TK-Col GF

Gel Filtration Prepacked Columns

Product Manual

Content

1. Product Introduction

TK-Col GF series gel filtration prepacked columns are ready-to-use gel filtration columns with TA-GF series media (TA-GF30, TA-GF75, TA-GF200) loaded into TK-EC columns (TK-EC 16/70, TK-EC 16/100, TK-EC 26/70, TK-EC 26/100, TK-EC50/70, TK-EC50/100). TK-Col series gel filtration columns are widely used in laboratory process development, small amount of sample preparation and assay, suitable for the separation and purification of peptides, recombinant proteins, antibodies and other biomolecules, etc. TK-Col series gel filtration columns have the following features:

- Ready-to-use
- Fast flow rate
- High resolution
- Stable column bed volume
- Good physicochemical tolerance

2. Technical parameters

Table 1 TA-GF30/75/200 Technical Parameters

Product name		TA-GF30	TA-GF75	TA-GF200
Chemical composition		Highly cross-linked agarose and dextran		
Separation range	Linear molecules	0.4KD~7KD	0.5KD~30KD	1KD~100KD
	Spherical molecules	0.3KD~10KD	3KD~70KD	10KD~600KD
	Nucleic acid	<10bp	<50bp	<150bp
Particle size distribution range		24-44μm		
Average particle size		34μm		
Recommended Flow Rate		30~60cm/h（TK-EC26/100 ，h=80cm）		
Maximum Pressure		0.3MPa（3bar）		
pH Stability		3-12 (work), 1-14 (CIP)		
Chemical stability		Stabilized in common aqueous solutions: 0.5M NaOH, 1.0M acetic acid, 30% acetonitrile, 30% isopropanol, 8M urea, 6M guanidine hydrochloride, 50mM PB PH7.0, 2% SDS, 24% ethanol		
Storage conditions		20% ethanol, room temperature (2~30°C)		
Appearance		White slurry, can be layered if left to stand		
Precautions		Freezing may destroy the internal structure of the medium; 2% Benzyl Alcohol is only used for foreign shipments or customer-specified		

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

Product name	Resin	Separation range (Da)	Average particle size	Column bed height	Column bed volume (ml)	Recommended flow rate (ml/min)	Maximum Pressure	Storage
TK-Col 16/60 GF30	TA-GF30	<10,000	34μm	60±1cm	120	1-3	0.3MPa（3bar）（43.5psi）	20% ethanol or 2% benzyl alcohol (for international transportation), room temperature (4~8°C is better)
TK-Col 26/60 GF30	TA-GF30	<10,000	34μm	60±1cm	320	3-8		
TK-Col 16/60 GF75	TA-GF75	3,000~70,000	34μm	60±1cm	120	1-3		
TK-Col 26/60 GF75	TA-GF75	3,000~70,000	34μm	60±1cm	320	3-8		
TK-Col 16/60 GF200	TA-GF200	10,000~600,000	34μm	60±1cm	120	1-3		
TK-Col 26/60 GF200	TA-GF200	10,000~600,000	34μm	60±1cm	320	3-8		
TK-Col 16/90 GF200	TA-GF200	10,000~600,000	34μm	90±1cm	480	1-3		
TK-Col 26/90 GF200	TA-GF200	10,000~600,000	34μm	90±1cm	480	3-8		

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.

3.3 Balancing

- The recommended flow rate is generally used to rinse the column with an equilibration buffer. The choice of equilibration buffer depends on the stability of the sample, and the type and pH of the buffer do not have a significant effect on the effectiveness of gel filtration. However, agarose contains a small amount of sulfate and carboxylate groups and in order to minimize non-specific adsorption of basic protein samples, it is recommended that at least 0.15 M NaCl be added to the equilibration buffer. The pH and conductivity of the buffer to be exported should be the same as that of the buffer before entering the column to indicate that the column is well equilibrated, usually 2-5 column volumes are required.

- Use the recommended flow rate to flush the chromatographic column with equilibrium buffer. The pH and conductivity of the buffer to be exported are consistent with that of the buffer before entering the chromatographic column that means the chromatographic column is well equilibrated, generally 2~5 column volumes are needed.
- Depending on the type of column, the recommended flow rates are generally chosen within the range of flow rates, see Table 2.

3.4 Sampling

- In order to prevent the sample from clogging the column, the sample needs to be filtered with 0.45μm microporous filter membrane before loading, and can be appropriately diluted if the viscosity is too high, and the protein concentration should not exceed 70mg/ml.
- Load the sample onto the chromatography column through the sample loading ring of the chromatography system and other devices, the volume of the sample will vary according to the difference between the target material and the impurity. If the difference between the target and the impurity is relatively small, the sample volume is 1-5% of the column volume, and if the difference between the target and the impurity to be removed is relatively large (e.g., removal of inorganic salts or buffer exchange in the sample), the sample volume is 10-25% of the column volume.

3.5 Separation

- Continue to flush the chromatography column with equilibration buffer, collecting the different components of the effluent based on UV absorption, until no more biomolecules are effluent, typically requiring 1 to 2 column volumes.

3.6 Rebalancing

- Rinse the column with equilibrium buffer and wait until the pH and conductivity of the outlet buffer are the same as the equilibrium buffer prior to entering the column before proceeding to the second sample, and so on.

3.7 Regeneration

- Flush the column with a solution containing 1M NaCl for 1 to 2 column volumes to rinse out nonspecifically adsorbed impurities.

3.8 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

3.9 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

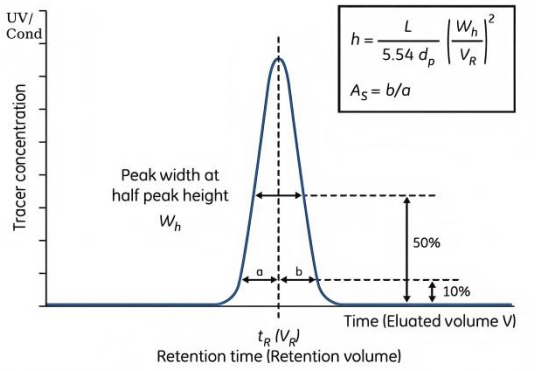
N=theoretical plate number

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



3.10 Evaluation of results

h=HETP/d_{50v}

d_{50v} = median particle size volume distribution (cm)

In general, a value of HETP that is less than three times the average particle size of the medium and an asymmetry factor As between 0.8 and 1.8 indicates good column efficiency. (For TA-GF chromatography resin the number of plates per meter should be greater than 10,000). For unsatisfactory results, it is necessary to analyze the reasons and reload the column.

4. Cleaning and regeneration

TK-Col series gel filtration preloaded 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 solution can inhibit the growth of bacteria, but does not have the effect of sterilization and pyrogen removal, it is recommended that TA-GF30, TA-GF75 and TA-GF200 media can be washed with 0.5M NaOH at the recommended flow rate on the loaded columns prior to and during use to reduce the risk of microbial contamination.

TA-GF30, TA-GF75 and TA-GF200 media are sold with 20% ethanol or 2% benzyl alcohol as preservation solution.

TA-GF30, TA-GF75 and TA-GF200 media are stored in 20% ethanol in a closed container at 2-30°C. To prevent ethanol evaporation and microbial growth, it is recommended that the preservative solution be replaced with a fresh one every 3 months.

6. Destruction and recycling

TA-GF30, TA-GF75 and TA-GF200 are difficult to degrade in nature and incineration is recommended to protect the environment.

7. Ordering Information

Table 5 Article number and packaging

Product	Item No.	Norm
TK-Col 16/60 GF75	Y6005	1 piece
TK-Col 26/60 GF75	Y6006	1 piece
TK-Col 16/60 GF200	Y6007	1 piece
TK-Col 26/60 GF200	Y6008	1 piece
TK-Col 16/60 GF30	Y6009	1 piece
TK-Col 26/60 GF30	Y6010	1 piece
TK-Col 16/90 GF200	Y60107	1 piece
TK-Col 26/90 GF200	Y60108	1 piece