

#### (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**

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	
	(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  $5\sim10$  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  $5\sim10$  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  $5\sim10$  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  $4\sim30^{\circ}$ C ( $4\sim8^{\circ}$ C is preferred).

#### 7. Preparation of buffer

**Equilibrium liquid:** 0.14M NaCl, 0.0027M KCl, 0.01M Na<sub>2</sub>PO<sub>4</sub>, 0.0018M KH<sub>2</sub>PO<sub>4</sub>, 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°C) 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  $4\sim30^{\circ}$ C ( $4\sim8^{\circ}$ C is preferred).

### **Trouble shootings**

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

Poor elution condition, fast elution speed and abrupt elution gradient.  The target compound degrades.  Bad loading effect of column resin. Non-specificity absorption of impurity materials.  The top of separation column has a large volume of sample.  There is microbial grow in the media.  Speed of sample loading is too fast.  Protein or lipids accumulate in the media.  Determine the stability of target compound.  Reload or re-purchase.  Add appropriate additive to reduce the non-specific absorption.  Reload the column or reduce the volume of the sample.  Correctly store the media after used.  Reduce the flow speed of sample loading.  Protein or lipids accumulate in the media.  Decrease of loading  Re-couple with new media	/th Edition, revised in April	, 2017	
High viscosity of sample.    Dilute the sample properly with equilibrium liquid to decrease the viscosity.		Sample has not been pretreated.	
High viscosity of sample.    High viscosity of sample.   equilibrium liquid to decrease the viscosity.		Sumple mas not seen premouncal	filtered before loading.
Insufficient washing.  Increase the washing volume until the baseline smooth and keep consistence with equilibrium liquid.  Wash the media timely and effective.  Poor elution condition, fast elution speed and abrupt elution gradient.  The target compound degrades.  Bad loading effect of column resin. Non-specificity absorption of impurity materials.  The top of separation column has a large volume of sample.  There is microbial grow in the media.  Speed of sample loading is too fast.  Protein or lipids accumulate in the media.  Protein or lipids accumulate in the media.  Ligand dropped off due to excessive use.  Conformation of sample changed in the process of ultrasonic lysis or processive use.  Conformation of sample changed in the process of ultrasonic lysis or processive use.  Perform the ultrasonic lysis or processive use.			Dilute the sample properly with
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expression and cannot combine		expression and cannot combine	conditions.
well with ligand.		well with ligand.	Conditions.

# 7th Edition, revised in April, 2017

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	Leakage occurred or a large	Check whether there is leakage or
or being dry	volume of bubbles was introduced.	bubble, reload the column.
Flow of the column is exceedingly slow	Protein or lipids accumulate in the	Wash the media or filter membrane
	media.	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.