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**（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）**

# GST Focurose 4B (GST-4B)

Catalog No: E-CM-AF05

This manual must be read attentively and completely before using this product.

May you have any problems, please contact our Technical Service Center for help.

Phone: 240-252-7368(USA) 240-252-7376(USA)
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

Please read this manual carefully before use to ensure the performance and successful operation. If you have any questions, please contact our Technical Support.

## Product introduction

GST-4B is suitable for separation and purification of GST-tagged protein, Glutathione S-transferase (GST) or Glutathione dependent protein.

## Advantages

1. Quick and easy (one-step purification).
2. Fast flow rate, high yields and easy to be implemented.
3. The mild elution conditions can perfectly keep the biological activity of protein.

## Performance index

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| Ligand | Glutathione |
| Matrix | 4% agarose |
| Particle size range | 45-165 μm |
| Average particle size | 90 μm |
| Binding capacity | 15-25mg (GST-tagged protein)/mL (media) |
| pH stability | 4-12 |
| Chemical stability | All of the common used solution, such as 1M Acetate(pH 4.0), 1M NaOH, 70% Ethanol, 8M Urea, 6M Guanidine hydrochloride. |
| Flow rate | 75-100 cm/h<br>(XK16/40. Column bed height: 30 cm) |
| Storage buffer | 20% Ethanol |
| Storage temperature | 4~30℃ |

## Operation (take 1 mL column and 5 mL column for example)

**1. Wash (water)**

Wash the media with 5~10 CV (column volume) of purified water with a flow rate of 0.5 mL/min (1 mL) or 2.0 mL/min (5 mL).

Note: This operation is used to remove the 20% ethanol in media.

**2. Equilibration**

Balance the media with 5~10 CV of equilibrium liquid with a flow rate of 0.5 mL/min (1 mL) or 2.0 mL/min (5 mL) until the baseline turns stable and then set to zero.

Note: This procedure is used to balance the media. Make sure that the pH and component in media are in accordance with sample.

**3. Sample application**

Make sure that the pH of sample solution is between 6.5~8.0, then apply the sample with flow rate of 0.2 mL/min (1 mL) or 1.0 mL/min (5 mL) after centrifugation and filtration. Wash with the equilibrium liquid until the baseline tends to zero.

Note: The binding capacity of proteins varies with the type of lysates, the properties of target proteins, flow rate, temperature, and pH. Low flow rates often increase the binding efficiency of samples.

**4. Elution**

Elute with 5~10 CV of eluent at with a flow rate of 0.5 mL/min (1 mL) or 2.0 mL/min (5 mL) and collect the eluted solution.

Note: Low flow rate may increase the concentration of target protein in the eluted solution.

**5. Wash with water**

Wash the media with 5~10 CV of purified water with a flow rate of 0.5 mL/min (1 mL) or 2.0 mL/min (5 mL).

Note: This procedure is used to remove the eluent in media.

**6. Storage**

Wash the media with 5~10 CV of 20% ethanol with a flow rate of 0.5 mL/min (1 mL) or 2.0 mL/min (5 mL) and the store the media.

Note: 20% ethanol can prevent the growth of microorganism. Media preserved with 20% ethanol can be stored at 4~30℃ (4~8℃ is preferred).

**7. Preparation of buffer**

**Equilibrium liquid:** 0.14M NaCl, 0.0027M KCl, 0.01M $Na_2PO_4$, 0.0018M $KH_2PO_4$, adjust the pH to 7.3. Store the prepared equilibrium liquid at room temperature.

Note: The pH of equilibrium liquid must be ensured to be 7.3. If the of pH sample solution <6.5 or pH>8.0, the reaction between sample and media is weak or even invalid.

**Eluent:** 0.05M Tris-HCl, 0.01M GSH, adjust the pH to 8.0. Store the prepared eluent at room temperature.

Note: Prepare fresh eluent before use. Or add GSH into advance prepared 0.05M Tris-HCl (pH=8.0, store at 4℃) and adjust the pH to 8.0 (pH will decline after adding GSH).

## Cleaning

The excellent performance of media (e.g. loading ability, mobility, column efficiency, etc.) can be recovered after cleaning the strong coupling substance (e.g. some strong coupling protein, denatured protein, lipids, etc.).

It is recommended to wash the media after used for each 5 times. The exact washing frequency should be adjusted according to the cleanliness of the purified sample.

1. Wash the media with 2~5 CV of 1.0%Triton X-100 or 70% ethanol, then wash the media with 5~10 CV of purified water immediately.
   Note: This procedure is used to remove the hydrophobic binding substances.
2. Wash the media with 2~5 CV of 8M urea or 6M guanidine hydrochloride, then wash the media with 5~10 CV of purified water immediately.
   Note: This procedure is used to remove the precipitates or denatured substances accumulated in the media.
3. Store the media after washed with 5~10 CV of 20% ethanol.
   Note: 20% ethanol can prevent the growth of microorganism. Media preserved with 20% ethanol can be stored at 4~30℃ (4~8℃ is preferred).

## Trouble shootings

| Problem | Possible cause | Suggestion |
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| The target compound does not combine with the media or the combining amount is low when purifying | Overloading of sample volume. | Decrease the sample volume. |
| | Speed of sample loading is too fast. | Reduce the flow speed of sample loading. |
| | Protein or lipids accumulate in the media. | Wash the media timely and effectively. |
| | Sample inactivates in the process of ultrasonic lysis. | Perform the ultrasonic lysis under relatively mild conditions. |
| | pH of sample or equilibrium liquid is not in the right range. | Make sure that the pH of sample or equilibrium liquid is between 6.5~8.0. |
| | The expression condition is too severe that the conformation of target changed and cannot combine with the media. | An empty vector is suggested to set as control of expression and purification. |
| | Targets accumulates. | Add 1-10 mM DTT before lysis. |
| No target compound was collected or only a small amount of target compound was collected | The target compound does not combine with the media or the combining amount is low. | Confirm whether the target combine with media or not. |
| | Unsuitable elution condition. | Increase the GSH concentration to 20-40 mM and make sure that the pH of eluent is between 8.0~9.0 |
| | Insufficient elution time. | Decrease the flow rate and prolong the retention time of eluent. |
| | Insufficient elution volume. | Increase the elution volume. |
| | The target compound accumulates in the elution buffer. | Check the stability of target compound in the elution buffer (salt concentration, pH, etc.) Add some additives into the eluent, such as 0.1%Triton X-100 or 2% N-octyl glucoside. |

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| Low purity of target compound | Sample has not been pretreated. | Samples must be centrifuged or filtered before loading. |
| | High viscosity of sample. | Dilute the sample properly with equilibrium liquid to decrease the viscosity. |
| | Insufficient washing. | Increase the washing volume until the baseline smooth and keep consistence with equilibrium liquid. |
| | Impurity protein or lipids accumulate in the media. | Wash the media timely and effective. |
| | Poor elution condition, fast elution speed and abrupt elution gradient. | Adjust the elution condition. |
| | The target compound degrades. | Determine the stability of target compound. |
| | Bad loading effect of column resin. | Reload or re-purchase. |
| | Non-specificity absorption of impurity materials. | Add appropriate additive to reduce the non-specific absorption. |
| | The top of separation column has a large volume of sample. | Reload the column or reduce the volume of the sample. |
| | There is microbial grow in the media. | Correctly store the media after used. |
| Decrease of loading volume | Speed of sample loading is too fast. | Reduce the flow speed of sample loading. |
| | Protein or lipids accumulate in the media. | Wash the media timely. |
| | Ligand dropped off due to excessive use. | Re-couple with new media |
| | Conformation of sample changed in the process of ultrasonic lysis or expression and cannot combine well with ligand. | Perform the ultrasonic lysis or expression under relatively mild conditions. |

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| The chromatographic peak rises slowly | The media was loaded too tight. | Reload the column. |
| The chromatographic peak trails | The media was loaded too loose. | Reload the column. |
| The column bed cracks or being dry | Leakage occurred or a large volume of bubbles was introduced. | Check whether there is leakage or bubble, reload the column. |
| Flow of the column is exceedingly slow | Protein or lipids accumulate in the media. | Wash the media or filter membrane timely. |
| | Protein precipitates in the media. | Adjust the content of equilibrium liquid and wash buffer to maintain the stability of target compound and combining efficiency of media. |
| | There is microbial grow in the media. | Filter and degas all the reagents. Samples must be centrifuged or filtered before loading. |