GST-tag Protein Purification Gel, 45-135 µm

Cat No: AG-GST-4500

Description

GST-tag protein agarose gel is made from a highly cross-linked 4% agarose gel as a matrix, which is chemically covalently bound to reduced glutathione through a 12-atom spacer arm, and is used to purify GST-tag recombinant proteins expressed by fusion of various expression systems.

Characteristics

Matrix	Highly crosslinked 4% agarose gel
Ligand	Glutathione (GSH)
Particle size	45~135 µm
Concentration	The gel volume accounts for 50% of the suspension volume
Pressure flow rate	80 ~ 150 cm/h (0.3 MPa, 3 bar)
Carrying capacity	> 30 mg GST protein
Storage buffer	1× PBS containing 20% ethanol
Shelf life	Stable storage at 2 ~ 8°C for two years

Operation process

1. Buffer preparation

For buffers such as binding/washing buffers and elution buffers, please prepare or purchase our GST-tag protein purification kit.

2. Sample preparation

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

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

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

Enzyme inhibitors, etc.

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

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

3. 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**. 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 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** to the chromatographic column, collect the washing liquid 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.

4. 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.

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Problem solving

Problem	Reason	Solution
The production of the target protein is low or undetectable	The protein may be an inclusion body, but the supernatant has no protein	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 gel	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 are more impurity	The target protein is	Add appropriate protease

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bands in the eluent	degraded by 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.

Ordering Information

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