GST-tag Protein Purification Kit

Cat No: AKIT-GST-2

Description

GST-tag Protein Purification Kit is composed of GST-tag protein agarose magnetic bead and optimized prefabricated buffer and 50 mL magnetic rack, which is used for efficient and rapid purification of GST label 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 magnetic bead	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
Magnetic rack	50 mL double row four well

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 10 X GSH solution 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 fusion protein

1) **GST-tag protein agarose magnetic beads** were thoroughly mixed, 2 mL magnetic bead suspension was taken, placed in a 50 mL centrifuge tube, and 10 mL **binding/washing buffer (1 ×)** was added, after fully mixed, magnetic separation was performed, supernant was discarded, and the above steps were repeated once.

2) The prepared fusion protein supernatant containing GST-tag was added to the treated magnetic beads, and after mixing, the centrifuge tube was placed on the mixing machine and incubated at room temperature for 1 ~ 2 h. (Can also be incubated at 2 ~ 8°C for 2 ~ 4 h or overnight)

3) After incubation, the centrifuge tube is placed on the magnetic rack for magnetic separation, the supernatant is absorbed, and the supernatant is placed at 2 ~ 8°C as a flow through for subsequent testing. 15 mL **binding/washing buffer (1 ×)** was added to the centrifuge tube and mixed in the mixer for 10 ~ 15 min, and then magnetic separation was performed to absorb the supernatant (reserved for sampling and testing). Repeat the preceding steps four times.

4) Add 1 mL **eluent buffer**, blow and mix for 10 ~ 20 times, and collect Ep tubes with superclear to 1.5mL through magnetic rack. Repeat the operation to collect 5 ~ 10 tubes of eluent respectively.

5) SDS-PAGE test

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 shake it on a shaking table. The results can be observed after dyeing for 10 ~ 30 min.

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

3. Regeneration and storage of magnetic beads

Megnetic bead regeneration steps please refer to or directly buy our company GST-tag protein purification regeneration kit.

After the regeneration of the magnetic bead, it can be used immediately. If it is not used immediately, it is necessary to add equal volume 20% ethanol and store it 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 effectively eluted from the magnetic bead	Extend the elution time, or 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 magnetic beads	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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