BeaverBeads™ GSH

Product Introduction

BEAVER GST fusion protein purified beads are a new functional material designed for efficient and rapid purification of glutathione S-transferase (GST) fusion protein. It can extract the target protein with high purity from the biological samples in one step using magnetic separation method and it greatly simplifies the purification process and improves the efficiency of purification. This method is suitable for scientific research and industrial field to purify GST fusion protein.

Compared with the traditional column chromatography purification method, Beaverbeads method does not need to carry out high-speed long time centrifugation and filter filtration to the crude protein samples, or control the flow rate, and does not need the expensive chromatography equipment. The specific binding of the sample to the magnetic beads, washing and elution of target protein become very simple, fast and easy to operate. For skilled operators, high purified protein can be obtained within 1 h, and parallel processing of large scale samples can be easily achieved to save researchers time and cost.

Product Information

Note:

1：The amount of magnetic beads protein binding is related to the target protein characteristics, where only reference values are given.

Product name	BeaverBeads™ GSH
Bead size	30μm~150μm
GSH ligand content	20~30 μmol/mL（100% beads）
GST fusion protein binding capacity	Up to 10 mg/mL （100% beads）
Suspension concentration	10%（v/v）magnetic beads suspension
Preserving fluid	20%（v/v）ethanol
Chemical stability	At room temperature can tolerate 1 h of 70% ethanol, 6 M hydrochloride, 0.1 M hydroxide, 0.1 M acetic acid
Shelf Life	2 years when stores at 2 ~ 8 ℃,

2：1 mL magnetic beads suspension contain 100 μL of magnetic beads.

Scope of Application

It is suitable for the separation and purification of glutathione S-transferase (GST) fusion protein, glutathione transferase and other proteins that are compatible with glutathione.

Operational Steps

The binding performance of the target protein and the magnetic beads will directly affect the purification efficiency of the target protein. The preparation of various buffers will also affect the recovery and purity of the target protein to a certain extent. Therefore, before the large-scale protein is purified, the user should design the experiment and screen out the buffer for the target protein, including Binding / Washing Buffer (Buffer A) and Elution Buffer (Buffer B). The following provides a strong binding of the GST tag protein purification process for the user reference.

1. Preparation of buffer solution

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: 0.1 M Tris solution 50 mL, 0.307 g reduced glutathione, then adjusted to pH 8.0 with 0.1 M hydrochloric acid then add deionized water to 100 mL.

Note: 1. Reduced glutathione is easy to be oxidized, **Buffer B requires current use to prepare.**

2. Different GST fusion protein combines magnetic beads at different level. For most of the GST fusion protein, using 10 mM reduced glutathione Buffer B can elute the target protein; while for some GST fusion protein with strong binding capacity, operator can prolong the elution time, increase elution times, or increase the concentration of GSH in Buffer B;

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

2. Sample processing

This User's Manual provides below three sample processing:

(1) Escherichia coli, yeast and other intracellular protein expression: dilute the expression cells with appropriate amount of Buffer A, then add protease inhibitors (eg. the final concentration of 1 mM PMSF); ice bath ultrasound to lysis cell to make crude protein samples. If the sample is too viscous, adding the appropriate amount of nuclease to the crude sample and place it for 30 min on ice to degrade the 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 protein supernatant and dilute with equal amount of Buffer A to balance to make crude protein samples.

(3) Animal cells intracellular expression protein: Take appropriate amount of animal cells, wash with plenty of PBS once and discard the supernatant; resuspend with 1% (v / v) Triton X-100 or 1% (v / v) NP-40 Buffer A; add protease inhibitor (eg. the final concentration of 1 mM PMSF); then place on the ice for 10 min to make crude protein samples.

3. Beaverbeads pre-processing

In general, the application amount of the bead is calculated by the target protein yield and the magnetic bead binding information. For example, using E.coli to express a target protein. 1g wet weight of the fungus is obtained from 250ml fermentation broth. So the target protein production estimates at 5~10mg. Users will need 10ml 10% magnetic beads suspension for the purification of target protein. Below are detail operation processes:

(1)Put BEAVER magnetic beads on the vortex mixer to mix, and then use a pipette to take 10 mL of magnetic beads suspension into a centrifuge tube.

(2)Put the centrifuge tube on the magnetic separator. Remove the supernatant when the solution is clear.

(3)Put 5~10ml Buffer A into the above centrifuge tube and cover the lid tightly, swirl for 15 sec to make the beads resuspended. Then put the centrifuge tube on the magnetic separator* and remove the supernatant. Repeat this step twice.

（*Note: In the process of the magnetic separation and to reduce the loss of magnetic beads, cover the centrifuge tube lid tightly when the solution is clear. Keep the centrifuge tube in the magnetic separator and flip the separator and centrifuge tube up and down several times to make the lid's residual beads washed. Put it aside to let the solution become clear again; the same as below）

4.The binding of target protein and Beaverbeads

(1) Use 10 mL Buffer A to suspend 1 g wet weight fungus and then crush and crack to form a crude protein sample;

(2) Add the crude protein sample to a centrifuge tube which is containing pretreated beads, and then cover the lid;

(3) Place the centrifuge tube in a vortex mixer for 15 sec and then place on a rotary mixer for 20 to 30 min at room temperature (if necessary, it could be rotated for one hour at a low temperature of 2 to 8 ° C to prevent degradation of the target protein);

(4) Place the centrifuge tube on a magnetic separator for beads separation, and remove the supernatant to a new centrifuge tube for subsequent detection. Take out the centrifuge tube from the magnetic separator for following washing steps.

5. BeaverBeads washing

(1) Add 5 ~ 10 mL Buffer A to the centrifuge tube with beads, rotate for 2 min, then make beads magnetic separation and remove the washing liquid to the new centrifuge tube for later sampling test;

(2) Add 5 ~ 10 mL Buffer A to the above centrifuge tube to make the beads resuspended, and then transfer them to a new centrifuge tube to avoid contamination of the target protein with nonspecific adsorbate on the wall of the original centrifuge tube; then make beads magnetic separation and remove the supernatant to the cleaning solution collection tube.

6.The elution of target protein

(1) Add 2 ~ 5 mL Buffer B (the user can change the elution volume based on the target protein concentration) to the centrifuge tube, cover the centrifuge tube lid, and then place the centrifuge tube on the rotary mixer, rotating at room temperature for 2 min; make magnetic separation, then collect the elution into a new centrifuge tube to make the purified target protein sample;

(2) If necessary, repeat the above steps once and collect the sample into a new centrifuge tube to test if the target protein elutes completely.

7.The cleaning and preservation of Beaverbeads

After a simple cleaning, the used beads can be used again or store long periods of time. Users can choose different cleaning methods according to the usage of the beads, which is shown in the following cases:

Case 1: When usage is less and the binding ability is not obviously reducing, you can use high pH and low pH buffer switch-washing method.

(1) High pH Washing (alkali washing): add used beads to 10 mL of Buffer C (0.1 M Tris-HCl, 0.5 M NaCl, pH 8.5) solution, vortex swirl for 60 s, make beads magnetic separation, then remove supernatant;

(2) Low pH Washing (acid washing): add 10 mL Buffer D (0.1 M sodium acetate, 0.5 M NaCl, pH 4.5), vortex swirl for 60 sec, make beads magnetic separation, then remove the supernatant;

(3) Repeat step (1) - (2) 2 times, a total of 3 times washing.

Case 2: When usage is large and the binding ability is significantly reducing due to the accumulated binding of precipitated, denatured or nonspecific adsorbate proteins. To remove the precipitate or denatured protein, follow below wash steps:

(1) Wash with 5 mL 6 M guanidine hydrochloride twice, vortex swirl for 60 sec each time, make beads magnetic separation, then remove supernatant;

(2) Then wash three times with 10 mL 1 x PBS, vortex swirl for 60 sec each time, make beads magnetic separation, then remove supernatant.

Case 3: To remove the hydrophobic binding material, follow below wash steps:

(1) Wash three times with 5 mL 70% ethanol or 0.1% nonionic surfactant, swirl for 60 sec each time, make beads magnetic separation, then remove supernatant;

(2) Then wash with 10 mL 1 x PBS 3 times, vortex swirl for 60 sec each time, make beads magnetic separation, then remove supernatant.

Note：After completion of Case 1 or Case 2, if the user needs to continue to use the beads for protein purification, wash with Buffer A for 2 to 3 times. If not, then wash the beads 2 to 3 times with 20% ethanol, then add 20% (v / v) ethanol to the beads making total volume to 10 mL and store at 2 ~ 8 ° C.

Optimization of Protein Purification Process

The above procedure is suitable for the purification of most GST fusion proteins. Depending on the different binding properties between the target protein and the GST fusion protein purified beads, the purification process can be optimized from the following aspects to improve the recovery and purity of the target protein.

1. **A reference method to improve target protein recovery：**

(1) Prolong the incubation time of the protein solution with the Beaverbeads;

(2) Add 1 ~ 10 mM DTT to the sample and buffer helps to improve the binding of some GST fusion proteins to Beaverbeads;

(3) Add a little protease inhibitor to prevent degradation of the target protein;

(4) Increase the amount of Beaverbeads;

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

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

2. A reference method to improve the purity of target protein:

(1) Avoid vigorous ultrasonic rupture which may cause breakage of GST tag and the protein;

(2) Add a little protease inhibitor during the purification process to prevent degradation of the target protein;

(3) Add 0.1% Tween20 or 2% NP-40 to the sample solution and buffer to reduce the adsorption of nonspecific proteins;

(4) Extend the washing time, increase the washing times;

(5) Use gradient concentration of reduced glutathione to elute target protein

Note

(1) Be sure to read this user's manual carefully before using this product for the first time;

(2) The freezing, drying and high-speed centrifugation and other operations should be avoided during the use and preservation of the beads ;

(3) Before using this product, be sure to fully oscillate the beads to maintain a uniform suspension;

(4) Please use pipette tip and centrifuge tube with good quality to avoid the stick of the wall or BEAVER beads loss caused by tube leakage during the mixing process;

(5) In the process of mixing the beads and the solution, if the solution is viscous, the magnetic beads cannot be resuspended by just turning around the centrifuge tube, using short-term whirlpool mixing to make the BEAVER beads fully resuspended;

(6) Users can retain the removed supernatant 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. When using different kinds of proteins, it is recommended to use new beads to prevent cross contamination;

(8) This product must be used with magnetic separation equipment.

(9) This product is for research use only.

Cat No.	Product name	Specification	Concentration
70601-5	BeaverBeads™ GSH	5 mL	10%（v/v）
70601-100	BeaverBeads™ GSH	2×50 mL	10%（v/v）
70601-1000	BeaverBeads™ GSH	4×250 mL	10%（v/v）

For support, please visit: www.beaverbio.com/support or email: Service@beaverbio.com