GST Protein Purification Magnetic Particles

Description

GST fusion protein purification magnetic bead is a new functional material designed for efficient and rapid purification of glutathione mercaptotransferase (GST) fusion protein, which can be directly purified from biological samples in one step by magnetic separation, greatly simplifying the purification process and improving the purification efficiency. It is suitable for convenient purification of GST fusion protein in scientific and industrial fields.

Compared with the traditional column chromatography purification method, the magnetic beads are used to purify GST fusion protein, which does not require multiple high-speed centrifugation and filtration membrane filtration of crude protein samples for a long time, no flow rate control, and no expensive chromatographic equipment. The specific binding of samples to magnetic beads, washing and elution of target proteins become very simple, fast and easy to operate. For skilled operators, high-purity target proteins can be obtained within 1 h, and parallel processing of high-throughput and large-scale samples can be easily achieved, saving time and costs for researchers.

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

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Characteristics

Constituent: GST

Particle size: 30~150 μm

GST ligand content: 20~30 μmol/mL (100% magnetic beads)

GST fusion protein binding amount: ≥ 5mg/mL (100% magnetic beads)

Concentration: 10% (V/V)

Preservation solution: 20% (V/V) ethanol

Chemical stability: At room temperature, it can tolerate 70% ethanol, 6 M guanidine hydrochloride, 0.1 M sodium hydroxide, 0.1 M acetic acid for 1 h

Storage temperature: 4°C ~ 30°C (long-term storage, recommended 4°C ~ 8°C)

Quality guarantee period: Stable storage at 2-8°C, shelf life of 2 years

Note: 1. The amount of protein binding is related to the properties of the target protein, and the value here is only for reference; 2. 1 mL magnetic bead suspension contains 100 µL magnetic beads.

Scope of application

It is suitable for the separation and purification of glutathione mercaptotransferase (GST) fusion protein, glutathione transferase and other proteins with glutathione affinity.

Advantages

1. Protein purification is fast

Simple operation, one step purification can obtain high purity protein;

Convenient and fast, one hour easy to achieve protein purification.

2. Protein purification is flexible

Multiple samples can be processed simultaneously to achieve high throughput;

The protein concentration and volume can be easily controlled, which is convenient for subsequent sample refining;

The scale of purification can be easily controlled;

It has good purification effect on trace and low abundance samples.

3. Protein purification economy

Short purification process, high protein production and activity;

The equipment is simple, reducing the cost of equipment purchase and maintenance, and the threshold of protein purification is greatly reduced;

The purification time is short and the time cost is saved;

Multi-sample processing can be carried out at the same time to realize automatic operation and save labor cost;

It can be reused.

Operation process

The binding properties of target protein and magnetic beads will directly affect the purification efficiency of target protein, and the preparation of various buffers will also affect the recovery and purity of target protein to a certain extent. Therefore, before large-scale protein purification, users

should design their own experiments to screen out buffers suitable for target proteins, including Binding /Washing Buffer (Buffer A) and Elution Buffer (Buffer B). The following provides a purification process of GST label protein with strong binding force for users' reference.

1. Buffer solution preparation

Buffer A: 140 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4, pH 7.4

Buffer B: 50 mM Tris-HCl, 10 mM reduced glutathione, pH 8.0. **Preparation method**: Take 0.1 M Tris solution 50 mL, add 0.307 g reduced glutathione, then adjust the pH to 8.0 with 0.1 M hydrochloric acid, add deionized water to 100 mL. Can be scaled up and down equally.

Note:

1. Reduced glutathione is easily oxidized, Buffer B requires immediate use (please dissolve as needed to prevent oxidation, do not dissolve all at once);

2. Different GST fusion proteins have different degrees of binding to magnetic beads. For most GST fusion proteins, Buffer B containing 10 mM reduced glutathione can elute the target protein; For a few GST fusion proteins with strong binding ability, the elution time and elution times can be extended appropriately, or the concentration of reduced glutathione in Buffer B can be increased.

3. 1~5 mM EDTA, 1~10 mM DTT, 0.1~1.0% Triton X-100, 0.1~1.0% Tween 20 can be added to Buffer A and Buffer B to improve the stability of target proteins.

2. Sample handling

This User Manual provides the following three sample handling methods:

(1) E. coli, yeast and other intracellular expression proteins: the expression cells were diluted with appropriate Buffer A and added with protease inhibitors (such as PMSF with a final concentration of 1 mM); The ice bath ultrasonic lysis of cells, which is a crude protein sample. If the sample is too thick, an appropriate amount of nuclease can be added to the crude sample as needed and placed on ice for 30 min to degrade nucleic acid. In addition, if the target protein content is low, it is recommended to centrifuge the crude protein sample.

(2) Extracellular expression protein: Take extracellular expression supernatant, dilute and balance with an equal amount of Buffer A, that is, the crude protein sample.

(3) Intracellular expression of protein in animal cells: Appropriate amount of animal cells were taken, washed once with appropriate amount of PBS, and the supernatant was discarded; Resuspension with Buffer A containing 1% (V/V) Triton X-100 or 1% (V/V) NP-40; Add protease inhibitors (e.g., PMSF with a final concentration of 1 mM); The crude protein sample is placed on ice for 10 min.

3. Magnetic bead pretreatment

In general, the amount of magnetic beads used is calculated by the user based on the target protein yield and magnetic bead load information. For example, Escherichia coli is used to express a target protein, and 1 g of wet weight bacteria is harvested from 250 mL fermentation solution. The target protein yield is estimated to be 5-10 mg through pre-experiment, and the user needs to take 10 mL of 10% magnetic bead suspension for the purification of the target protein. The following is an example to explain in detail:

(1) The magnetic bead product is fully mixed on the vortex mixer, and 10 mL magnetic bead suspension is taken in the centrifugal tube with a pipette;

(2) Place the centrifugal tube on the magnetic separator, and remove the supernatant after the solution is clarified;

(3) Add 5~10 mL Buffer A to the centrifugal tube with magnetic beads, close the lid, and swirl for 15 s to re-suspend the magnetic beads. Place the centrifugal tube on the magnetic separator, magnetic separation, remove the supernatant, repeat washing 2 times.

(Note: In the process of magnetic separation, in order to reduce the loss of magnetic beads during use, after the solution is clarified, close the centrifugal tube cover, keep the centrifugal tube still on the magnetic separator, and turn the magnetic separator and the centrifugal tube up and down several times, so that the clarified solution can be washed with the residual magnetic beads on the centrifugal tube cover, and let it stand for a moment, so that the solution can be re-clarified; Same as below.)

4. The target protein binds to the magnetic bead

(1) The bacteria with 1 g wet weight was suspended with 10 mL Buffer A, and the crude protein sample was obtained after crushing and cracking;

(2) The crude protein sample was added into the centrifuge tube equipped with pretreatment magnetic beads, and the cover of the centrifuge tube was tightly closed;

(3) The centrifuge tube was placed in a vortex mixer for 15 s, and then placed on a rotating mixer for 20~30 min at room temperature (if necessary, it could be rotated at a low temperature of 2~8°C for about 1 h to prevent the degradation of the target protein);

(4) Place the centrifugal tube on the magnetic separator for magnetic separation, and remove the supernatant to the new centrifugal tube for subsequent detection. Remove the centrifugal tube from the magnetic separator for subsequent washing steps.

5. Magnetic bead washing

(1) Add 5~10 mL Buffer A into the centrifuge tube equipped with magnetic beads, rotate the mixture for 2 min, magnetic separation, and remove the cleaning solution to the new centrifuge tube for sampling and testing;

(2) Add 5~10 mL Buffer A to the centrifuge tube equipped with magnetic beads to re-suspend the magnetic beads and transfer the magnetic bead suspension to the new centrifuge tube to avoid the non-specific adsorption protein on the wall of the original centrifuge tube to contaminate the target protein; Magnetic separation, remove the supernatant to the cleaning solution collection pipe.

6. Target protein elution

(1) Add 2~5 mL Buffer B (the user can change the elution volume to adjust the target protein concentration as required) into the centrifuge tube, cover the centrifuge tube tightly, and then place the centrifuge tube on the rotating mixer, and rotate mixing for 2 min at room temperature; Magnetic separation, eluent collected into a new centrifuge tube, that is, the purified target protein sample;

(2) If necessary, the above steps can be repeated once to collect the sample into a new centrifuge tube to test whether the target protein is completely eluted.

7. Magnetic beads cleaning and preservation

After simple cleaning treatment after use, magnetic beads can continue to be used for subsequent purification operations, and can also be stored for a long time. Users can choose different cleaning methods according to the use of magnetic beads, mainly in the following cases:

Case 1: When the number of repeated use is small and the binding ability is not significantly reduced, the high pH buffer and low pH buffer can be used for cleaning

(1) High pH washing (alkali washing) : 10 mL Buffer C (0.1 M Tris-HCl, 0.5 M NaCl, pH 8.5) is added to the magnetic bead after use, and the vortex oscillates for 60 s, magnetic separation, and the supernatant is removed.

(2) Low pH washing (pickling) added 10 mL Buffer D (i.e. 0.1 M sodium acetate, 0.5 M NaCl, pH 4.5), swirled for 60 s, magnetic separation, removal of supernatant;

(3) Repeat alkaline washing, pickling alternately cleaning 2 times, a total of 3 times.

Case 2: The ability of magnetic beads to bind target proteins decreased significantly due to precipitation, denaturation or accumulation of non-specific adsorption proteins. To remove precipitated or denatured proteins, wash as follows:

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(1) Wash with 5 mL 6 M guanidine hydrochloride twice, swirl for 60 s each time, magnetic separation, remove the supernatant;

(2) Wash with 10 mL 1×PBS for 3 times, each vortex oscillation 60 s, magnetic separation, remove the supernatant.

Case 3: Remove hydrophobic binding substances, can be washed as follows:

(1) Wash with 5 mL 70% ethanol or non-ionic surfactant with concentration of 0.1% for 3 times, swirl for 60 s each time, magnetic separation, remove the superserum;

(2) Wash with 10 mL 1×PBS for 3 times, each vortex oscillation 60 s, magnetic separation, remove the supernatant.

Note: After the cleaning operation of Case 1 or Case 2 is completed, if the user needs to continue using the magnetic beads for protein purification, it is necessary to wash them with Buffer A 2-3 times first. If you do not need to continue to use, wash the magnetic beads with 20% ethanol for 2 to 3 times, and then add 20% (V/V) ethanol to the magnetic beads to make the total volume of 10 mL, stored at 2 to 8°C.

Optimization of protein purification process

The above operation process is applicable to the purification of most GST fusion proteins. According to the different binding properties of target proteins and GST fusion protein purification magnetic beads, users can optimize the purification process from the following aspects to improve the recovery and purity of target proteins.

1. Reference methods to improve the recovery rate of target protein:

(1) Prolonging incubation time of protein solution and magnetic beads;

(2) The addition of 1-10 mM DTT in the sample and buffer helped to improve the binding of some GST fusion proteins to magnetic beads;

(3) Add appropriate protease inhibitors to prevent target protein degradation;

(4) Increase the amount of magnetic beads;

(5) Prolong the elution time of the target protein or increase the elution frequency;

(6) Using freshly prepared Buffer B to ensure the target protein elution efficiency.

2. Reference methods to improve the purity of the target protein:

(1) Avoid severe ultrasonic breakage that causes GST tag to break with the target protein;

(2) Add appropriate protease inhibitors in the purification process to prevent the degradation of the target protein;

(3) Adding 0.1% Tween20 or 2% NP-40 to the sample solution and buffer can reduce the adsorption of non-specific proteins;

(4) Extend the washing time and increase the number of washing times;

(5) Glutathione eluted target protein was reduced by gradient concentration.

Notes

(1) Please read this user manual carefully before using this product for the first time;

(2) Freezing, drying and high-speed centrifugation should be avoided during the use and preservation of magnetic beads;

(3) Before using this product, be sure to fully oscillate to keep the magnetic beads in uniform suspension;

(4) Please choose a good quality pipette suction head and centrifugal tube to avoid leakage of magnetic beads during the wall attachment or mixing process;

(5) In the process of mixing the magnetic beads with the solution, if the solution is thick and cannot be re-suspended by turning the centrifugal tube, the pipette can be used to repeatedly suck or short-time vortex mixing to fully re-suspended the magnetic beads;

(6) The user can retain the supernatant removed by magnetic separation according to actual needs for sampling and testing in order to analyze the purification process and optimize the protein purification process;

(7) This product can be reused, when reused, it is recommended to purify the same protein, and when purifying different kinds of protein, it is recommended to use new magnetic beads to prevent cross-contamination;

(8) This product needs to be used with a magnetic separator;

(9) This product is for research use only.

Ordering Information

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