TA-GST 4B

Affinity Chromatography Resin

Product Manual

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1. Product Introduction

TA-GST 4B is a medium made by coupling glutathione to a 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 in modern genetic engineering for the expression of fusion proteins, which is conducive to the solubility, expression and maintenance of the activity of the proteins. GST tags are commonly used in modern genetic engineering to express fusion proteins, which is conducive to the solubility and maintenance of protein activity. Glutathione S-Transferase and its fusion proteins from different sources can be purified in one step to obtain high-purity target proteins, and the chromatography medium has high pressure resistance, fast flow rate, and mild operating conditions, which is conducive to the maintenance of protein activity.

Table 1 TA-GST 4B technical parameters

Appearance	White slurry, layered when placed
Base frame	4% agarose
Particle size distribution range	45~165μm
Functional groups	Glutathione with 10 atom arms
Binding capacity	＞5mg GST/mL filler
Pressure resistance	0.16 bar
Chemical Stability	Stable in all commonly used water-soluble buffers: 1M acetic acid, 0.1M NaOH, 70% ethanol, 6M guanidine hydrochloride (2 hours at room temperature)
pH stability	4~13
Temperature resistance	2~30℃, cannot be frozen
Storage	2~30℃, 20% ethanol or 2% benzyl alcohol
Flow rate	<75cm/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 4B 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).
- Load the column at a flow rate that is 1.3 times the used flow rate (maximum not to exceed 75 cm/h).
- Open the lower head of the column and start the peristaltic pump or chromatography system according to the flow rate set above. Set the alarm pressure less than 0.1 bar, if the pressure exceeds 0.1 bar during column loading, the flow rate needs to be reduced 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), press the column head to 5mm below the marked position of the gel surface, 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

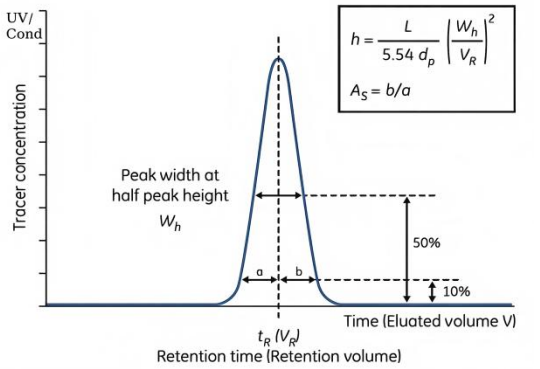
N=theoretical plate number

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 4B media can be treated with 70% ethanol for 12h before and during use to reduce the risk of microbial contamination.

TA-GST 4B media are sold with 20% ethanol or 2% benzyl alcohol as preservation solution. After use, TA-GST 4B 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 4B 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 4B	Y5057	25mL
	Y5058	100mL
	Y5059	500mL
	Y5060	1L
	Y5061	5L
	Y5062	10L
	Y5063	20L