# TK-Col GST 4B

# TK-Col 16/10 GST 4B

# TK-Col 26/10 GST 4B

## Affinity Chromatography Prepacked Columns

# Product Manual

# Content

## 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.

TK-Col GST 4B pre-packed columns are ready-to-use affinity chromatography columns filled with TA-GST 4B media in TK-EC 1 ml ,TK-EC 4.9 ml ,TK-EC 5 ml ,TK-EC 20 ml chromatography vacutainer columns. TK-Col 16/10 GST 4FF pre-packed columns are ready-to-use affinity columns filled with TA-GST 4B media in TK-EC 16/20 chromatography columns; TK-Col 26/10 GST 4B pre-packed columns are ready-to-use affinity columns filled with TA-GST 4B media in TK-EC 26/20 chromatography columns. This series of columns eliminates the hassle of loading the columns by the customer and the risk of poor column performance. This type of pre-packed columns is widely used for laboratory process development, small amount of sample preparation, and suitable for the separation and purification of biomolecules such as GST-tagged proteins. It has the following features:

- Ready-to-use
- Volume stabilization of the column bed
- Good physical and chemical resistance

## 2. Technical parameters

**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 |

**Table 2: Technical parameters for each prepacked column (see end page for item number)**

| Product name | Prepacked resin | Prepacked column volume ml | Inner diameter× Column bed height mm×mm | Recommended flow rate + ml/min | Storage | Pressure resistance | Sieve plate aperture (μm) |
|---|---|---|---|---|---|---|---|
| **TK-Col GST 4B** | TA-GST 4B | 1 | 7×25 | <0.5 | 2-8°C, 20% ethanol or 2% benzyl alcohol (for international shipments). | 0.3MPa（3bar） | 10 |
| | | 4.9 | 8×100 | <1.0 | | | |
| | | 5 | 16×25 | <2.5 | | | |
| | | 20 | 16×100 | <2.5 | | | |
| **TK-Col 16/10 GST 4B** | | 19.1-21.1 | 16×100（±5） | <2.5 | | | |
| **TK-Col 26/10 GST 4B** | | 50.4-55.7 | 26×100（±5） | <10 | | | |

## 3. Methods of use

- *TK-Col 16&26 series chromatography columns are made of glass and should be handled gently to prevent breaking or affecting the column efficiency.*
- *To avoid clogging the column, all samples and buffers need to be filtered through 0.45um membrane.*
- *In order to get a good separation effect, avoid too much temperature difference between the buffer and the column.*
- *Keep the column out of direct sunlight.*
- *Chromatography columns can be used in a chromatography cooler, but the flow rate needs to be reduced appropriately.*

### 3.1 Connecting the column to the chromatography system

- Open the package and take out the column
- Check whether the column is intact, and whether the column has been dried out during transportation, if any of the above situations occurs, please contact Chutian Microsphere sales representative in time.
- Fix the column next to the chromatography system and pay attention to the flow direction of the column.
- Start the chromatography system, make sure the air bubbles in the chromatography system are drained, and set the alarm pressure of chromatography system to 0.3MPa, then adjust and keep the flow rate running at 0.2ml/min.
- After the chromatography system is purged of air bubbles, open the upper and lower plugs of the chromatography column and connect the chromatography column under low flow rate operation.

### 3.2 Pretreatment of chromatography columns

- Rinse, the chromatography column is stored in 20% ethanol or 2% benzyl alcohol (for international transportation) during transportation, first rinse off the storage solution with 2 column volumes of distilled water.
- Sterilization, for sample safety, it is recommended to rinse 2 column volumes with 0.5M NaOH before rinsing 2 column volumes with distilled water for the first use.

### 3.3 Equilibration of Chromatographic Columns

- Use the recommended flow rate to flush the chromatographic column with equilibrium buffer, the pH and conductance of the buffer to be exported are consistent with that of the buffer before entering the column that is to say that the column is well equilibrated, and generally 2~5 column volumes are needed.
- Buffer: generally use neutral buffer, such as 20 mM PB, 0.15 M NaCl, pH 7.3.

### 3.4 Flow rate

- According to the type of chromatographic column, generally choose the recommended flow rate within the range of flow rate, for the height of the column in the 10~15cm high case can be selected on the sample flow rate selection <50cm/h, equilibrium, cleaning, elution can be appropriate to increase the flow rate, but do not exceed the flow rate of 75cm/h. The larger the column height, the slower the flow rate. (See Table 2)

### 3.5 Sampling

- Sample and sample volume: The pH and conductivity of the sample need to be adjusted to be consistent with the binding buffer, and in order to prevent the sample from clogging the column, the sample needs to be filtered with a 0.45 μm microporous filtration membrane before sampling, and the volume of the sample is determined according to the impurity content in the sample and the binding loading of the medium.

### 3.6 Rinse

- Rinse with equilibration buffer until the UV absorption value drops to the appropriate value.

### 3.7 Eluent

Reduced glutathione is commonly used for elution, e.g., 50 mM Tris, 10 mM reduced glutathione, pH 8.0. 1-10 mM DTT can be added to the buffer, which can increase the purity of the target.

### 3.8 Regeneration and rebalancing

Regeneration: Regular in-situ cleaning prevents the build-up of contaminants and maintains a stable working condition. An in-situ wash should be performed after each use to ensure reproducible results. The general regeneration method is as follows: 2 column volumes of high pH buffer (0.1M Tris-HCl, 0.5M NaCl, pH 8.5) and low pH buffer (0.1M sodium acetate, 0.5M NaCl, pH 4.5) alternately washed three times; 10 column volumes of binding buffer to equilibrate the column.

Re-equilibration: Equilibrate the column with equilibration buffer and set aside.

### 3.9 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 3: 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 |

### 3.10 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

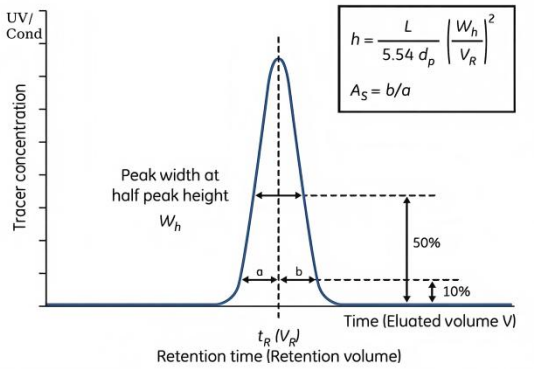
N=theoretical plate number

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




### 3.11 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.

## 4. 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 during use (if the contamination is more serious, it is recommended to carry out in-situ cleaning after each use to ensure the reproducibility of the results).

Regeneration: 2 column volumes of high pH buffer (0.1M Tris-HCl, 0.5M NaCl, pH 8.5) and low pH buffer (0.1M sodium acetate, 0.5M NaCl, pH 4.5) were washed alternately three times; 10 column volumes of binding buffer to equilibrate the chromatography column.

If the medium is used for a period of time and the protein binding capacity decreases due to excessive impurities deposited on the surface, the medium needs to be cleaned as follows:

Precipitate 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.

## 5. 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.

## 6. Destruction and recycling

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

## 7. Ordering Information

**Table 4 Article number and packaging**

| Product | Item No. | Norm |
|---|---|---|
| **TK-Col GST 4B** | Y6239 | 1×1ml |
| | Y6241 | 5×1ml |
| | Y6240 | 1×5ml |
| | Y6242 | 5×5ml |
| | Y624203 | 1×4.9ml |
| | Y624204 | 1×20ml |
| **TK-Col 16/10 GST 4B** | Y6243 | 1pac. |
| **TK-Col 26/10 GST 4B** | Y6244 | 1pac. |