TD-G25 C/M/F/SF

Dextran Gel

Chromatography Resin

Product Manual

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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.

TD-G25 is divided into the following four types according to the particle size: TD-G25 C i.e. coarse particles, TD-G25 M i.e. medium particles, TD-G25 F i.e. fine particles, and TD-G25 SF i.e. super-fine particles.

Table 1 TD-G25 technical parameters

Product name		TD-G25 C	TD-G25 M	TD-G25 F	TD-G25 SF
Particle size distribution range (dry)		100~300μm	50~150μm	20~80μm	20~50μm
Particle size distribution range (wet)		160~500μm	80~240μm	30~130μm	30~80μm
Average particle size (wet)		320μm	140μm	90μm	50μm
Maximum flow rate (cm/h)		650	430	240	110
Separation range	**Linear molecules**	100D~5kD			
	Spherical molecules	1kD~5kD (10~45 amino acids)			
	nucleotide	<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			

2. Methods of use

2.1 Dry Powder Resin Dissolution

- Since the medium is supplied as a dry powder, it needs to be dissolved first and then used after loading the chromatography column
- Calculate the amount of TD-G25 dry powder needed based on the volume of the column.

  Amount of dry powder (g) = (column volume x 1.15) ÷ 4.5.
- Pour the medium into 5 times the weight of the dry powder in 0.1M NaCl at 80~100℃ and stir slightly, dissolve for 1h, or at room temperature for 4h (note that do not use the magnetic stirrer to stir in the process of dissolution, the use of the magnetic stirrer will lead to the rupture of the medium particles), and it is better to degas the medium under negative pressure after dissolution at room temperature.
- After the completion of swelling (high temperature swelling need to wait until the cooling to room temperature), remove part of the upper layer of clear liquid, so that the volume of the sedimentary gel accounted for 50% to 75% of the total volume, stirred well and standby.

2.2 Chromatography column loading

- The column loading buffer has little effect on the column loading effect, you can use the buffer used for chromatography or water as the loading solution.
- Take the cleaned TK-EC chromatography column, use purified water to drain the air bubbles in the lower sieve through the discharge port of the chromatography column, keep about 1cm high liquid at the bottom of the column, tighten the lower plug, and adjust the column so that it is perpendicular to the ground.
- Slowly pour the stirred gel suspension into the column at one time (use a column loader if necessary), be careful not to bring in air bubbles, and use a stirring rod to stir it again after pouring.

  Note: Column loader: an empty column tube of the same diameter as the TK-EC column.
- Fill the column loader with loading liquid, connect the column loader to the chromatography system, turn on the flow rate, empty the air bubbles in the hose, turn off the flow rate, and then tighten the top cap of the column loader.

  NOTE: This procedure is for TK-EC 50 and lower chromatography columns only.
- Pour in and stir again with a glue stirring stick, then rinse the media particles on the column from top to bottom along the inner wall with the column loading liquid, and allow the media to settle naturally until there is about 1cm of clarified liquid on the suspension. Install the upper column head and connect the upper column head to the chromatography system or peristaltic pump. Adjust the adapter so that it is lowered into contact with the clarifying liquid and tighten the seal when it is fully immersed in the clarifying liquid. Ensure that the valve at the top of the column is open and slowly lower the adapter until all air bubbles are removed.

  NOTE: This procedure is for TK-EC 100 and above columns only.Flushing the inner wall reduces the risk of media particles sticking between the seal and the column wall and avoids the risk of leakage.
- Set the loading flow rate, due to the hardness of TD-G25 series gel, the flow rate is almost proportional to the pressure, it will not damage the media at a pressure of less than 5 Bar, the loading of the column should be completed at the highest possible flow rate, take the TK-EC50 with a column height of 30cm as an example for reference as follows:

Diethylammonium chloride	TD-G25 C	TD-G25 M	TD-G25 F	TD-G25 SF
Suggested flow rate cm/h	600	400	200	100

- Open the lower head of the column and start the peristaltic pump or chromatography system according to the flow rate set above.Set the pressure inside the column less than the maximum pressure resistance of the chromatography column, if overpressure in the process of loading the column, you need to reduce the flow rate appropriately.
- Wait for the gel suspension to finish settling needs to be kept for another 30min or more, mark the position of the gel surface, and then stop the pump.
- Remove the column loader (if there is one), load the upper column head, lower the column head to the position of about 0.5cm on the gel surface, continue to press the column according to the above flow rate until the gel surface is clear and stable, mark the column height when the gel surface is stable.
- Stop the pump, open the valve/plug on the column head, close the valve/plug at the bottom of the column, slightly relax the sealing ring of the column head, press the column head down to about 0.3 ~ 0.5cm below the marked position, tighten the sealing ring of the column head, close the valve/plug of the column head, and the column loading is completed.

2.3 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 2: 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

2.4 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

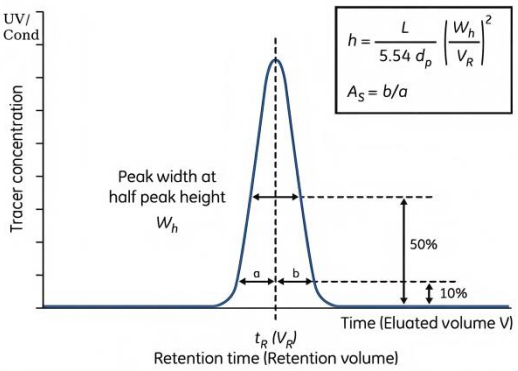
N=theoretical plate number

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



2.5 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.

2.6 Chromatographic methods

① **Buffer selection:** generally use aqueous buffer, the pH and conductivity of the buffer have little effect on the separation effect, the main consideration is the stability of the sample in the buffer.

② **Flow rate:** run at the recommended flow rate, the higher the column height, the slower the flow rate.

③ **Sample preparation:** To prevent the sample from clogging the column, the sample should be filtered through a 0.45μm microporous membrane before loading.

④ **Equilibration:** Use the recommended flow rate to flush the column with equilibration buffer. The equilibration of the column is complete when the pH and conductivity of the buffer at the outlet are the same as those of the buffer prior to entering the column, which generally requires 2 column volumes.

⑤ **Sample loading:** The sample is loaded onto the column directly or by the sample loading ring of the chromatography system. The volume of the sample loaded varies 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.

⑥ **Separation:** Continue to flush the column with equilibrium buffer and collect the different components until no more biomolecules flow out, usually requiring 1~1.5 column volumes.

⑦ **Re-equilibration:** Flush the column with equilibration buffer and wait until the pH and conductivity of the outlet buffer are essentially the same as the equilibration buffer before entering the column, then a second sample can be taken, and so on.

2.7 Factors affecting the separation effect

TD-G25 series gel filtration media based on the principle of molecular sieve separation of substances with differences in size, the type of buffer and pH will not normally affect the separation, in the case of abnormal separation can be analyzed from the following aspects:

- Ionic interaction: Dextran chains contain a small number of terminal carboxyl groups, which will interact ionically with the isolated material at very low ionic strengths, which can be eliminated at salt concentrations greater than 50 mM in the buffer.
- Hydrophobic effect: the crosslinking agent contains a small amount of ether oxygen, the use of phenol: acetic acid: water = 1:1:1 solution, as well as urea or potassium thiocyanate can offset this effect, when the salt concentration in the buffer more than 0.5M and in low pH will lead to enhanced hydrophobic effect, you can add organic solvents such as ethanol, acetonitrile and so on in the buffer to eliminate this effect.

3. Examples of applications

Example1: TD-G25 M desalination

Chromatography column: TK-EC 50/40;

Loading height: 25 cm;

Chromatography medium: TD-G25 M;

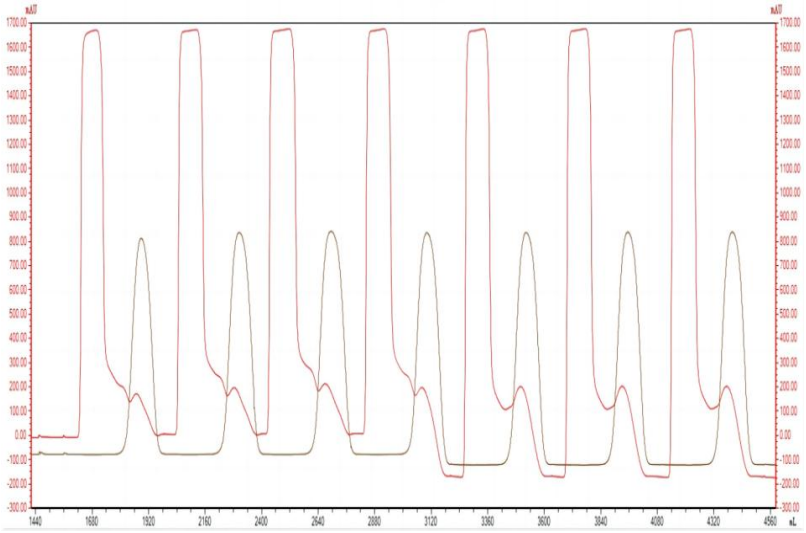
Column bed volume: CV=500mL;

Sample: mussel protein, 45KDa, 15g/L;

Equilibrium solution: 5% acetic acid (mass fraction);

Flow rate: 20 ml/min;

Sample volume: 20CV%.



Example2: TD-G25 F desalination

Chromatography column: TK-EC 50/60; loading height: 50cm;

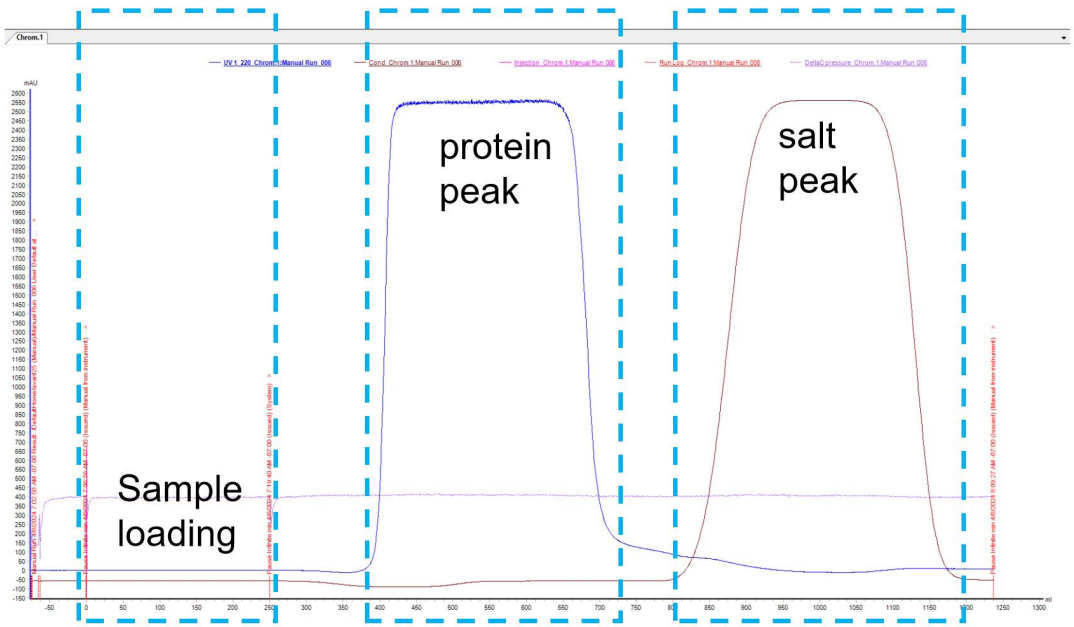
Chromatography medium: TD-G25 F; column bed volume: CV=1000mL;

Sample: Recombinant collagen (50KDa, 5g/L) + 20mM sodium acetate + 500mM sodium chloride, pH=5.0;

Equilibrium solution: 20mM sodium acetate, pH 5.0;

Flow rate: 20ml/min;

Sample volume : 25% CV



4. Cleaning and regeneration

TD-G25 series resin may have a decrease in column efficiency and deterioration in separation effect after a period of use, the following process can be used for cleaning and regeneration.

① Rinse 2 column volumes with purified water.
② Flush 1 column volume with 1M NaCl.
③ Flush 1 column volume with 0.2M NaOH.
④ Rinse 4 column volumes with purified 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

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

7. Ordering Information

Table 4 Article number and packaging

Product	Item No.	Norm	Product	Item No.	Norm
TD-G25 C	Y1054	25g	TD-G25 F	Y1068	25g
	Y1055	100g		Y1069	100g
	Y1056	500g		Y1070	500g
	Y1057	1kg		Y1071	1kg
	Y1058	5kg		Y1072	5kg
	Y1059	25kg		Y1073	25kg
	Y1060	1L		Y1074	1L
TD-G25 M	Y1061	25g	TD-G25 SF	Y1075	25g
	Y1062	100g		Y1076	100g
	Y1063	500g		Y1077	500g
	Y1064	1kg		Y1078	1kg
	Y1065	5kg		Y1079	5kg
	Y1066	25kg		Y1080	25kg
	Y1067	1L		Y1081	1L