GSH Magnetic Beads for GST Tag Protein Purification

Notice!

Magnetic beads should be stored at 4°C, do not freeze.
Do not dry the magnetic beads.

1. Package Information

Component	Cat#: B23700	Cat#: B23701	Cat#: B23702	Cat#: B23703	Cat#: B23704
GSH Magnetic Beads for Gst Tag Protein Purification	500 ul	5 mL	10 mL (5 mL X2)	25 m (5mL X5)	50 mL (5 mL X10)

1. Diameter of the bead is about 50 μm~100 μm.
2. Amount of GSH Ligands is 20~30 μmol/mL
3. Binding capacity to proteins is about 5~10 mg/mL (pure beads). The protein binding capacity is provided as a reference. True binding capacity is correlated with the nature of the target proteins.
4. Working temperature is between 4℃~30℃.
5. GSH Magnetic Beads for Gst Tag Protein Purification is stable in 70% Ethanol, 6M Guanidine Hydrochloride, 0.1M NaOH and 0.1M Acetic Acid at room temperature for 1h.

2. Storage Information

GSH Magnetic Beads for Gst Tag Protein Purification is provided as 10% (v/v) beads suspension (1 mL suspension contains 100 μL beads) in the storage buffer of 20% Ethanol. This product can be stored at 4℃~30℃, and it is suggested to be stored at 4℃for long-term stability.

3. Notices for This Product

1. Read the User Manual carefully before the first use.
2. Avoid freezing, drying and high-speed centrifugation when using and storing the beads.
3. Shake the beads thoroughly to keep them a homogeneous suspension before use.
4. Choose well-qualified pipette tips and centrifuge tubes in case of the beads loss from adherence or leakage when mixing.
5. When mixing the beads and the solutions, aspirate the beads with the pipette repeatedly or vortex briefly, if the solution is too thick to resuspend manually.
6. To analyze the purification process and optimize the protein purification procedure, keep the removed supernatant (elution) assay for protein detection if necessary.
7. The product can be used multiple times, as long as the same kinds of proteins are being assayed, when purifying a different kind of protein sample, choose the new beads to prevent the interactive contamination.
8. The product should be used with the magnetic separator.
9. The product can be stably stored at 4℃ for two years.
10. The product can only be used for research purposes.

4. Reference Protocol for Gst-tag Protein Purification

The binding capability of the target proteins and the beads has a direct effect on the purification efficiency, as does the preparation of the buffer on the recovery ratio and the purity of the target proteins. Therefore, users should design a preliminary experiment before a large-scale purification to select the appropriate buffer, which is recommended to include binding buffer (Buffer A) and elution buffer (Buffer B). A sample protocol depicting a GST fusion protein purification procedure with a relatively strong binding capability is provided below.

4.1. Preparation of Buffer

Buffer A (Binding Buffer): 140 mM NaCl, 2.7 mM KCl, 10 mM Na2HPO4, 1.8 mM KH2PO4, pH 7.4

Buffer B (Elution Buffer): 50 mM Tris- HCl, 10 mM GSH, pH 8.0. Preparation Method: Add 0.307g GSH to 100 mL 0.1M Tris- HCl, then adjust pH to 8.0 with 0.1 M HCl and add deionized water to a total volume of 100 mL.

Note:

1. GSH is oxidized quickly, so prepare Buffer B immediately before use.
2. The binding capability between various GST- fusion proteins and the beads is different. Most GST- fusion proteins can be eluted by Buffer B which contains 10 mM GSH. A few GST- fusion proteins which have a relatively strong binding capability, may require prolonged elution, increased time of the elution, or increased concentration of GSH in Buffer B.
3. To increase the stability of the target proteins, users may add 1~5 mM EDTA, 1~10 mM DTT, 0.1~ 1% Triton X- 100, or 0.1~ 1% Tween 20 to Buffer A and Buffer B.

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4.2. Preparation of Samples

This User Manual provides preparation of three samples as follows:

1. Intracellular- expressed proteins in bacteria, yeasts and other cells: dilute the cells with Buffer A and add a protease inhibitor (like BiotoolTM Protease Inhibitor Cocktail), lysis cells in an ultrasonic ice bath to generate the crude protein samples. Once the samples are homogenized, add nucleases to the crude samples and place on ice for 30 min to degrade nucleic acids. If the yield of the target proteins is expected to be low, centrifuge the crude proteins to concentrate sample.
2. Extracellular- expressed proteins: harvest the supernatant containing the extracellular- expressed proteins and add equal amounts of Buffer A to generate the crude protein samples.
3. Intracellular- expressed proteins in animal cells: wash animal cells once with PBS, scrape into a tube and centrifuge. Discard the supernatant and resuspend the cells with Buffer A containing 1% (v/v) Triton X- 100 or 1% (v/v) NP- 40, add a protease inhibitor, lastly, place the tube on ice for 10 min to generate the crude protein samples.

4.3. Pretreatment of Beads

The amount of beads used is determined by users' calculations according to the target protein yield and the beads' capacity. For example, for a protein expressed in E.coli, 1g-wet weight of microbes can be obtained from 250 mL broth. The yield of the target protein will come to 5~10 mg by the experimental estimation, and users will need 10 mL 10% beads suspension for that target protein purification. Details of this example are presented as follows.

1. Homogenize the Biotool GSH Magnetic Beads fully with a vortex, and pipette 10mL beads suspension to a centrifuge tube.
2. Place the tube on the magnetic separator, discard the supernatant when the liquor becomes clear.

Note:

Make sure that every time you remove the supernatant by pipetting or pouring only when the tube is still attached to the magnetic separator!

3. Add 5~10 mL Buffer A to the tube, close the tube cap tightly, oscillate the tube on a vortex for 15s to resuspend the beads; place the tube on the separator for a magnetic separation* and discard the supernatant.
4. Repeat step (3) twice.

Attention *: in order to decrease the loss of beads during magnetic separation, close the centrifuge tube cap tightly and keep the tube on the separator until the liquid becomes clarified. Next, invert the separator and the tube for several times by hand to wash the beads adherent to the cap by the clear liquid, let the tube stand on the bench top until the liquid settles and becomes clear again.

4.4. Binding of Target Protein and Beads

1. Suspend 1g- wet weight of microbes with 10 mL Buffer A, and lysis. This is the crude protein samples.
2. Apply the samples to the tube containing the pretreated beads and close the tube cap tightly.
3. Vortex the tube for 15 s, then mix lightly and agitate it at room temperature for 20~30 min (if necessary, mix the tube at 4~ 8℃ for 1 h to prevent the target protein degradation).
4. Place the tube on the magnetic separator for separation, then pipette the supernatant to a new centrifuge tube. Take the tube off the magnetic separator and proceed to washing steps.

4.5. Washing of Beads

1. Add 5~10 mL Buffer A to the centrifuge tube containing the beads, shake them for 2 min, perform a magnetic separation and pipette the elute to a new tube (keep elution for a following test).
2. Add 5~10 mL Buffer A to the centrifuge tube containing the beads to suspend them, pipette the beads suspension to a anew tube to avoid nonspecific-adherent proteins in the previous tube contaminating the target ones; perform a magnetic separation and pipette the supernatant to the elute tube.

4.6. Elution of Target Proteins

1. Add 2~5 mL Buffer B to the tube containing the beads (users may change the elution volume to adjust the concentration of the target proteins if necessary), close the tube cap tightly and mix and shake them at room temperature for 2 min, perform a magnetic separation and collect the elution into a new tube. This is the purified target protein samples.
2. If necessary, repeat the elution step (1) once again and collect the samples into a new tube to examine whether the target proteins have been eluted thoroughly.

4.7. Washing and Storage of Beads

The beads can be used for purification again after washing treatment. Additionally, for a longtime storage, users may choose different washing methods according to the usage situation of the beads.

Situation 1: When the beads are not repeatedly used for many times, or the binding capability of the beads doesn't decrease obviously, users may wash the beads by an alternative mode with high pH and low pH.

1. Add 10 mL Buffer C (0.1 M Tris- HCl, 0.5 M NaCl, pH 8.5) to the used beads, place the tube on a vortex for 60 s, perform a magnetic separation and discard the supernatant.
2. Add 10 mL Buffer D (0.1 M Sodium Acetate, 0.5 M NaCl, pH 4.5), place the tube on a vortex for 60 s, perform a magnetic separation and discard the supernatant.
3. Repeat step (1) and (2) above twice.

Situation 2: When the beads are repeatedly used for many times, the binding capability of the beads and the target proteins will decrease, due to the precipitation, denaturation or accumulation of the nonspecific adherent proteins. Users may adopt the following methods to wash the precipitates or denatured proteins from the beads:

1. (1) Wash the beads with 5mL 6 M Guanidine Hydrochloride twice, place the tube on a vortex for 60 s each time, perform a magnetic separation and discard the supernatant.
2. Wash the beads with 10 mL 1×PBS for three times, place the tube on a vortex for 60s each time, perform a magnetic separation and discard the supernatant.

In order to remove the hydrophobic- conjugated substances, wash the beads as follows:

1. Wash the beads with 5 mL 70% Ethanol or 0.1% non-ionic surfactant for three times, place the tube on a vortex for 60 s each time, perform a magnetic separation and discard the supernatant.
2. Wash the beads with 10 mL 1×PBS for three times, place the tube on a vortex for 60 s each time, perform a magnetic separation and discard the supernatant.

After finishing the washing operations for Situation 1 and 2, if users need to use the beads again for protein purification, wash the beads with Buffer A for 2~3 times as in the protein purification steps. If storing them for a brief time, wash the beads with 20% Ethanol for 2~3 times, then add 20% (v/v) Ethanol to the beads to a total volume of 10 mL and store them at 4~8℃.

5. Trouble Shooting

Q1: How to increase the Recovery Ratio of Target Proteins?

A1: You can try the following measures:

1. Prolong the incubation of the proteins and the beads.
2. Add 1~10 mM DTT to the samples and buffer, which helps increase the binding capability of certain GST- fusion proteins and the beads.
3. Add various protease inhibitors to prevent the protein degradation.
4. Increase the amount of beads.
5. Prolong the elution of the proteins or increase times of the elution.
6. Use the just- prepared fresh Buffer B to ensure efficient elution of the target proteins.

Q2: How to increase the purity of target proteins?

A2: You can try the following measures:

1. Avoid ultrasonic lysis to keep the GST tag and the target protein intact.
2. Add various protease inhibitors to prevent the protein degradation.
3. Add 0.1% Tween 20 or 2% NP- 40 to the samples and buffer to decrease the adsorption of the nonspecific proteins.
4. Prolong the washing of the proteins and increase times of the washing.
5. Elute the target proteins by GSH gradient concentration.