GST-tag Protein Purification Kit

Product Description

Glutathione S-transferase (GST) is a key enzyme in the glutathione binding reaction and catalyzes the initial step of the glutathione binding reaction. GST is often used to construct fusion proteins in the field of genetic engineering research, because the GST fusion protein expression system has the characteristics of efficient expression of the target protein, good solubility of the expression product, and easy separation and purification of the product.

GST-tagged recombinant proteins can be specifically bound to GST-tag Purification Resin by GST, while other proteins cannot be bound, and GST-tagged recombinant proteins can be eluted by excess reduced glutathione (GSH) solution, thereby achieving GST-tagged protein isolation and purification. Since the purification process is always maintained under mild and non-denaturing conditions, the obtained target protein can maintain its own biological activity, so it can be used for routine structural and functional studies, antibody production, and protein-protein interactions, protein-nucleic acid interactions, etc.

This product is a simple, rapid, efficient and highly specific kit for the purification of GST-tagged proteins, which provides the relevant reagents and affinity chromatography column empty column tubes required for the purification of GST-tagged proteins, which brings great convenience to the purification of GST-tagged proteins. Compared with most similar products currently on the market, non-specific protein binding is lower, pressure tolerance is stronger, and GSH conjugation is more stable.

This product can purify up to 10 GST-tagged recombinant proteins, with a maximum purification volume of approximately 12 mg per protein. For GST-tagged recombinant proteins with a molecular weight of 50 kD, a maximum of 10 proteins of approximately 4-6 mg can be purified in one package. Without the use of an affinity chromatography column empty column tube, this kit is sufficient for 500 small purifications of GST-tagged proteins with reference to these instructions.

Composition and storage conditions

Components	K4201-10 mL
GST-tag Purification Resin	10 mL
Lysis buffer	180 mL
Eluting buffer (GSH required)	60 mL

GSH	184 mg
Affinity Chromatography Column (3 mL, empty)	10 sets
Store GST-tag Purification Resin at 4°C and the other components at -20°C for 1 year.	

Experimental manipulation

1. Preparation of 10X GSH solution and elution buffer

1.1 Preparation of 10X GSH solution: 184 mg of GSH is dissolved and mixed with 6 mL of Eluting buffer (GSH required) to produce 10X GSH solution. The prepared 10X GSH solution is stored at -20°C and is effective for at least one year.

1.2 Preparation of elution buffer: Elution buffer is mixed in a ratio of 9:1 to Eluting buffer (GSH required): 10X GSH solution (step 1.1). For example, 9 mL of Eluting buffer (GSH required) is mixed with 1 mL of 10X GSH solution, and the mixed solution is the elution buffer. Since GSH is easily oxidized in solution and fails, the elution buffer should be freshly prepared, and the prepared elution buffer should be stored at 4°C and effective within two weeks. The components of the elution buffer were 50 mM Tris, 150 mM NaCl, 10 mM GSH, pH 8.0.

2. Large-scale purification of GST-tagged proteins (this method is suitable for purification of large protein samples, such as bacterial volumes of 150 mL and above)

2.1 For fresh or thawed bacterial pellets, resuspend the cells by adding Lysis buffer at a ratio of 4 mL (2-5 mL acceptable) per gram of bacterial pellet wet weight. If necessary, add the appropriate amount of protease inhibitor cocktail to the Lysis buffer before lysing the bacteria.

2.2 Sonication of bacteria on ice. The ultrasonic power was 200-300 W, and each sonication was 10 s, with an interval of 10 s, and a total of 6 sonications. *[Note] The specific method of ultrasonic treatment must be explored and optimized according to the specific model of ultrasound instrument.*

2.3 Centrifuge at 10,000 g at 4°C for 20-30 min, collect bacterial lysate supernatant and place in an ice water bath or on ice. 20 μL of the supernatant can be reserved for subsequent testing. *[Note] The supernatant is filtered with a 0.22 μm or 0.45 μm membrane for further purification. If insoluble impurities are mixed in the supernatant, the purity of the protein obtained in subsequent purification will be seriously affected.*

2.4 Discard the stock solution by centrifugation at 1 mL of GST-tag Purification Resin at 4°C (1,000 g×10 s), add 0.5 mL of Lysis buffer to the gel to resuspend and equilibrate the gel, centrifuge at 4°C (1,000 g×10 s) to discard the liquid, repeat the equilibration 1-2 times and discard the liquid. Approximately 4 mL of bacterial lysate supernatant was added to it and slowly rocked for 60 minutes at 4°C on a side or horizontal shaker. (The amount of GST-tag Purification Resin used is related to the amount of protein expression, generally 1 mL of GST-tag Purification Resin binds 4-5

mg of protein)

2.5 Load a mixture of Lysis buffer and GST-tag Purification Resin into the Affinity Chromatography Column (3 mL, empty) provided with this kit. *[Note] You can also take 1 mL of GST-tag Purification Resin mixed well, then equilibrate it with 0.5 mL Lysis buffer for 2-3 times, and then add about 4 mL of bacterial lysate to supernatant, and then collect the flow-through solution and repeat the column 3-5 times to fully bind the target protein. Mixing and then loading the column is more cumbersome to operate, but it is more conducive to the full binding of GST-tagged proteins to the resin.*

2.6 The lid at the bottom of the cartridge was opened, and the liquid in the column was drained under the action of gravity, and approximately 20 μL of flow-through fluid was collected for subsequent analysis.

2.7 Wash the column 5 times, add 0.5-1 mL of Lysis buffer each time, and collect approximately 20 μL of the through-column liquid for subsequent analysis and detection. The Bradford method (K4103) can be used to measure the protein content quickly and easily in each wash and eluate during column washing and the next step of elution, so that the number of washes and elutions can be increased or decreased. *Note: If the purity of the protein obtained in the future is not high enough, the number of column washes can be increased by 2-3 times.*

2.8 Elute the protein of interest 6-10 times with 0.5 mL of elution buffer each (step 1.2). Collect each eluate separately into a different centrifuge tube. The resulting eluate is a sample of purified GST-tagged protein.

Notes

1. This kit can be used not only for the purification of GST-tagged recombinant proteins expressed by Escherichia coli, but also for GST-tagged recombinant proteins expressed in mammalian cells, insect cells, baculovirus and other expression systems.
2. GST-tagged recombinant protein has the advantages of high solubility when expressed in Escherichia coli, and it is easy to maintain the intact activity of the target protein. Many eukaryotic proteins are expressed in the form of inclusion bodies in E. coli, but when they are fused to GST in E. coli, a considerable number can achieve soluble expression, which facilitates subsequent purification.
3. If there is a site-specific protease recognition site between the GST tag and the protein of interest, such as PreScission Protease (K1101) or TEV Protease (K1098), the GST tag can be excised with the corresponding protease.
4. In general, the addition of a GST tag to the N-terminus of the protein of interest preserves the enzymatic activity of GST, facilitating the purification of GST-tagged recombinant proteins using GST-tag Purification Resin.

5. GST-tag Purification Resin uses a highly cross-linked 6% agarose gel matrix and uses further optimized adapter and GSH conjugation technology to bind GST-tagged recombinant proteins with high capacity and specificity. The gel has a particle diameter of 45-165 μm and can tolerate a maximum pressure of 0.025 MPa, or about 5.8 psi. The recommended flow rate for protein purification with a fixed flow rate is 0.5 mL/min. Store in 20% ethanol, 5 ml of the total volume of 10 mL is gel and 5 ml is liquid, and the gel should be fully resuspended before aspiration.
6. GST-tag Purification Resin has a large binding capacity for GST-tagged recombinant proteins, and its maximum binding capacity to GST is 5-6 mg protein/ml gel, which is comparable to that of similar products of internationally renowned brands. The maximum binding amount in actual use depends on the molecular weight of the GST-tagged recombinant protein to be purified, the larger the molecular weight, the greater the maximum binding capacity, and the smaller the molecular weight, the smaller the maximum binding capacity. The actual maximum purification per mL of gel is approximately 8-12 mg for GST-tagged recombinant proteins with a molecular weight of 50 kD and 12-18 mg per mL of gel for GST-tagged recombinant proteins with a molecular weight of 100 kD. However, for proteins with the same molecular weight, the maximum capacity of binding will also be different due to the different characteristics of the protein itself.
7. Do not freeze GST-tag Purification Resin at -20ºC or lower.
8. GST-tag Purification Resin should always keep the gel moist during storage and purification.
9. A variety of affinity chromatography column empty column tubes (PC2051/ PC2052/ PC2053/ PC2054/ PC2055/ PC2056) with different capacities can be used with this product.
10. If centrifugation does not completely remove insoluble matter from the protein sample, the sample solution can be filtered through a 0.45 μm filter.
11. Purification of GST-tagged proteins should always be maintained under native conditions, and if the fusion protein is expressed as inclusion bodies, urea and guanidine hydrochloride need to be removed by dialysis and protein refolding after dissolving the inclusion bodies with 8 M urea or 6 M guanidine hydrochloride before purification with this product.
12. Purification of protein samples should be completed as soon as possible after collection and should always be placed at 4ºC or in an ice bath to slow down protein degradation. In order to effectively inhibit protein degradation, an appropriate amount of protease inhibitor cocktail can be added to the lysate, such as K1024/K1009 protease inhibitor cocktail (for bacterial and fungal extraction), or K1007/K4002 protease inhibitor cocktail (general-purpose).
13. This product is for scientific research purposes only.

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