Version: 02

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Product Name: Glutathione MagBeads

Cat. No.: L00895

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1. Product Description

1.1 Intended Use

The GenScript Glutathione MagBeads are developed for quick and efficient small-scale purification of recombinant glutathione-S-transferase (GST)-tagged fusion proteins from bacteria, yeast and mammalian crude cell lysates.

1.2 Principle

GenScript's Glutathione MagBeads are superparamagnetic beads pre-coupled with reduced glutathione (GSH). For purification, samples containing the target proteins are added to the Glutathione MagBeads. The GST-tagged proteins bind to GSH on the MagBeads. The beads are then washed and the GST- tagged proteins are eluted. Magnetic separation simplifies protein purification process by eliminating the need for centrifugation, minimizes sample loss and removes the excessive steps of a centrifugation-based purification process.

1.3 Description of Material

Material Supplied

The GenScript Glutathione MagBeads are magnetic agarose beads, 45-100 µm in diameter. The beads are supplied as 25% slurry in phosphate buffered saline (PBS), pH 7.4, containing 20% ethanol. The Glutathione MagBeads have a binding capacity of more than 20 mg GST-tagged protein per 1 mL of settled beads (e.g. 4 mL of 25% slurry).

Additional Material Required

Mixing/rotation device, magnetic separation rack, microcentrifuge tubes, pipettes and pipette tips.

Additional Buffers Needed

Binding/Wash Buffer: 1×PBS, pH 7.4

Elution Buffer: 10 mM reduced glutathione (GSH) in 0.05 M Tris-HCl, pH 8.0

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2. Instructions For Use

The protocol uses 100 µL of Glutathione MagBeads (400 µL of 25% slurry), which may be scaled up or down for individual samples.

2.1 Preparation of Cell Lysate

Prepare the cell lysate from a culture expressing the GST-tagged protein of interest using a method of your choice, such as French Press or sonication.

2.2 Preparation of the MagBeads

1. Completely resuspend the beads by shaking or vortexing the vial.
2. Transfer 100 µL of the beads into a clean microcentrifuge tube.
3. Place the tube on a magnetic separation rack to collect the beads. Remove and discard the supernatant.
4. Add 1 mL of Binding/Wash Buffer to the tube and invert the tube several times to mix. Use the magnetic separation rack to collect the beads and discard the supernatant. Repeat this step twice.
5. Proceed to Binding of GST Fusion Protein (Section 2.3).

2.3 Binding of GST-tagged Protein

1. Resuspend the beads in 100 µL of Binding/Wash Buffer.
2. Add the cell lysate containing the GST-tagged proteins to the tube and gently invert the tube to mix.
3. Incubate the tube with mixing (on a shaker or rotator) for 60 – 120 minutes. For samples with low expression levels (< 0.1 mg/mL), it is recommended to incubate for longer than 120 minutes.
4. Use the magnetic separation rack to collect the beads and discard the supernatant. If necessary, keep the supernatant for analysis.
5. Add 1 mL of Binding/Wash Buffer to the tube and mix well, use the magnetic separation rack to collect the beads and discard the supernatant. Repeat the wash step three times.
6. Proceed to elution of GST-tagged protein (Section 2.4).

2.4 Elution of GST-tagged Protein

1. Add 100 µL of Elution Buffer to the tube, mix well, and incubate for five minutes at room temperature (or at a lower temperature if the protein is unstable at room temperature) with occasional mixing.
2. Use the magnetic separation rack to collect the beads and transfer the supernatant that contains the eluted protein into a clean tube.
3. Repeat the elution step twice to recover target protein as completely as possible.

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3. Troubleshooting

Review the information below to troubleshoot your experiments using the GenScript Glutathione MagBeads.

Problem	Cause	Solution
The yield of the purified fusion protein is low or undetectable.	Not enough MagBeads used.	Increase the amount of the MagBeads used.
	Insufficient target protein present in the cell lysate.	Increase the amount of cell lysate.
	The target protein has degraded.	Add appropriate protease inhibitors such as PMSF to the cell lysate and Binding/Wash buffer.
	The target protein contains denatured GST.	Use mild cell lysis methods, such as adding lysozyme, so that GST is not denatured.
	The fusion protein is not efficiently eluted from the MagBeads.	Increase elution time or the concentration of reduced glutathione (GSH) to 15 mM or higher in the Elution Buffer.
		Adjust the pH of the Elution Buffer to 8.0-9.0.
		Add Triton X-100 (0.1%, final concentration) or n - Octylglucoside (2%, final concentration) or NaCl (0.1 - 0.2 M, final concentration) to the Elution Buffer.
Multiple non - specific bands observed in the eluted sample.	The target protein has degraded.	Add appropriate protease inhibitors such as PMSF to the cell lysate and Binding/Wash buffer.
	Host proteins, such as chaperonins, may interact with the fusion protein.	Add DTT (5 mM, final concentration) in the Binding/Wash Buffer.
		Add Chaperonin Buffer (2 mM ATP, 10 mM MgSO4, 50 mM Tris-HCl) to the cell lysate and incubate at 37 ℃ for 10 minutes prior to the purification.
	Non-specific proteins are binding to the MagBeads	Use more stringent wash conditions. Detergents such as 1% Triton X - 100, 1% Tween - 20, 0.03% SDS, or 0.1% NP-40 may be used.

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4. General Information

4.1 Storage and Stability

This product is stable until the expiration date stated on the COA, when stored unopened at 2–8°C. **Do not freeze**. Keep the MagBeads in liquid suspension during storage and all handling steps. Drying will cause loss of binding capacity and result in reduced performance. Completely resuspend the beads before use. Observe sterile technique to avoid bacterial/fungal contamination.

4.2 Technical Support

Please contact GenScript for further technical information (see contact details). Certificate of Analysis/Compliance and the latest revision of the package insert/instructions for use is available at https://www.genscript.com/product/documents.

4.3 Warning and Limitations

This product is for research use only. Not intended for any animal or human therapeutic or diagnostic use unless otherwise stated. This product contains 20 % EtOH as a preservative. Flammable liquid and vapor. Flash point 38°C. R-10 flammable. Material Safety Data Sheet (MSDS) is available at https://www.genscript.com/product/documents.

4.4 Related MagBeads Products

Cat. No.	Product Name
L00273	Protein A MagBeads
L00274	Protein G MagBeads
L00295	Ni-Charged MagBeads
L00672	Protein A MagBeads MX
L00673	Protein G MagBeads MX
L00695	AmMag™ Protein A Magnetic Beads
L00776	AmMag™ Ni Magnetic Beads
L00894	Protein A/G MagBeads MX

For research and manufacturing use. Direct human use, including taking orally and injection are forbidden.

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