TA-4B & TA-6B

Agarose Gel Filtration Chromatography Resin

Product Manual

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Content

1. Product Introduction

TA-4B and TA-6B are gel filtration media made of 4% and 6% agarose respectively through emulsification, rinsing and sieving, used for the separation and purification or detection of biomolecules such as polysaccharides, nucleic acids, and viruses, and are often used as a base for affinity, hydrophobicity, and ion-exchange chromatography media.The gel structure of TA-4B and TA-6B is fixed by hydrogen bonding, and the substrate is relatively soft and poor in compression resistance.and are not resistant to autoclaving.

Table 1 TA-4B and TA-6B Technical Parameters

Product name		TA-4B	TA-6B
Agarose concentration		4%	6%
Separation range	**Linear molecules**	30kd~5000kd	10kd~1000kd
	Spherical molecules	60kd~20000kd	10kd~4000kd
	Nucleic acid	45bp~850bp	15bp~190bp
Particle size distribution range		45-165μm	45-165μm
Average particle size		90μm	90μm
Recommended maximum flow rate		20cm/h (TK-EC16/60, H=50cm. 20°C, water)	25cm/h (TK-EC16/60, H=50cm. 20°C, water)
Maximum pressure		0.16bar	0.2bar
pH Stability		4-9 (working), 4-9 (CIP)	
Chemical stability		Stable in common aqueous solutions: 4M urea, 70% ethanol	
Sterilization		0.5M NaOH, 2h	
Temperature of use		2~30°C	
Storage conditions		20% ethanol, 2~30°C (4~8°C is better)	
Appearance		White paste, can be layered by placing	
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-4B (or TA-6B) needed based on the volume of the chromatography columns

  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 suitable container, add the column loading solution until the concentration of the gel suspension is 50~75%, stir well and set aside.
- Take a cleaned TK-EC chromatography column, use purified water to drain the air bubbles in the lower screen through the chromatography column drain port, 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 (TA-4B: 20cm/h, TA-6B: 25cm/h) and start the pump to press the column until the gel surface stabilizes. If the pressure is overpressurized during column loading (TA-4B: 0.16bar, TA-6B: 0.2bar), the flow rate needs to be reduced appropriately.Mark the height of the column bed when it is stable.
- Remove the column loader (if available), lower the column head to about 0.5cm above the gel surface, set the flow rate (TA-4B: 40cm/h, TA-6B: 60cm/h) and continue to press the column 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 down the column head to about 0.3 ~ 0.5cm below the marked position, tighten the sealing ring of the column head, close the valve/plug at the column head, and the column loading 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

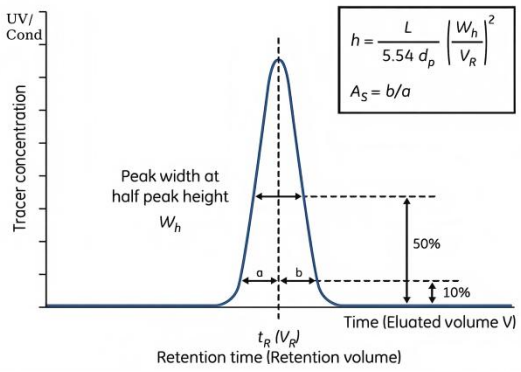
N=theoretical plate number

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)

The h-value calculated by the above formula is less than 3, and the asymmetry factor is 0.8~1.5 then it is judged to be qualified. For unsatisfactory column efficiency the reason needs to be analyzed and the column reloaded.

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 with 0.45 μm microporous membrane and the pH and conductivity of the sample should be adjusted to match the equilibrium buffer (pH and conductivity can be adjusted by dilution, ultrafiltration, and replacement of the buffer with TD-G25) before the sample is loaded.
- **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 before entering the chromatography column, which means that the equilibration of the chromatography column is complete, and

generally requires 2~5 column volumes.

- **Sample loading:** Load the sample onto the chromatographic column through the loading ring and other devices of the chromatographic system, the volume of sample loading will be different according to the size of the difference between the target material and the impurities, generally 1~5% of the column volume of the sample volume is loaded, according to the effect of the separation can be adjusted appropriately to the volume of the sample, the sampling.
- **Separation:** continue to flush the chromatographic column with equilibrium buffer, collect the different components flowing out, until no more biomolecules flow out, generally need 1~1.5 column volume.
- **Regeneration:** Flush the column with equilibrium buffer for 2~3 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. Cleaning and regeneration

TA-4B and TA-6B in some processes, there are denatured proteins, lipids, strong hydrophobic proteins, etc. can not be eluted during the regeneration process, or after a period of time after the use of a possible decline in column efficiency, backpressure increases, the separation effect of the deterioration of the color of the chromatographic medium, etc., can be used for the following process for the in-situ cleaning (CIP) for the different types of impurities and contaminants recommended cleaning conditions are as follows:

- Removal of tightly bound proteins: flush 1 column volume with 1M NaCl.
- Removal of strongly hydrophobic proteins and precipitated proteins: Wash 1 column volume with 0.5M NaOH, then rinse with 5~10 column volumes of purified water immediately.
- Removal of lipoproteins and lipids: Wash with 2~3 column volumes of 70% ethanol or 30% isopropanol, then rinse with 5~10 column volumes of pure water.

Note: 70% ethanol or 30% isopropanol should be degassed before use; reverse cleaning can be used in case of serious clogging.

4. 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-4B and TA-6B media can be used to wash the chromatography columns at the recommended flow rate with 0.5M NaOH to reduce the risk of microbial contamination prior to and during the use of TA-4B and TA-6B media.

TA-4B and TA-6B are sold with 20% ethanol or 2% benzyl alcohol as preservation solution.TA-4B and TA-6B are stored in 20% ethanol at 2~30℃ 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 TA-4B and TA-6B are difficult to degrade in nature, incineration is recommended for environmental protection.

6. Ordering Information

Table 3 Article number and packaging

Product	Item No.	Norm
TA-4B	Y1001	200ml
	Y1002	1L
	Y1003	5L
	Y1004	10L
	Y1005	20L
TA-6B	Y1006	200ml
	Y1007	1L
	Y1008	5L
	Y1009	10L
	Y1010	20L