TK-Col FF

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. It offers high loading capacity, excellent resolution, controllable conditions, and scalability, making it widely used in pharmaceuticals, chemicals, metallurgy, food processing, and other industries.

IEX FF ion exchange media feature a highly cross-linked agarose backbone that preserves the excellent hydrophilicity and pore structure of natural polysaccharide compounds, offering outstanding compatibility with biomacromolecules. With an average particle size of 90 μ m, IEX FF surfaces contain charged groups, where different charge types determine distinct ion exchange characteristics. IEX FF primarily includes the following media:

TA-Q FF Strong Anion Exchange Media

TA-SP FF Strong cation exchange resin

TA-DEAE FF Weak anion exchange resin

TA-CM FF Weak cation exchange medium

TK-Col FF pre-packed columns are ready-to-use ion exchange chromatography columns featuring IEX FF 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 and the risk of suboptimal column efficiency. These pre-packed columns are widely used for 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:

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

2. Technical parameters

Table 1 Technical Parameters

Resin	TA-Q FF	TA-SP FF	TA-DEAE FF	TA-CM FF
Appearance	White slurry, layered on placement			
Framework	6% highly cross-linked agarose			
Media Type	Strong anion	Strong cation	weak anion	weak cation
Functional	Quaternary amine	Sulfopropyl	Diethylaminoethyl	carboxymethyl
Ion Carrying Capacity	180~250μmolCl^-/ml	180~250μmol H^+/ml	110~160μmolCl^-/ml	90~130μmol H^+/ml
Average particle size	90μm			
Maximum Pressure	0.3 MPa			
Operating pH Range	2~12	4~13	2~12	4~13
Chemical stability	Common aqueous phase solution, 1M NaOH, 6M guanidine hydrochloride, 8M urea, 30% isopropanol, 70% ethanol			
pH Stability	2~14 (CIP); 2~12 (working)	3~14 (CIP); 4~13 (working)	2~14 (CIP); 2-12 (working)	2~14 (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	60-300cm/h			
Storage	2~30℃, 20% ethanol or 2% benzyl alcohol	2~30℃, 20% ethanol or 2% benzyl alcohol, 0.2M NaAc	2~30℃, 20% ethanol or 2% benzyl alcohol	

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

Product name	Resin	Column bed volume (ml)	Inner diameter × column bed height mm×mm	Recommended flow rate (ml/min)	Storage	Maximum Pressure	Column material	Screen aperture（μm）
TK-Col Q FF	TA-Q FF	1	7×25	0.2-2	20% ethanol	0.3MPa（3bar）（43.5psi）	Polypropylene	10
		4.9	8×100	0.2-2.5				
		5	16×25	1-10				
		20	16×100	1-10				
TK-Col SP FF	TA-SP FF	1	7×25	0.2-2	20% ethanol containing 0.2 M NaAc			
		4.9	8×100	0.2-2.5				
		5	16×25	1-10				
		20	16×100	1-10				
TK-Col CM FF	TA-CM FF	1	7×25	0.2-2	20% ethanol			
		4.9	8×100	0.2-2.5				
		5	16×25	1-10				
		20	16×100	1-10				
TK-Col DEAE FF	TA-DEAE FF	1	7×25	0.2-2	20% ethanol			
		4.9	8×100	0.2-2.5				
		5	16×25	1-10				
		20	16×100	1-10				

3. Methods of use

- The chromatography column is made of glass and should be handled gently to prevent it from breaking or affecting the column efficiency.
- To avoid clogging the column, all samples and buffers need to be filtered with 0.45um membrane.
- In order to get a good separation effect, avoid too much temperature change between the buffer and the chromatography column.
- Place the chromatography column in a place without direct sunlight.
- The chromatography column 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 chromatography column.
- Check whether the chromatography column is intact, and whether the chromatography column has been dried out by air intake during transportation, if any of the above situations occurs, please contact the sales representative of Truking Micro-sphere in time.
- Fix the chromatography column next to the chromatography system, pay attention to the flow direction of the chromatography column.
- Start the chromatography system, make sure the air bubbles in the chromatography system are drained, and set the alarm pressure of the 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 chromatographic columns

- Rinse, the chromatography column is stored in 20% ethanol or 2% benzyl alcohol (for international shipments) 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 and then 2 column volumes with distilled water for the first use.
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3.3 Balancing

Buffer: Select buffer salts whose buffer ions do not interact with the resin ligands. Equilibration buffers should be low-salt (less than 5 mS/cm) and high/low pH (typically: cation exchange resin: 1 pH unit below the target protein's isoelectric point; anion exchange resin: 1 pH unit above the target protein's isoelectric point) to facilitate binding, while also considering sample stability in the buffer. Elution buffer is 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 entering the column, typically requiring 2 – 5 column volumes.

Table 3: Buffers Suitable for Anion Exchange Chromatography

pH range	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 8.6-9.6	bis-Tris propane	20	Cl^-	6.65； 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) 50(pH8.8)	Cl^-	8.88
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 range	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 rate

- Depending on the type of chromatography column, flow rates within the recommended flow rate range are generally selected, with slower flow rates for higher column heights.

3.5 Sample Preparation

• To prevent sample clogging, filter samples through a 0.45 μ m microporous membrane prior to loading. Adjust sample pH and conductivity to match the equilibration buffer (achieved via dilution, ultrafiltration, or buffer replacement using TD-G25). Determine loading volume based on sample substance concentration and ion-exchange resin binding capacity.

3.6 Rinsing

Rinse with equilibration buffer until UV absorbance decreases to an appropriate level.

3.7 Elution

Elution: Employ linear or stepwise gradients to increase elution strength in the mobile phase, eluting substances with varying binding strengths from the column. Collect different fractions and detect the target compound's position.

3.8 Regeneration

Regeneration: Flush the column with a high-salt solution (e.g., 2M NaCl).

Rebalancing: After flushing with the equilibration buffer, a second loading can be performed. Repeat as needed.

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 3: 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

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

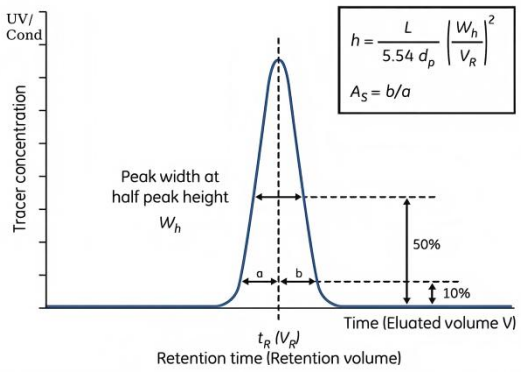
N=theoretical plate number

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



4. Cleaning and regeneration

TK-Col series gel filtration pre-packed columns in use for a period of time there may be a decline in column efficiency, the separation effect deteriorates, the need for cleaning and regeneration, generally 5 ~ 10 cycles need to do a thorough regeneration (regeneration frequency depends on the contamination status of the columns), regeneration needs to be based on the nature of the contaminants using the appropriate regeneration reagents.

- First flush 1 column volume with buffer containing 1M NaCl
- To remove denatured proteins: 2 column volumes were backflushed with 1M NaOH at a flow rate of 20cm/h.

Note : Denatured proteins can also be removed by protease using 1mg/ml of gastric enzyme dissolved in 0.1M acetic acid solution containing 0.5M NaCL.

- Removal of lipids or lipoproteins: 70% ethanol or 30% isopropanol at a flow rate of 40 cm/h for 4 column volumes (to prevent air bubbles can be used to gradually increase the proportion of organic solvents in a gradient); or 1% non-ionic decontaminants
- Inorganic contaminants: rinse 2 column volumes with 0.5M acetic acid
- Rinse 4 column volumes with distilled water

5. Sterilization and storage

Since 20% ethanol or 2% benzyl alcohol preservation solutions lack bactericidal and pyrogen-removing properties, it is recommended that all TK-Col IEX FF pre-packed columns be treated with 1M NaOH for 0.5-1 hour or longer to achieve sterilization and pyrogen removal.

TK-Col IEX FF pre-packed columns should be stored in 20% ethanol (or 10 mM NaOH) containing 0.2 M sodium acetate. To prevent ethanol evaporation and microbial growth, it is recommended to replace the ethanol solution with fresh 20% ethanol every three months for used columns.

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6. Destruction and recycling

Since the packing material in TK-Col IEX FF series pre-packed columns is difficult to degrade naturally, incineration is recommended for environmental protection.

7. Ordering Information

Table 7 Article number and packaging

Product	Item No.	Norm
TK-Col Q FF	Y6339	1×1ml
	Y6013	5×1ml
	Y6014	1×5ml
	Y6015	5×5ml
	Y601503	1×4.9ml
	Y601504	1×20ml
TK-Col DEAE FF	Y6340	1×1ml
	Y6016	5×1ml
	Y6017	1×5ml
	Y6018	5×5ml
	Y601803	1×4.9ml
	Y601804	1×20ml
TK-Col CM FF	Y6341	1×1ml
	Y6019	5×1ml
	Y6020	1×5ml
	Y6021	5×5ml
	Y602103	1×4.9ml
	Y602104	1×20ml
TK-Col SP FF	Y6388	1×1ml
	Y6022	5×1ml
	Y6023	1×5ml
	Y6024	5×5ml
	Y602403	1×4.9ml
	Y602404	1×20ml