TK-Col Hard MC/MA

TK-Col 16/10 Hard MC/MA

TK-Col 26/10 Hard MC/MA

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 Mix-IEX series matrix is a highly cross-linked, extremely rigid agarose with a composite ionic group as its ligand. Beyond conventional ion exchange interactions, it incorporates hydrophobic and hydrogen bonding forces. This medium type accommodates high-flow-rate operations with stable physical and chemical properties. It tolerates high salt concentrations during binding phases. Throughout purification and regeneration processes, the medium's capacity and performance remain unaffected by external buffers, making it ideal for large-scale industrial production.

- The TH base frame features 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 Mix-IEX series primarily includes the following two types of media:
  - TH-MC Composite Weak Cation Exchange Media
  - TH-MA Composite Strong Anion Exchange Media

TK-Col Hard MC/MA prepacked columns are ready-to-use ion exchange chromatography columns with TH-MC/MA media in TK-EC 1ml ,TK-EC 4.9 ml ,TK-EC 5ml ,TK-EC 20ml chromatography columns. TK-Col 16&26 Hard MC/MA prepacked columns are ready-to-use ion exchange columns with TH-MC/MA series media loaded into TK-EC16 or TK-EC26 chromatographic columns, eliminating the trouble of loading the columns by themselves and the risk of poor column efficiency. This type of prepacked column is widely used in laboratory process development, small amount of sample preparation, and is 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-MC	TH-MA
Appearance outside	Spherical, white paste-like substance, which separates into layers when	
Base frame	High-rigidity agarose	
Media Type	Compound weak cation	Compound strong anion
Ion load	70~90μmol H^+/ml	90~120μmol Cl^-/ml
Average particle size	75μm	
Pressure resistance	0.5MPa	
Operating pH range	2~12	3~12
Chemical stability	Common aqueous solutions: 1 M sodium hydroxide, 6 M hydrochloric acid guanidine, 30% isopropyl alcohol, 70% ethanol	
pH stability	3~14 (CIP); 3~12 (working)	2~14 (CIP); 3~12 (working)
Temperature tolerance	Operating temperature: 4–40°C. Must not be frozen.	
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℃，20% ethanol or 2% benzyl alcohol	

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

Product Name	Resin	Prepacked column volume (ml)	Inner diameter × Column bed height mm × mm	Recommended flow rate ml/min	Pressure resistant	Storage	Screen aperture (μm)
TK-Col Hard MC	TH-MC	1	7×25	<3	0.5MPa（5bar）（72.5psi）	2~30℃，20% ethanol or 2% benzyl alcohol	10
		4.9	8×100	<4			
		5	16×25	<16			
		20	16×100	<16			
TK-Col Hard MA	TH-MA	1	7×25	<3			
		4.9	8×100	<4			
		5	16×25	<16			
		20	16×100	<16			
TK-Col 16/10 Hard MC	TH-MC	19.1~21.1	16×100（±5）	3-16			
TK-Col 26/10 Hard MC	TH-MC	50.4~55.7	26×100（±5）	8-44			
TK-Col 16/10 Hard MA	TH-MA	19.1~21.1	16×100（±5）	3-16			
TK-Col 26/10 Hard MA	TH-MA	50.4~55.7	26×100（±5）	8-44			

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 prepacked columns can be used in a chromatography cooler, but the flow rate needs to be reduced appropriately.*

3.1 Connect 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

- 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

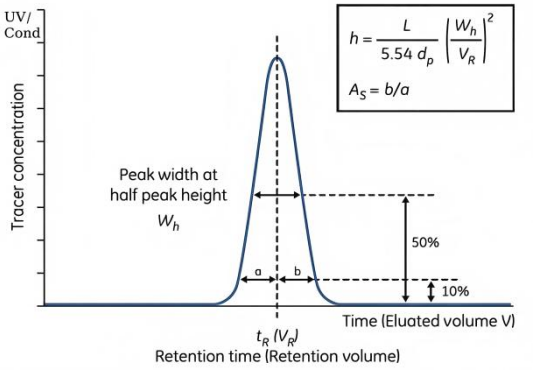
The units for VR and Wh should be consistent.

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 MC/MA pre-packed columns can be treated with 1M NaOH for more than 0.5-1h to achieve sterilisation and pyrogen removal.

TK-Col TH MC/MA 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 TH MC/MA 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 Name	product number	wrap
TK-Col Hard MC	Y6351	1×1ml
	Y6054	5×1ml
	Y6055	1×5ml
	Y6056	5×5ml
	Y605603	1×4.9ml
	Y605604	1×20ml
TK-Col Hard MA	Y6352	1×1ml
	Y6057	5×1ml
	Y6058	1×5ml
	Y6059	5×5ml
	Y605903	1×4.9ml
	Y605904	1×20ml
TK-Col 16/10 Hard MC	Y6102	one stick
TK-Col 26/10 Hard MC	Y6103	one stick
TK-Col 16/10 Hard MA	Y6104	one stick
TK-Col 26/10 Hard MA	Y6105	one stick