabling adaptation to high-flow operations. Its physical and chemical properties remain stable throughout purification and cleaning/regeneration processes, with the medium's loading capacity and performance unaffected by external buffers. This makes it more suitable for large-scale industrial production.

- TH IEX possesses the following characteristics:
  - High flow rate, large volume processing capacity, short operating cycles, and improved yield
  - High-rigidity substrate with superior pressure resistance
- The TH IEX series primarily includes the following three 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 featuring TH IEX series media packed into TK-EC 1ml, TK-EC 4.9ml, TK-EC 5ml, and TK-EC 20ml empty chromatography columns. This eliminates the hassle of self-packing columns and the risk of suboptimal column efficiency. These pre-packed columns are widely used in laboratory process development and small-scale sample preparation, suitable for the separation and purification of biomolecules such as peptides, recombinant proteins, and antibodies. They feature the following characteristics:

- Ready to use
- Stable column bed volume
- High flow rate
- High dynamic binding capacity
- Excellent physicochemical tolerance

## 2. Technical parameters

**Table 1: Technical Parameters for Various Media**

| medium | TH-Q | TH-DEAE | TH-S | TH-SP |
|---|---|---|---|---|
| Appearance | Spherical, white paste-like substance that separates into layers when left to stand. | | | |
| Base frame | Highly rigid agarose containing long-chain glucan | | | |
| 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 | | | |
| Withstand 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 hydrochloric acid guanidine, 30% isopropanol, 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 | Operating temperature: 4–40°C. Do not freeze. Autoclavable at 121°C (add 0.1 M salt) (Anion: NaCl; Cation: NaAc) | | | |
| payload | Different proteins vary significantly, with binding capacities ranging from a few milligrams to over a hundred milligrams per milliliter of medium—all within the normal range. | | | |
| Recommended flow rate | 90~500cm/h | | | |
| Storage | 2~30℃，20% ethanol or 2% benzyl alcohol | | 2~30℃，20% ethanol or 2% benzyl alcohol，0.2M NaAc | |

**Table 2: Technical Parameters of Pre-Packed Columns (Part Numbers See Last Page)**

| Product Name | Resin | Prepacked column volume (ml) | Inner diameter × Column bed height mm × mm | Recommended flow rate ml/min | Withstand pressure | Storage | Screen aperture（μm） |
|---|---|---|---|---|---|---|---|
| **TK-Col Hard Q** | TH-Q | 1 | 7×25 | <3 | 0.5MPa（5bar）（72.5psi） | 20% ethanol | 10 |
| | | 4.9 | 8×100 | <4 | | | |
| | | 5 | 16×25 | <16 | | | |
| | | 20 | 16×100 | <16 | | | |
| **TK-Col Hard DEAE** | TH-DEAE | 1 | 7×25 | <3 | | | |
| | | 4.9 | 8×100 | <4 | | | |
| | | 5 | 16×25 | <16 | | | |
| | | 20 | 16×100 | <16 | | | |
| **TK-Col Hard S** | TH-S | 1 | 7×25 | <3 | | 20% ethanol containing 0.2 M sodium acetate | |
| | | 4.9 | 8×100 | <4 | | | |
| | | 5 | 16×25 | <16 | | | |
| | | 20 | 16×100 | <16 | | | |
| **TK-Col Hard SP** | TH-SP | 1 | 7×25 | <3 | | | |
| | | 4.9 | 8×100 | <4 | | | |
| | | 5 | 16×25 | <16 | | | |
| | | 20 | 16×100 | <16 | | | |

## 3. Methods of use

- *To prevent clogging of the chromatography column, all samples and buffers must be filtered through a 0.45μm membrane.*
- *To achieve optimal separation, avoid significant temperature differences between the buffer and the chromatography column.*
- *Store the chromatography column away from direct sunlight.*
- *Pre-packed columns may be used in chromatography refrigerators, but the flow rate should be appropriately reduced.*

### 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<br>5.1-6.1 | Succinic acid | 50 | $Na^+$ | 4.21；<br>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

- Rinse the chromatography column with a high-concentration salt solution (e.g., 2M NaCl).
- Rebalance: After rinsing with the equilibration buffer, proceed with the second sample loading. Repeat as necessary.

### 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

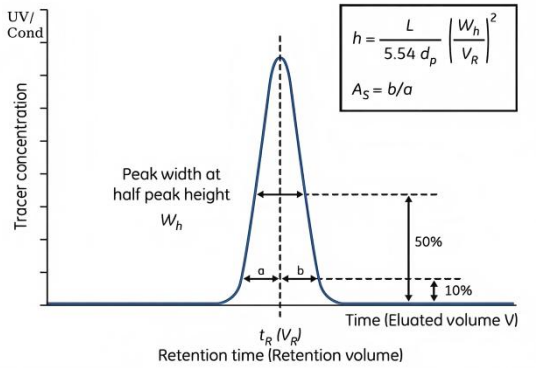
The units for VR and Wh should be consistent.

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 TH IEXAll pre-installed columns can be treated with 1M NaOH for 0.5–1 hour or longer to achieve sterilization and pyrogen removal.

TK-Col TH IEX SP/S Pre-packed columns are stored in 20% ethanol containing 0.2M sodium acetate (or 10mM NaOH). To prevent ethanol evaporation and microbial growth, used columns should be replaced with fresh 20% ethanol solution every three months.

Other TK-Col TH IEX pre-packed columns should be stored in 20% ethanol (or 10 mM NaOH). To prevent ethanol evaporation and microbial growth, used columns should be replaced with fresh 20% ethanol solution every three months.

## 6. Destruction and recycling

Due to the difficulty in naturally degrading the packing material within the TK-Col TH IEX series pre-packed columns, incineration is recommended to protect the environment.

## 7. Ordering Information

**Table 7: Part Numbers and Packaging**

| Pseudolaric acid | product number | wrap |
|---|---|---|
| TK-Col Hard Q | Y6348 | 1×1ml |
| | Y6045 | 5×1ml |
| | Y6046 | 1×5ml |
| | Y6047 | 5×5ml |
| | Y604703 | 1×4.9ml |
| | Y604704 | 1×20ml |
| TK-Col Hard DEAE | Y6349 | 1×1ml |
| | Y6048 | 5×1ml |
| | Y6049 | 1×5ml |
| | Y6050 | 5×5ml |
| | Y605003 | 1×4.9ml |
| | Y605004 | 1×20ml |
| TK-Col Hard S | Y6350 | 1×1ml |
| | Y6051 | 5×1ml |
| | Y6052 | 1×5ml |
| | Y6053 | 5×5ml |
| | Y605303 | 1×4.9ml |
| | Y605304 | 1×20ml |
| TK-Col Hard SP | Y6541 | 1×1ml |
| | Y6542 | 5×1ml |
| | Y6543 | 1×5ml |
| | Y6544 | 5×5ml |
| | Y6545 | 1×4.9ml |
| | Y6546 | 1×20ml |