GST-tag Protein Purification Kit (Gel Method)

Cat No: AKIT-GST-1

Description

GST-tag Protein Purification Kit (Gel Method) is composed of GST-tag protein agarose gel, optimized prefabricated buffer and 2 chromatographic column empty column kit for efficient and rapid purification of GST-tag fusion protein.

For custom sizes, formulations or bulk quantities please contact our customer service department.

Website: www.abvigen.com **Phone: +1 929-202-3014 Email:** info@abvigenus.com

Kit Composition

GST-tag protein agarose gel	10 mL (50% v/v)
Binding/washing buffer (10 ×)	100 mL
Eluting buffer (GSH to be added)	10 mL
Reduced glutathione GSH	184 mg/ bottle
Protein quick stain solution	50 mL
SDS-PAGE Sample Loading buffer (5 ×)	10 mL
Affinity chromatography column empty column set	2 sets

Note:

1. Preparation of binding/washing buffer (1 ×) before use: mix an appropriate amount of binding/washing buffer according to the ratio of 9:1, such as 9 mL ultra-pure water and 1mL binding/washing buffer (10 ×), the mixed solution is the binding/washing buffer (1 ×).

2. Preparation of GSH solution (10 ×) and elution buffer

2.1 Preparation of GSH solution (10 ×) : Dissolve the 184 mg GSH provided by the kit with 6mL of the elution buffer provided by the kit (**to be added with GSH**) and mix well, that is, GSH solution (10 ×). The prepared GSH solution (10 ×) -20°C, to avoid repeated freezing and thawing, effective within one year.

2.2 Preparation of elution buffer: Mix an appropriate amount of ultra-pure water and GSH solution (10 ×) at the ratio of 9:1, for example, mix 9 mL ultra-pure water and 1mL GSH solution (10 ×), and the

mixed solution is the elution buffer. Because GSH is easy to be oxidized in the solution and fails, the elution buffer should be used in the present, and the prepared elution buffer should be stored in sub-packaging at -20°C, avoid sub-packaging and storage, and be effective within two weeks.

Operation Process

1. Sample preparation

Take Escherichia coli expression system, 500 mL induced bacterial solution as an example.

1) Centrifuge at 4°C for 30 min (4000 x g) to collect bacteria and discard supernatant.

2) Re-suspend the bacteria with a pre-cooled **binding/washing buffer (1 ×)**, adding the appropriate amount of inhibitors, such as protease inhibitors (PMSF) or other protease inhibitors, if needed.

3) Use ultrasonic crushing method to break the bacteria on the ice until the sample is broken completely.

4) Centrifuge at 4°C for 20 min (12,000 x g), separate supernatant and precipitation, and filter for impurity removal. Retain samples of supernatant and precipitation for subsequent testing.

2. Purification of recombinant GST-tag protein

1) Gently resuspension **GST-tag protein agarose gel**.

2) 2 mL of **GST-tag protein agarose gel** was absorbed and added to the chromatographic column, and the **GST-tag protein agarose gel** was balanced with 10 mL **binding/washing buffer (1 ×)**. Repeat the preceding steps one more time.

3) Close the lower exit of the chromatographic column, add the prepared supernatant containing GST-tag protein to the chromatographic column, and then cover the upper entrance of the chromatographic column tightly, and it is recommended to seal with a sealing film. Place on the mixer and incubate at room temperature for 1 ~ 2 h. (Can also be incubated at 2 ~ 8°C for 2 ~ 4 h or overnight)

4) After the end of incubation, open the upper and lower inlet and outlet of the chromatographic column, and collect the supernatant after all the supernatant flows out of the chromatographic column, and put it at 2 ~ 8°C as a flow through for subsequent detection.

5) Immediately add 10 mL **binding/washing buffer (1 ×)** to the chromatographic column, collect the detergent and place it at 2 ~ 8°C for subsequent testing. Repeat the preceding steps four times.

6) Add 1 mL **eluent buffer** and collect eluent with 1.5 mL Ep tube. Collect 5 to 10 tubes respectively.

7) SDS-PAGE detection

The resulting sample (including run-off, washing solution and eluent) and the original sample were tested for purification using SDS-PAGE. Add an appropriate amount of **protein fast dyeing solution** to immerse the PAGE glue, and then put it on the shaking table, dyeing 10 ~ 30 min to observe the results.

Note: The target protein should be dialysis or ultrafiltration to remove free glutathione before storage, and then frozen at -80°C.

3. Gel regeneration and storage

For gel regeneration steps, please refer to or purchase our GST-tag protein purification and regeneration kit directly.

After the gel is regenerated, it can be used immediately. If it is not used immediately, the equal volume of 20% ethanol should be added and stored at 2 ~ 8°C.

Problem Solving

Problem	Reason	Solution
The production of the target protein is low or undetectable	The protein may be an inclusion body, and there is no protein in the supernatant	The lysate can be detected by electrophoresis to determine whether the supernatant contains the target protein, and the inclusion body protein needs to be purified according to the inclusion body protein.
	The target protein may be inactivated	Use mild ultrasonic crushing conditions or other cracking conditions, such as lysozyme.
	The target protein is degraded by protease	Add appropriate protease inhibitors, such as PMSF, to the cleavage step or washing step.
	The target protein cannot be	Extend the elution time, or

	effectively eluted from the gel	increase the concentration of reduced glutathione in the eluent to 15 mM or more
		Triton X-100 (final concentration 0.1%), octyl-glucoside (final concentration 2%), or NaCl (final concentration 0.1 ~ 0.2 M) were added to the elution.
There were more impurity bands in the eluent	The target protein is degraded by protease	Add appropriate protease inhibitors such as PMSF in the cleavage step or washing step.
	Excessive ultrasound treatment can cause some proteins to bind to fusion proteins	Use mild ultrasonic crushing conditions or other cracking conditions.
	Some proteins bind nonspecifically to fusion proteins or gels	Optimization of washing conditions: Adding detergents such as 1% Triton X-100, 1%Tween-20, 0.03% SDS or 0.1% NP-40 can reduce non-specific adsorption. Increasing the salt concentration in the washing solution can also reduce the non-specific adsorption.

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Ordering Information

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