TD-G50 C/M/F/SF

Dextran Gel

Chromatography Resin

Product Manual

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Content

1. Product Introduction

TD-G50 is a gel filtration medium made of dextran as raw material and chlorinated propylene oxide as cross-linking agent. It has hydrophilic and porous characteristics and is commonly used for buffer replacement, desalination and removal of small molecules from biological samples such as proteins, polysaccharides, and nucleic acids, as well as for the separation and purification of polypeptides and oligonucleotides, and for the separation and purification of antibiotics, chemically-synthesized medicines, and natural products.It can also be used for the purification of nano-materials in chemical industry and sewage treatment in environmental protection etc.

TD-G50 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-G50 is divided into the following four types according to the particle size: TD-G50 C i.e. coarse particles, TD-G50 M i.e. medium particles, TD-G50 F i.e. fine particles, and TD-G50 SF i.e. super-fine particles.

Table 1 TD-G50 technical parameters

Product name		TD-G50 C	TD-G50 M	TD-G50 F	TD-G50 SF
Particle size distribution range (dry)		100~300μm	50~150μm	20~80μm	20~50μm
Particle size distribution range (wet)		210~620μm	100~300μm	35~200μm	40~100μm
Average particle size (wet)		420μm	200μm	130μm	70μm
Maximum flow rate (cm/h)		650	430	240	110
Separation range	**Linear molecules**	500D~10kD			
	Spherical molecules	1kD~30kD (15~270 amino acids)			
	nucleotide	<15bp			
Water value (ml/g dry powder)		5			
Volume of soluble gel per gram of dry powder		10ml (water) 9ml (0.5M NaCl)			
Appearance		White or off-white powdery solid			
Pressure resistance		0.5MPa			
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-G50 dry powder needed based on the volume of the column.
- Amount of dry powder (g) = (column volume x 1.15) ÷ 9.
- Pour the medium into 10 times the weight of the dry powder in 0.1M NaCl at 80~100℃ with slight stirring and dissolve for 1h, or at room temperature for 4h (note that do not use the magnetic stirrer for stirring during 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 the 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, stir 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 screen through the drainage port of the chromatography column, keep about 1cm high of liquid at the bottom of the column, tighten the lower plug, and adjust the column so that it is perpendicular to the ground.
- Pour the stirred gel suspension slowly 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: empty column tubing of the same diameter as the TK-EC chromatography column.

- Connect the upper column head to the chromatography system or peristaltic pump, drain the air bubbles under the screen of the upper column head (for the chromatography column with diameter less than 10cm, you can use the peristaltic pump or the earwash ball to suck out the air bubbles under the screen after turning the column head upward), put the column head into the chromatography column, shake the column head so that the air bubbles can be discharged from the edge of the column head, and then tighten the sealing knob. For the diameter of ≥10cm chromatography column, firstly, do not tighten the sealing ring too much, press down the shaft head to let the liquid inside the column back out through the column head in order to discharge the air bubbles inside the column head, and then tighten the sealing knob.

- Set the flow rate of loading column, due to the hardness of TD-G50 series gel, the flow rate and pressure are almost proportional to each other, under the pressure of less than 5Bar will not cause damage to the medium, should be as large as possible flow rate to complete the loading of the column, take TK-EC50, column height 30cm as an example for reference as follows:

Product name	TD-G50 C	TD-G50 M	TD-G50 F	TD-G50 SF
Recommended flow rate cm/h	600	450	260	180

- 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), lower the column head to a position about 0.5cm above the gel surface, continue to press the gel once according to the flow rate above, mark the position of the gel surface.
- Stop the pump, open the valve/plug on the plunger head, close the valve/plug on the bottom of the plunger, slightly loosen the plunger head seal, lower the plunger head to about 0.3 ~ 0.5cm below the marked position, tighten the plunger head seal, close the plunger head valve/plug, and the loading of the plunger 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

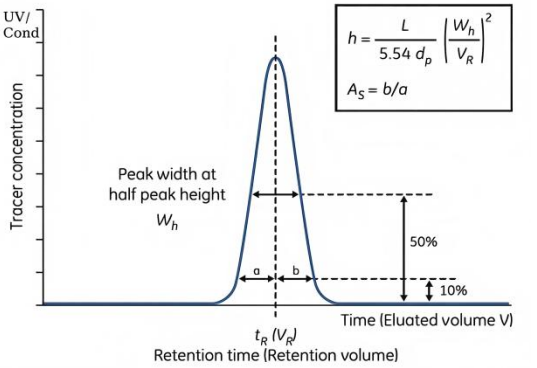
N=theoretical plate number

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-G50 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. Cleaning and regeneration

TD-G50 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.

4. Sterilization and storage

The solubilized TD-G50 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-G50 is stored in a cool and dry place in airtight condition to prevent moisture absorption; dissolved TD-G50 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.

5. Destruction and recycling

Since TD-G50 is difficult to degrade in nature, incineration is recommended to protect the environment.

6. Ordering Information

Table 4 Article number and packaging

Product	Item No.	Norm	Product	Item No.	Norm
TD-G50 C	Y1104	25g	TD-G50 F	Y1118	25g
	Y1105	100g		Y1119	100g
	Y1106	500g		Y1120	500g
	Y1107	1kg		Y1121	1kg
	Y1108	5kg		Y1122	5kg
	Y1109	25kg		Y1123	25kg
	Y1110	1L		Y1124	1L
TD-G50 M	Y1111	25g	TD-G50 SF	Y1125	25g
	Y1112	100g		Y1126	100g
	Y1113	500g		Y1127	500g
	Y1114	1kg		Y1128	1kg
	Y1115	5kg		Y1129	5kg
	Y1116	25kg		Y1130	25kg
	Y1117	1L		Y1131	1L