GST Fusion Protein Purification Kit

Catalog No.: BRC0021

Product Introduction

EnkiLife GSH-magnetic beads are a novel functional material designed for the efficient and rapid purification of glutathione S-transferase (GST) fusion proteins. Through magnetic separation technology, target proteins can be purified directly from biological samples in a single step, dramatically simplifying the purification process and improving efficiency. This makes it ideal for both research and industrial applications requiring convenient GST fusion protein purification.

Compared to traditional column chromatography, EnkiLife GSH-magnetic beads beads eliminate the need for multiple time-consuming high-speed centrifugation steps, filter membrane filtration, flow rate control, or expensive chromatography equipment. The specific binding of samples to beads, washing, and target protein elution are simple, fast, and easy to operate. Skilled users can obtain highly pure target protein within 1 hour and easily achieve high-throughput, large-scale parallel sample processing, saving both time and cost for researchers.

Product Information

Parameter	Specification
GSH-magnetic bead	5mL
Buffer A	100mL
Reduced Glutathione	307 mg, weigh as needed
Buffer B	50 mM Tris-HCl Buffer, pH 8.0±0.1 (100 mL)
Bead Size	30–150 μm
GSH Ligand Density	20–30 μmol/mL (100% bead volume)
Suspension Concentration	10% (V/V) bead suspension
Storage Solution	20% (V/V) ethanol
Chemical Stability	Stable for 1 hour at room temperature in: 70% ethanol, 6 M guanidine-HCl, 0.1 M NaOH, 0.1 M acetic acid
Shelf Life	2 years at 2–8°C

*Binding capacity varies depending on target protein characteristics; value provided is for reference only.

*Magnetic bead concentration: 100 μL beads per 1 mL suspension

Scope of Application

Suitable for purification of:

- Glutathione S-transferase (GST) fusion proteins
- Glutathione transferases

- Other proteins with affinity for glutathione

Operating Protocol

The binding efficiency of target protein to beads directly affects purification yield. Buffer composition also influences protein recovery and purity. Therefore, before large-scale purification, users should optimize buffers (Binding/Washing Buffer A and Elution Buffer B) for their specific target protein.
The following protocol is provided as a reference for a strongly binding GST-tagged protein:

Buffer Preparation

Buffer A (Binding/Washing Buffer): 140 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4, pH 7.4 (Essentially PBS)
Buffer B (Elution Buffer): Dissolve 0.307 g reduced glutathione in 100 mL of 50 mM Tris-HCl (pH 8.0±0.1). Prepare fresh as needed. Scale proportionally.
Important Notes:

1. Reduced glutathione oxidizes easily. Prepare Buffer B fresh before each use. Do not dissolve all glutathione at once.
2. GST fusion proteins exhibit varying binding strengths. For most proteins, 10 mM GSH in Buffer B is sufficient for elution. For strongly bound proteins, extend elution time, increase elution cycles, or raise GSH concentration.
3. You may add 1–5 mM EDTA, 1–10 mM DTT, 0.1–1.0% Triton X-100, or 0.1–1.0% Tween-20 to Buffers A and B to enhance protein stability.

Sample Preparation

Choose the appropriate method based on your expression system:
A. Intracellular Expression (E. coli, yeast):

1) Resuspend cell pellet (e.g., 1 g wet weight) in appropriate volume of Buffer A + protease inhibitors (e.g., 1 mM PMSF final).
2) Lyse cells by sonication on ice.
3) If lysate is viscous, add DNase (e.g., 10 µg/mL) and incubate on ice for 30 min to degrade nucleic acids.
4) Centrifuge if necessary to clarify.

B. Secreted Expression: Collect culture supernatant and dilute 1:1 with Buffer A.
C. Mammalian Intracellular Expression:

1) Harvest cells, wash once with PBS, discard supernatant.
2) Resuspend in Buffer A containing 1% (V/V) Triton X-100 or NP-40 + protease inhibitors.
3) Incubate on ice for 10 min.

Bead Pretreatment

Bead volume depends on target protein yield and bead binding capacity.
Example: 250 mL E. coli culture → 1 g wet cell weight → ~5–10 mg target protein → Use 10 mL of 10% bead suspension (contains 1 mL beads).

1. Vortex the MagGSH bead stock vigorously to ensure homogeneous suspension.
2. Transfer 10 mL of bead suspension to a centrifuge tube.
3. Place tube on magnetic separator until solution clears. Remove supernatant.
4. Add 5–10 mL Buffer A, vortex 15 sec, perform magnetic separation, and remove supernatant.

Repeat wash twice.

Target Protein Binding

1. Resuspend 1 g wet cell pellet in 10 mL Buffer A, lyse, and clarify to obtain crude protein sample.
2. Add crude sample to pretreated beads. Cap tube securely.
3. Vortex 15 sec, then rotate at room temperature for 20–30 min (or 1 h at 2–8°C to prevent degradation).
4. Perform magnetic separation. Transfer supernatant to a new tube for analysis.
5. Proceed to washing steps.

Bead Washing

1. Add 5–10 mL Buffer A, rotate 2 min, perform magnetic separation, and collect wash solution for sampling.
2. Add 5–10 mL Buffer A, resuspend beads, and transfer bead suspension to a new tube to avoid contamination from proteins adsorbed to the tube wall. Perform magnetic separation and discard supernatant.

Protein Elution

1. Add 2–5 mL Buffer B to beads (adjust volume to control protein concentration). Cap tube.
2. Rotate at room temperature for 2 min.
3. Perform magnetic separation. Collect eluate—this is your purified protein.
4. Optional: Repeat elution once more and collect separately to verify complete recovery.

Bead Cleaning and Storage

After use, beads can be cleaned for reuse or stored long-term. Choose cleaning method based on usage:

Method 1: Mild Cleaning (for minimal use cycles when binding capacity hasn't down)

1) Alkaline wash: Add 10 mL Buffer C (0.1 M Tris-HCl, 0.5 M NaCl, pH 8.5), vortex 60 sec, magnetically separate, remove supernatant.
2) Acidic wash: Add 10 mL Buffer D (0.1 M sodium acetate, 0.5 M NaCl, pH 4.5), vortex 60 sec, magnetically separate, remove supernatant.
3) Repeat alkaline/acid wash cycle 2 more times (total 3 cycles).

Method 2: Deep Cleaning (for heavily used beads with reduced binding capacity)

1) Wash twice with 5 mL 6 M guanidine-HCl (vortex 60 sec each) to remove precipitated/denatured proteins.
2) Wash three times with 10 mL 1×PBS.

Method 3: Removing Hydrophobic Contaminants

1) Wash three times with 5 mL 70% ethanol or 0.1% non-ionic detergent.
2) Wash three times with 10 mL 1×PBS.

Post-Cleaning:

- If reusing: Wash 2–3 times with Buffer A before next purification.
- If storing: Wash 2–3 times with 20% ethanol, then resuspend beads in 20% ethanol to original volume. Store at 2–8°C.

Protocol Optimization

To Improve Recovery:

1. Extend protein-bead incubation time.
2. Add 1–10 mM DTT to samples/buffers to enhance binding.
3. Use protease inhibitors to prevent degradation.
4. Increase bead volume.
5. Extend elution time/increase elution cycles.
6. Use freshly prepared Buffer B.

To Improve Purity:

1. Avoid harsh sonication that may cleave GST tag.
2. Add protease inhibitors throughout purification.
3. Add 0.1% Tween-20 or 2% NP-40 to reduce non-specific binding.
4. Extend washing time/increase wash cycles.
5. Use gradient GSH concentration for elution.

Important Notes

1. Read this manual carefully before first use.
2. Do NOT freeze, dry, or centrifuge beads—these cause irreversible aggregation.
3. Ensure beads are fully resuspended before each use.
4. Use high-quality, low-protein-binding tubes and pipette tips to prevent bead loss.
5. For viscous solutions, resuspend beads by repeated pipetting or brief vortexing if tube inversion is insufficient.
6. Save supernatants and wash fractions for process analysis and optimization.
7. Beads can be reused for purifying the same protein. Use fresh beads for different proteins to avoid cross-contamination.
8. A magnetic separator is required for operation (see Appendix).
9. For Research Use Only (RUO)—not for diagnostic or therapeutic applications.