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TRUKING MICRO-SPHERE

**Truking Micro-sphere Biotechnology Co.
Product manual**

Instruction Manual No.: 1010
Edition number: 01
Effective date: 2025.01.01

TK-EC 6.6 Series Lab-Scale Chromatography Empty Column

Product Manual



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1. Product Introduction

TK-EC 6.6 series laboratory medium-pressure chromatography columns are low and medium-pressure chromatography columns designed for users in the field of recombinant proteins, antibodies, vaccines, blood products and other biomacromolecules, as well as antibiotics, peptides, synthetic drugs, and natural products, and small molecules, and are suitable for low and medium-pressure chromatography medium on agarose, dextran, and polymer bases. It can be easily connected with all kinds of domestic and imported chromatography systems, which is made of high-precision borosilicate glass tubes and PEEK plastic, with good biocompatibility and chemical resistance, suitable for most of the aqueous solutions. The TK-EC series of laboratory-type chromatography columns have the following advantages:

- 1) Easy to use, easy to operate
- 2) Optional double column head, the height of the installed column has more flexible space
- 3) Inverted port interface design and high elasticity sealing ring effectively prevent leakage
- 4) Better resistance to organic solvents.
- 5) Uniform liquid flow distribution in the column head, easy to load high efficiency chromatographic columns.

Table 1 TK-EC 6.6 technical specifications

Product	Model	Inside Diameter (mm)	Pressure Resistance (bar)	Column Height (mm)	Column Bed Height Range (mm)	Bed Volume (mL)	Item Number
TK-EC6.6/10 (mm/cm) Dual adapter	6.6/100	6.6	35	100	0-60	0-2.0	A705102
TK-EC6.6/15 (mm/cm) Dual adapter	6.6/150	6.6	35	150	50-110	1.7-3.7	A705152
TK-EC6.6/20 (mm/cm) Dual adapter	6.6/200	6.6	35	200	84-164	2.8-5.6	A705150
TK-EC6.6/25 (mm/cm) Dual adapter	6.6/250	6.6	35	250	134-214	4.5-7.3	A705252
TK-EC6.6/30 (mm/cm) Dual adapter	6.6/300	6.6	35	300	184-264	6.3-9.0	A705332
TK-EC6.6/35 (mm/cm) Dual adapter	6.6/350	6.6	35	350	234-314	8.0-10.7	A705400

2. The structure of TK-EC 6.6 series laboratory-type chromatographic columns

Table 2 TK-EC 6.6 Structure

Operating parameter		
Operating temperature	4~40℃	
pH range	1~ 14	
Material Description		
Pipeline	ETFE OD:1/16	①
Tubing Fittings	PP	②
Adapter Adjustment Lever	PEEK	③
Adapter Adjustment Cap	POM	④
Upper fixing cap	POM	⑤
O-ring	FPM	⑥
Sieve plate (10μm)	PE	⑦
Glass tube	Borosilicate 3.3 glass	⑧
Lower fixing cap	POM	⑨

Chemical resistance

According to the chemical stability of the material related to the flow path, the product can withstand most acids, alkalis and most organic solvents; it does not tolerate strong acids, phenols, aromatic hydrocarbons and chlorinated hydrocarbons.

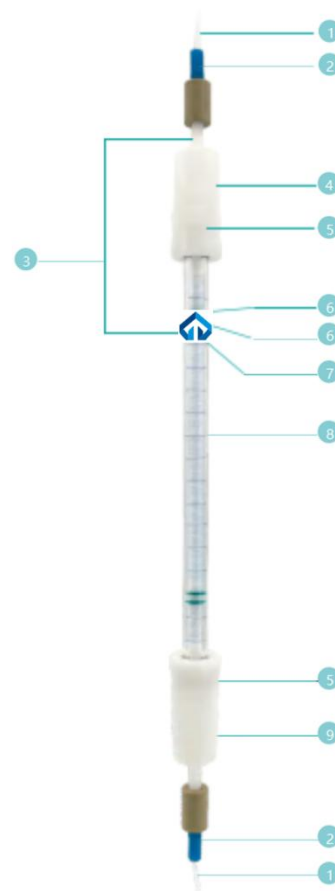


Fig.1 Structure of TK-EC 6.6

3. Chromatography column loading

3.1 Preparing the resin

Prior to loading the column, it is recommended to use the static precipitation method to determine the concentration of the gel suspension, and our packings are stored at 50% concentration in 20% geraniol. For example, the compression factor of the packing is 1.15, and we need to fill the column with a final column volume of 7mL, and according to the formula, we need 16mL of suspension.

$$\text{Volume of suspension} = \frac{\text{Column volume} \times \text{Resin Compression Factor}}{\text{Gel suspension concentration}}$$

Replace the 20% geraniol solution in the packing with the solution required for loading the column (e.g., water) by vacuum filtration, repeat the above steps three times, and finally re-suspend the packing with loading buffer, and volume to the desired volume using a measuring cylinder.

3.2 Inspection Columns

The installation of the column is done with the aid of a column loading system. All components must be in perfect condition. Check the glass parts, paying special attention to the ends, and replace the columns with missing ends.

3.3 Cleaning

The column and loader should be rinsed with 20% germanium before use. Check the sieve plate to ensure that the pore size of the selected sieve plate matches the particle size of the selected packing material, and make sure that the tubing of the bottom part and the top adapter are securely connected.

3.4 Installation

- (1) Secure the column with column clamps or a metal stand to ensure that the column tube is not tilted and is completely perpendicular to the ground, and to avoid broad, curved bands on the mounted column.
- (2) Add distilled water or 20% geraniol to the column to an elevation of at least centigrade, use a strong force to allow the liquid in the column to drain out of the bottom line, drain the bubbles, reserve at least 1 centigrade of elevation of liquid at the bottom of the column, and connect the conical fitting of the line with a plug.
- (3) Mix the suspension again to ensure that it is homogeneous, pour the suspension into the column tube as close to the wall as possible in one go with the aid of a glass rod, and fill the tube with column buffer.
- (4) Connect the adapter line to the equipment and run it at a low flow rate to drain the bubbles from the adapter line. Rotate the adjusting cap counterclockwise to rotate the retaining cap and adjusting cap to the bottom of the adjusting lever.
- (5) Open the bottom line plug, place the adapter vertically on the glass tube and tighten the retaining cap clockwise, making sure that the whole operation is done in a straight line. Be careful not to introduce bubbles.
- (6) Operate the equipment at the flow rate recommended in the packing instruction manual. The recommended column loading flow rate varies for different packings, and many packings will need to be loaded in two stages, first to accelerate settling at the recommended flow rate, and then to compact the packings at a higher flow rate (no more than 30% of the recommended flow rate), so please read the packing instruction manual before turning on the flow rate. Continue running until the gel surface stabilizes, read the scale and record.
- (7) Stop the device from flowing, screw the adapter clockwise down to 2-3 mm below the reading scale, continue to flow, and readjust the adapter's height if the surface of the adhesive changes. Installation of the column is complete.

NOTE: If loading the column at low temperatures, reduce the flow rate by half to prevent high viscosity of the loading buffer and excessive force.

- (8) If the column is not to be used for a while, the ends of the column can be closed with plugs using the bottom-up method, and the column can be removed to check whether there are any visible bubbles or gaps.

4. Column Efficacy Measurement

A loaded chromatography column can be evaluated by measuring the column efficiency. Flow rate, loading volume, and dead volume of the system all affect the effectiveness of the measurement. Due to the small column volume of the TK-EC 6.6 column, attention needs to be paid to the effect of the dead volume of the system on the

measurement of the column effectiveness, and it is necessary to choose thinner tubing (0.5 mm I.D. tubing is recommended), and to make the tubing between the loading loop to the column head and between the column outlet and the detector as short as possible.

4.1 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
Detection Data	UV 280 nm	Conductivity

4.2 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

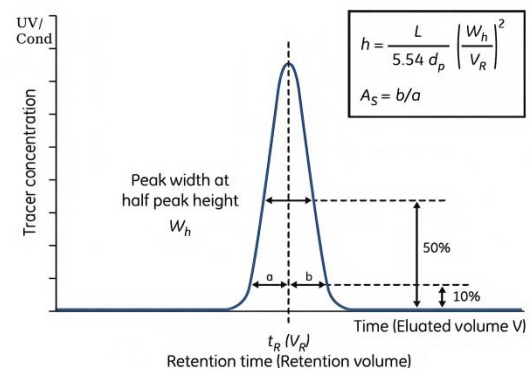
N = theoretical plate number

The units of V_R and W_h should be the same;

$$A_s = b/a$$

Where: a = first half peak width at 10% peak height

b = second half peak width at 10% peak height



4.3 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.

The following table lists standards for column efficiency and symmetry factors for common chromatography media:

Table 4 Criteria for column efficiency and symmetry factor

Particle size (μm)	Medium Base Frame	N/m	As
34	TA-HP	>10000	0.8-1.8
320	TD-C	>1000	0.8-1.8
140	TD-M	>2400	0.8-1.8
90	TD-F	>3700	0.8-1.8
50	TD-SF	>6600	0.8-1.8
90	TA-FF	>3700	0.8-1.8
90	TA-XL	>3700	0.8-1.8
75~90	TH	>4000	0.8-1.8
40	TH-Fine	>8000	0.8-1.8
200	TA-BB	>1700	0.8-1.8

5. Attention

Reverse cleaning can be used when a loaded column is severely clogged, but reverse flushing requires reducing the flow rate to less than half the normal flow rate.

6. Ordering Information

Table 5 Article number and packaging

Product	Item No.	Norm
TK-EC6.6/10 (mm/cm) Dual adapter	A705102	1
TK-EC6.6/15 (mm/cm) Dual adapter	A705152	1
TK-EC6.6/20 (mm/cm) Dual adapter	A705150	1
TK-EC6.6/25 (mm/cm) Dual adapter	A705252	1
TK-EC6.6/30 (mm/cm) Dual adapter	A705332	1
TK-EC6.6/35 (mm/cm) Dual adapter	A705400	1

Table 6 Accessories and Part Numbers

ACCESSORIES	Item No.	Package (pcs)
Pipe line 6.6	A9066	1
Pipe fitting 6.6	A9067	1
Adapter adjusting lever 6.6	A9068	1
Adapter adjustment cap 6.6	A9069	1
Upper fixing cap 6.6	A9070	1
O-ring seal 6.6	A9071	1
Sieve plate 6.6 (10um)	A9072	1
Lower fixing cap 6.6	A9073	1
Glass tube 6.6/10	A9074	1
Glass tube 6.6/15	A9075	1
Glass tube 6.6/20	A9076	1
Glass tube 6.6/25	A9077	1
Glass tube 6.6/30	A9078	1
Glass tube 6.6/35	A9079	1
6.6mm column loader	A9005	1