TA-GST 4FF

Affinity Chromatography Resin

Product Manual

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Content

1. Product Introduction

TA-GST 4FF is a medium made by coupling glutathione to highly cross-linked agarose gel, which is specially used for the isolation and purification of Glutathione S-Transferase (GST) and GST fusion proteins, and GST tags are commonly used for the expression of fusion proteins in modern genetic engineering, which is conducive to soluble expression and activity maintenance of proteins. GST tags are commonly used in modern genetic engineering to express fusion proteins, which is beneficial to the soluble expression and activity maintenance of proteins. For glutathione S-transferase and its fusion proteins from different sources, the target proteins can be purified in one step by using this medium, which is characterized by high pressure resistance, high flow rate, and mild operating conditions, which is conducive to the preservation of protein activity.

Table 1 TA-GST 4FF technical parameters

Appearance	White slurry, layered when placed
Base frame	4% agarose
Particle size distribution range	45~165μm
Functional groups	Glutathione with 10 atomic arms
Dynamic Binding Load	＞10mg GST/ml filler
Pressure resistance	0.3 MPa
Chemical stability	Stabilized in commonly used water-soluble buffers: 1M HAc, 70% ethanol, 6M guanidine hydrochloride (1 hour at room temperature)
pH stability	3~12
Storage	2~30℃, 20% ethanol or 2% benzyl alcohol
Pressure Flow Rate	~450cm/h (TK-EC16/10 H=5cm 25℃)
Recommended Flow Rate Range	Sampling flow rate: <100cm/h

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-GST 4FF 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.

- Media washing:Shake the media suspension well and measure the volume calculated by the above method, pour it into a funnel, draw off the liquid and wash it with about 3mL of purified water/mL of media, repeat the washing 3 times, each time when adding the washing liquid, you need to use a glass rod or stirring stick to stir, in order to wash off the original preservation liquid better.
- Preparation of Column Mounting Gel Suspension: Transfer the cleaned medium from the funnel to a beaker or other suitable container, add the column mounting solution until the concentration of the gel suspension is 50~75%, stir well and set aside.
- Take a cleaned TK-EC chromatography column (the diameter of TK-EC series chromatography columns ranges from 1cm to 45cm in various specifications to meet the different sizes of chromatography applications), drain the membrane air bubbles at the bottom of the column and keep about 1cm high liquid column at the bottom of the column, and adjust the column so that it is perpendicular to the ground.
- Pour the stirred gel suspension slowly into the chromatography column one at a time, taking care not to bring in air bubbles.
- 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 20cm, you can use the peristaltic pump or the 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 so that the air bubbles can be discharged from the edge of the column head, and then screw the sealing knob tightly. (For the chromatography column with diameter >30cm, 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).
- Set the flow rate (110 cm/h for TA-GST 4FF with a column height of 10-20 cm), open the bottom valve/plug of the column, turn on the flow rate, press the column at the set flow rate until the gel surface is clear and stable, and mark the position of the gel surface when it is stable.
- Remove the column loader (if any), install the upper column head, lower the column head to about 0.5cm above the gel surface, set the flow rate to 300cm/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 on the bottom of the column, slightly relax the sealing ring of the column head, press the column head down to about 0.3cm 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.

2.2 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

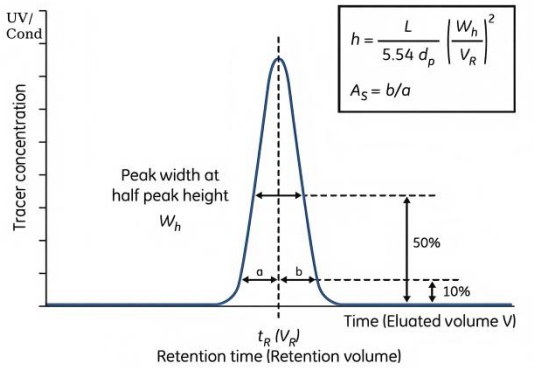
N=theoretical plate number

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 0.8~1.8 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

- **Sample solution:**
  - To avoid clogging the column, the sample solution should be centrifuged or filtered through a 0.45 μm filter prior to sampling.
  - The viscosity of the sample needs to be appropriate, high viscosity samples will cause uneven flow rate during the chromatography process and affect the mass transfer equilibrium.
- **Binding buffer:** Generally use neutral buffer, such as 20 mM PB, 0.15 M NaCl, pH 7.3.
- **Flow rate:** According to the height of the column, a flow rate of <75 cm/h is generally chosen, and a low flow rate is favorable for protein binding.
- **Sample preparation:** adjust the pH and conductance of the sample to be consistent with the equilibration buffer, and determine the volume of the sample according to the binding load of the medium and the content of the target in the sample.
- **Equilibration:** Wash the column with binding buffer until the UV absorption drops to an appropriate value.
- **Sampling:** Prepared samples are loaded according to the set conditions.
- **Wash:** Wash the column with an equilibration buffer until the UV absorption is close to the baseline.
- **Elution:** Reduced glutathione is commonly used for elution, e.g., 50 mM Tris, 10 mM reduced glutathione, pH 8.0. *1~10mM DTT can be added to the buffer, which can increase the purity of the target.*
- Regeneration: 2 column volumes of high pH buffer (0.1MTris-HCl, 0.5MNaCl, pH 8.5) and low pH buffer (0.1M sodium acetate, 0.5MNaCl, pH 4.5) were washed three times alternately; 10 column volumes of binding buffer equilibrated the chromatography column.

3. Cleaning and regeneration

As the number of times the chromatography medium is used increases, the accumulation of contaminants on the chromatography column also increases. Regular in-situ cleaning can effectively prevent the accumulation of contaminants and maintain the stable working condition of the chromatography medium. Customers can determine the frequency of in-situ cleaning according to the degree of contamination of the media in the process of use (if the contamination is more serious, it is recommended to carry out in-situ cleaning after each use, in order to ensure the reproducibility of the results). General cleaning methods are as follows:

- Precipitated or denatured material: wash with 2 column volumes of 6M guanidine hydrochloride followed by 5 column volumes of equilibration buffer.
- Hydrophobically bound substances: wash with 2 column volumes of 70% ethanol followed by 5 column volumes of equilibration buffer.

4. Sterilization and storage

Since 20% ethanol or 2% benzyl alcohol preservation solutions are not bacteriostatic or pyrogenic, it is recommended that TA-GST 4FF media can be treated with 70% ethanol for 12h before and during use to reduce the risk of microbial contamination.

TA-GST 4FF media are sold with 20% ethanol or 2% benzyl alcohol as preservation solution. After use, TA-GST 4FF should be stored in 20% ethanol in an airtight container 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.

5. Destruction and recycling

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

6. Ordering Information

Table 5 Article number and packaging

Product	Item No.	Norm
TA-GST 4FF	Y5050	25ml
	Y5051	100ml
	Y5052	500ml
	Y5053	1L
	Y5054	5L
	Y5055	10L
	Y5056	20L