TA-4FF & TA-6FF

Agarose Gel Filtration Chromatography Resin

Product Manual

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Content

1. Product Introduction

TA- 4FF and TA- 6FF are gel filtration media made of 4% and 6% agarose, respectively, emulsified, cleaned, crosslinked, and sieved. Due to their wide range of separation, they are used for the isolation and purification or detection of biological macromolecules such as polysaccharides, nucleic acids, and viruses, and are often used as a base for affinity, hydrophobic, and ion-exchange chromatography.TA-4FF and TA-6FF are cross-linked with bifunctional cross-linking agent on the basis of TA-4B and TA-6B gels, the degree of cross-linking is higher than that of TA-CL 4B and TA-CL 6B, and the physicochemical properties are more stable. These media can be sterilized by autoclaving at 121°C, and the flow rate is fast, which is suitable to be applied in large-scale separation and purification.

Table 1 TA-4FF and TA-6FF technical parameters

Product name		TA- 4FF	TA- 6FF
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 Flow Rate		90cm/h（TK-EC50/100，h=80cm）	110cm/h（TK-EC50/100，h=80cm）
Maximum Pressure		0.3MPa（3bar）	0.3MPa（3bar）
pH Stability		2-12 (working),2-14 (CIP, short-term)	2-12 (working),2-14 (CIP, short-term)
Chemical stability		Stable in common aqueous solutions: 2M NaOH, 8M urea, 6M guanidine hydrochloride, 1M acetic acid, 70% ethanol, 30% isopropanol	Stable in common aqueous solutions: 2M NaOH, 8M urea, 6M guanidine hydrochloride, 1M acetic acid, 70% ethanol, 30% isopropanol
Sterilization		Resistant to 121° C, 20min sterilization	Resistant to 121° C, 20min sterilization
Storage conditions		20% ethanol, room temperature (2~8° C is better)	20% ethanol, room temperature (2~8° C is better)

Appearance	White paste, can be layered when placed	White paste, can be layered when placed
Precautions	Freezing destroys the internal structure; 2% Benzyl Alcohol for foreign shipments only or customer specified	Freezing destroys the internal structure; 2% Benzyl Alcohol for foreign shipments only 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-4FF (or TA-6FF) 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 (when the height of the loaded column is 70~90cm, TA- 4FF is set to 110cm/h, TA- 6FF is set to 190cm/h), start the pump to press the column until the glue surface is stable (generally the glue surface is clear and stable after pressing the column for 1.5h), and mark the height of the column bed at the time of stabilization.
- Remove the column loader (if there is one), install the upper column head, lower the column head to a position about 0.5 cm above the glue surface, and continue to press the column according to the flow rate above until the glue surface is clear and stable, mark the position of the glue surface.

- Stop the pump, open the valve/plug on the column head, close the valve/plug on the column bottom, slightly relax the sealing ring of the column head, press down the column head to about 0.3 ~ 0.5cm below the marking position, tighten the sealing ring of the column head, close the valve/plug of the column head, and the installation of the column 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

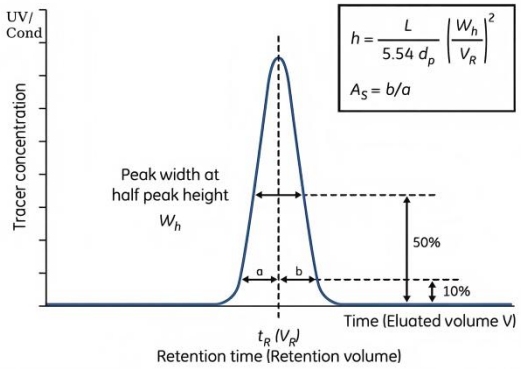
N=theoretical plate number

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 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 column volume of the sample volume, according to the effect of the separation can be appropriately adjusted the volume of sample loading, the maximum sample loading of 30% of the column volume.
- **Separation:** continue to rinse the 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 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

TA-6FF Removal of RNA in DNA purification

Chromatography column: TK-EC50/60

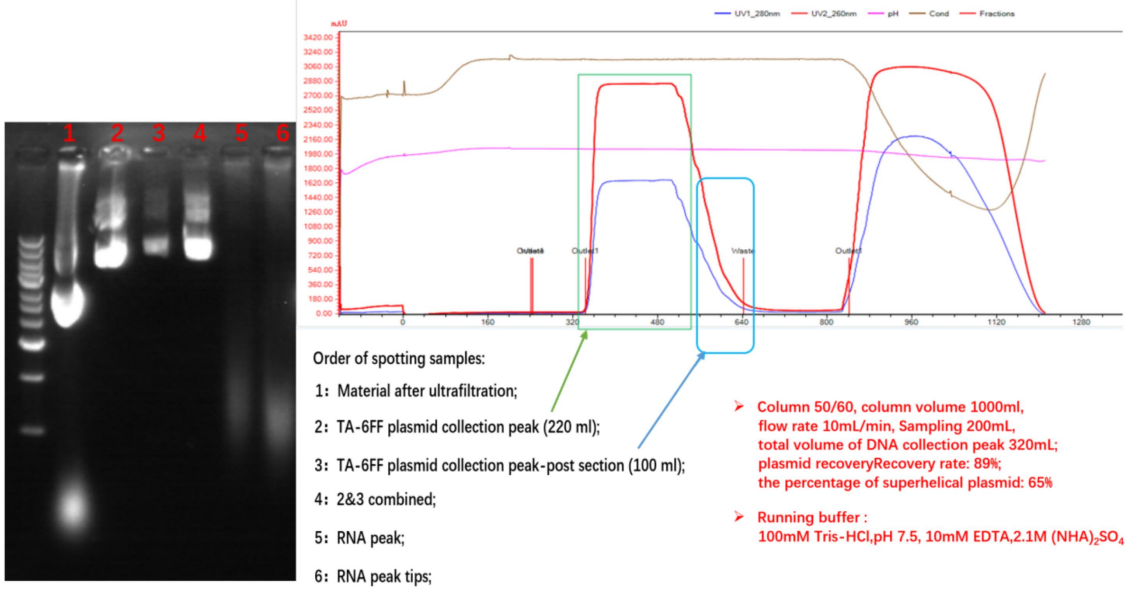
Column bed height: 50cm

Column bed volume: CV=1000ml

Buffer: 100mM Tris-HCl + 10mM EDTA + 2.1M $(NH_4)_2SO_4$, pH= 7.5

Sample volume: 200mL

Flow rate: 10mL/min



4. Cleaning and regeneration

TA-4FF and TA-6FF 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 the 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.

5. Sterilization and storage

Since 20% ethanol or 2% benzyl alcohol preservation solution can inhibit the growth of bacteria, but does not have sterilization, de- pyrogenic effect, it is recommended that before and during the use of TA-4FF and TA-6FF media, can be used to 0.5 ~ 1M NaOH according to the recommended flow rate of the chromatographic column has been loaded to reduce the risk of microbial contamination, can also be used in 121 ℃, 20min autoclave sterilization.

TA-4FF and TA-6FF are sold with 20% ethanol or 2% benzyl alcohol as preservation solution.

TA-4FF and TA-6FF are stored in 20% ethanol, airtight at 2~30°C. To prevent ethanol volatilization and microbial growth, it is recommended that the preservation solution be replaced with fresh preservation solution every 3 months.

6. Destruction and recycling

Since TA-4FF and TA-6FF are difficult to degrade in nature, incineration is recommended for environmental protection.

7. Ordering Information

Table 3 Article number and packaging

Product	Item No.	Norm
TA-4FF	Y1021	200ml
	Y1022	1L
	Y1023	5L
	Y1024	10L
	Y1025	20L
	Y1026	40L
TA-6FF	Y1027	200ml
	Y1028	1L
	Y1029	5L
	Y1030	10L
	Y1031	20L