TA-GF 30/75/200

Gel Filtration

Chromatography Resin

Product Manual

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Content

1. Product Introduction

TA-GF30, TA-GF75 and TA-GF200 are media with high cross-linking degree agarose as the backbone and cross-linked dextran as the packing medium, with an average particle size of 34 μm, combining the high selectivity of dextran and the physical properties of agarose, with high resolution, high hardness, small change of the column bed with the concentration of the buffer solution, stable chemistry, low non-specific adsorption, high recovery, easy to be scaled up, and a good choice for the fine purification stage.

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		

2. Methods of use

2.1 Chromatography column loading

Note: It is best to equilibrate the media suspension to room temperature before loading the column.

● Calculate the amount of TA-GF chromatography medium required based on the column volume of the planned column loading.

Settling volume required = column volume x 1.15 (i.e., compression ratio of approximately 1.15)

Volume of media suspension required = volume of settling media ÷ concentration of media suspension.

Note: The concentration of Truking Microsphere's original packaging media suspension is 80%, for non-original concentration of media suspension, customers can calculate the required volume according to the actual concentration of media suspension.

● Replacement solution: pour the gel suspension into a Büchner funnel, draw off the liquid and wash with about 3 times the volume of purified water, repeat the wash 3 times.

● Gel suspension preparation: Transfer the cleaned medium from the funnel to a beaker or other appropriate container, add the column loading liquid to a gel suspension concentration of 45~55%, stir well and set aside.

● Take the cleaned TK-EC chromatography column, use purified water to drain the air bubbles in the lower screen through the discharge 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 earwash ball to suck out the air bubbles under the screen after turning the column head upwards), put the column head into the chromatography column, shake the column head to make the air bubbles discharged from the edge of the head, and then tighten the sealing knob (for ≥10cm diameter chromatography column, firstly do not tighten the sealing ring too much, press down the head of the shaft to let the liquid inside the column back out through the column head to discharge the air bubbles inside the column head, and then tighten the sealing knob again).

● Set the flow rate (if the loading height is 60cm, set the loading flow rate to 30cm/h), open the bottom valve/plug of the chromatography column, start the pump to press the column until the gel surface is clear and stable, mark the position when the gel surface is stable.

● Remove the column loader (if there is one), install the upper column head, lower the column head to a position of about 0.5cm on the gel surface, and continue to press the column until the gel surface is clear and stable according to the 0.2MPa constant pressure mode, and 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 relax the sealing ring of the plunger head, press the plunger head down to about 0.3 ~ 0.5cm below the marked position, tighten the sealing ring of the plunger head, close the valve/plug of the plunger head, and the loading of the plunger is completed.

2.2 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

2.3 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

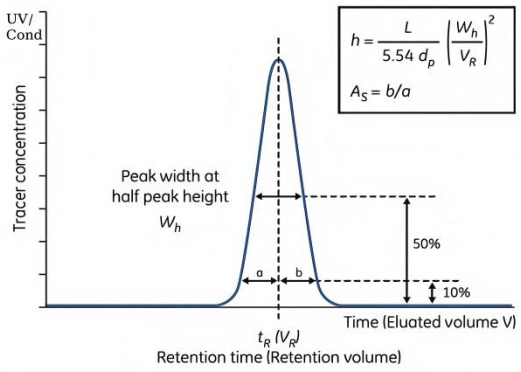
N=theoretical plate number

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



2.4 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 media 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.

2.5 Chromatographic methods

- **Chromatography pre-treatment:** 0.1~0.5 M NaOH can be used to treat the chromatography column for more than 2 hours to achieve the purpose of cleaning, disinfection and removal of heat source.
- **Sample Preparation:** To prevent the sample from clogging the column, the sample should be filtered through a 0.45 μm microporous membrane and the pH and conductivity of the sample should be adjusted to match the equilibration buffer (pH and conductivity can be adjusted by dilution, ultrafiltration, and replacement of the buffer with TD-G25) prior to loading. If the sample viscosity is too high, it can be diluted appropriately, and the protein concentration should not exceed 70 mg/ml.
- **Equilibration:** the column is rinsed with equilibration buffer using the recommended flow rate. the choice of equilibration buffer depends on the stability of the sample. the type and pH of the buffer do not have a significant effect on the effectiveness of gel filtration. however, agarose contains small amounts of sulfate and carboxyl groups. in order to minimize non-specific adsorption of alkaline protein samples it is recommended that a minimum of 0.15 M NaCl be added to the equilibration buffer. buffer to be exportedThe

pH and conductance of the buffer to be exported is the same as that of the buffer prior to entering the column, indicating that the column equilibration is complete, which generally requires 2 to 5 column volumes.

- **Sample loading:** Load the sample onto the chromatographic column through the sample loading ring and other devices of the chromatographic system, the volume of sample loading varies according to the difference in the size of the target material and impurities, generally 0.5-5% of the sample volume of the column volume, according to the separation effect can be adjusted appropriately by the volume of sample loading.
- **Separation:** Continue to flush the chromatography column with equilibrium buffer and collect the different components of the effluent until no more biomolecules are effluent, typically requiring 1 to 1.5 column volumes.
- **Regeneration:** Flush the column with equilibrium buffer for 2 to 3 column volumes.
- **Re-equilibration:** Wash the column with equilibration buffer, wait until the pH and conductivity are basically the same as that of the equilibration buffer, then the second sample can be taken, and so on.

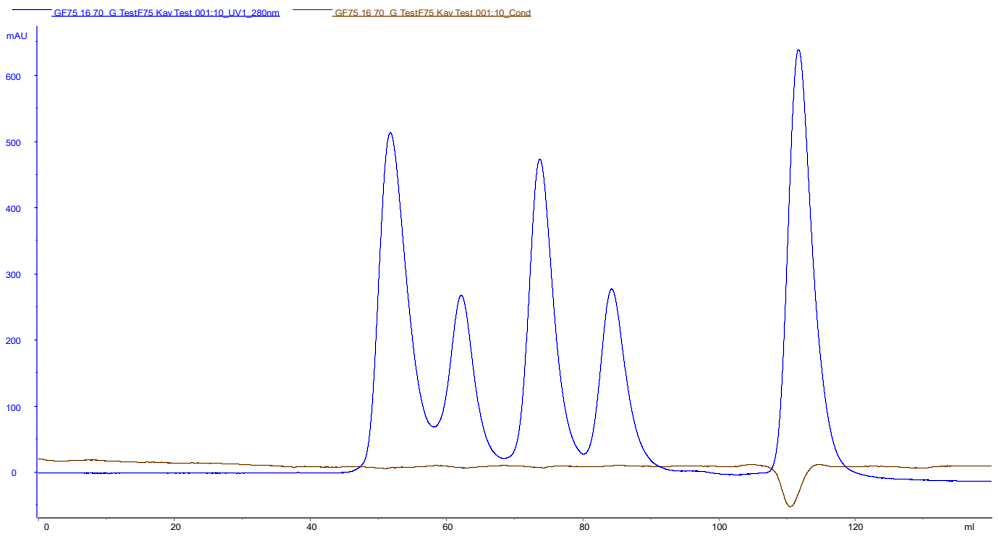
3. Application Cases

Example 1: TA-GF75 for protein isolation

Chromatography column: TK-EC 16/70; column height 61.2cm;

Chromatography medium: TA-GF75;

Proteins in order: IgG, ovalbumin, α-trypsinogen, cytochrome C and VB12.



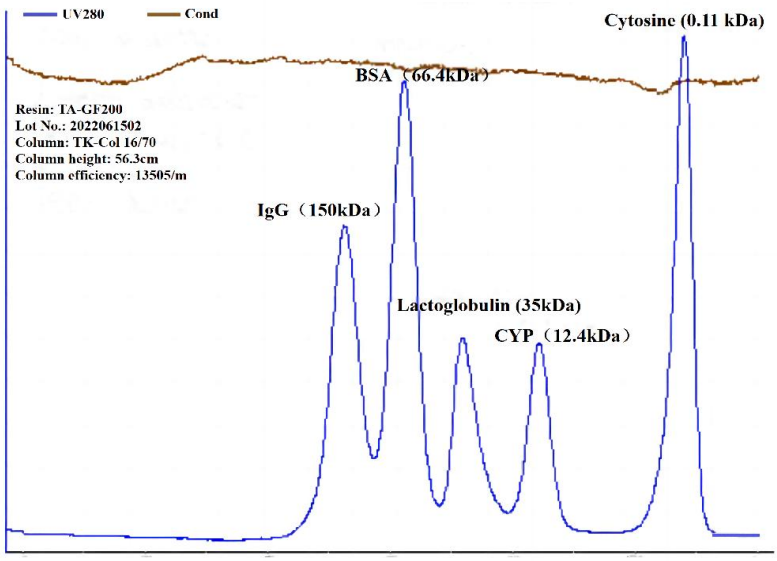
TA-GF75 used for protein separation chromatogram

Example 2: TA-GF200 for protein isolation

Chromatography column: TK-EC 16/70; column height 56.3cm;

Chromatography medium: TA-GF200;

Proteins in order: IgG, BSA, lactoglobulin, cytochrome CYP and cytosine.



TA-GF200 for separating protein chromatogram

4. Cleaning and regeneration

TA-GF30, TA-GF75 and TA-GF200 PG in some processes, there are denatured proteins, lipids, strong hydrophobic proteins, etc. in the regeneration process is not easy to elute, or after a period of time after the use of the column efficiency may be reduced, the back pressure increases, the separation effect of the deterioration of the color of the chromatographic medium, etc., can be used to the following process for the in situ cleaning. The frequency of regeneration depends on the contamination of the column, and it is generally recommended that the column be cleaned after every 5 cycles. Regeneration requires the use of appropriate regeneration reagents depending on the nature of the contaminant.

- First rinse 1 column volume with Buffer containing 1M NaCl
- **Removal of denatured proteins**
  - Two column volumes were backflushed using 0.5 M NaOH at a flow rate of 20 cm/h.

Note: Denatured proteins can also be removed by protease, using 1 mg/ml of gastrozyme dissolved in 0.1 M acetic acid solution containing 0.5 M NaCl.

- **Removal of lipids or lipoproteins**
  - Flush 4 column volumes with 70% ethanol or 30% isopropanol at a flow rate of 40 cm/h (a gradient can be used to gradually increase the proportion of organic solvent in order to prevent air bubbles).
  - Or use a 1% nonionic decontaminant

Note: 70% ethanol or 30% isopropanol should be degassed before use.

- **Removal of inorganic pollutant**
  - 2 column volumes were flushed using 0.5 M acetic acid.
- Rinse 4 column volumes with purified water

5. Sterilization and storage

Since 20% ethanol or 2% benzyl alcohol preservation solution can inhibit the growth of bacteria, but does not have bactericidal and de- pyrogenic effects, it is recommended that TA-GF30, TA-GF75 and TA-GF200 media can be used to reduce the risk of microbial contamination by using 0.5~1M NaOH to wash loaded chromatography columns at the recommended flow rate prior to and during use.

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, closed at 2~30°C. To prevent ethanol evaporation and microbial growth, it is recommended that the preservation solution be replaced with fresh preservation solution 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 3 Article number and packaging

Product	Item No.	Norm
TA-GF30	Y1039	200ml
	Y1040	1L
	Y1041	5L
	Y1042	10L
	Y1043	20L
TA-GF75	Y1044	200ml
	Y1045	1L
	Y1046	5L
	Y1047	10L
	Y1048	20L
TA-GF200	Y1049	200ml
	Y1050	1L
	Y1051	5L
	Y1052	10L
	Y1053	20L