TK-Col G25M
Desalting Pre-installation Column

Product Manual

Content

1. Product Introduction

TD-G25 series media is a gel filtration media made of dextran as raw material and epichlorohydrin as cross-linking agent, with hydrophilic and porous characteristics, commonly used in biological samples such as proteins, polysaccharides and nucleic acids, etc. buffer replacement, desalination and removal of small molecules, but also can be used for the separation and purification of polypeptides and oligonucleotides, as well as the separation and purification of antibiotics, chemically synthetically synthesized drugs and natural products, but also can be used for the purification of nanomaterials in the chemical industry and the environmental protection field, such as wastewater treatment.

TD-G25 separates molecules of different sizes according to the working principle of molecular sieve, based on the different retention time of molecules in the chromatographic column.

The TK-Col G25M series desalting pre-packed columns are ready-to-use columns featuring TD-G25M (middle-particle) media packed into TK-EC empty columns. This eliminates the hassle of self-packing chromatography columns and is commonly used for buffer exchange, desalting, and removal of small molecules from biological samples such as proteins, polysaccharides, and nucleic acids. The perfect integration of media and chromatography columns makes chromatography efficient and straightforward. The TD-G series pre-packed columns feature:

Ready-to-use

Stable bed volume

Excellent physicochemical tolerance

Flexible application methods

2. Technical parameters

Table 1 Technical Parameters

Product name		TD-G25 M
Particle size distribution range (dry)		50~150 μm
Particle size distribution range (wet)		80~240 μm
Average particle size (wet)		140μm
Maximum flow rate (cm/h)		430
Separation range	**Linear molecules**	100D~5kD
	Spherical molecules	1kD~5kD(10~45 amino acids)
	Nucleic acid	<8bp
Water value (ml/g dry powder)		2.5
Volume of soluble gel per gram of dry powder		4 to 6 ml (water) ≈4.5ml (0.5M NaCl)
Appearance		White or off-white powdery solid
Pressure resistance		0.5MPa（5bar）
pH Stability		2~13
Chemical stability		Stabilized in commonly used aqueous solutions: 0.2 M NaOH, 0.2 M HCl, 1 M HAc, 8 M urea, the 6M guanidine hydrochloride, 1% SDS, 24% ethanol, 30% propanol, 30% acetonitrile

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

Product name	TK-Col 16/10 G25	TK-Col 26/10 G25
Resin	TD-G25 M	TD-G25 M
Separation range (Da)	1,000~5,000	1,000~5,000
Average particle size	80~240μm	80~240μm
Column bed height	16/20	26/20
Column bed volume (ml)	20	53
Pressure resistance(MPa)	0.5	0.5
Recommended flow rate (ml/min)	<8	<21
Storage	2-30℃，20% ethanol or 2% benzyl alcohol (for international transportation),	

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

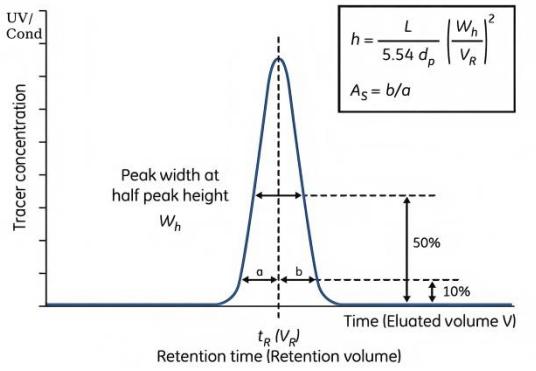
N=theoretical plate number

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



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

The solubilized TD-G25 can be autoclaved at 121°C for 30 min, or the loaded column can be treated with 0.5M NaOH for 30~60 min to reduce the risk of microbial contamination.

Dry powder TD-G25 is stored in a cool and dry place in airtight condition to prevent moisture absorption; dissolved TD-G25 is stored in 20% ethanol at 2~30°C in airtight condition, in order to prevent volatilization of ethanol as well as microbial growth, it is recommended to replace the fresh preservation solution every 3 months.

6. Destruction and recycling

TD-G25 is difficult to degrade in nature and incineration is recommended to protect the environment.

7. Ordering Information

Table 3 Article number and packaging

Product	Item No.	Norm
TK-Col 16/10 G25	Y6003	1 piece
TK-Col 26/10 G25	Y6004	1 piece