Glutathione MagBeads Cat. No. L00327

Technical Manual No. TM0260 **Version 08212013**

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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) fusion proteins from bacteria, yeast and mammalian crude cell lysates.

1.2 Principle

Add the sample containing the GST fusion proteins to the Glutathione MagBeads and allow the proteins bind to the MagBeads. Then the isolated proteins can be eluted off the beads. Magnetic separation eliminates changes of micro multiple tubes, minimizes the loss of sample and removes excessive steps of the centrifugation process.

1.3 Description of Material

Material Supplied
The GenScript Glutathione MagBeads are superparamagnetic beads, average 40µm in diameter, coupled with reduced glutathione (GSH). The beads are supplied as 25% slurry in phosphate buffered saline (PBS), pH 7.4, containing 20% ethanol. The Glutathione MagBeads has a binding capacity of 5 to 10 mg GST fusion protein per 1 ml settled beads (e.g. 4 ml 25% slurry).

Cat. No. L00327 Size: 8 ml.

Additional Material Required
Mixing/Rotation Device
Magnetic Separation Rack
Test tubes and pipettes
Buffers and solutions (see below)

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

2. Instruction For Use

The protocol uses 100 µl Glutathione MagBeads, this may be scaled up or down accordingly.

2.1 Preparation of Cell Lysate

Many different ways may be used for preparing a cell lysate containing expressed GST fusion proteins, such as French Press or sonication for *E.coli*.

2.2 Preparation of the MagBeads

1. Completely resuspend the beads by shaking or vortexing the vial.
2. Transfer 100 µl beads into a clean test tube.
3. Place the tube on a magnetic separation rack to collect the beads. Remove and discard the supernatant.
4. Add 1 ml 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 Fusion Protein

1. Resuspend the beads in 100 µl Binding/Wash Buffer.
2. Add the cell lysate containing GST fusion proteins prepared above to the tube and gently invert tube to mix.
3. Incubate the tube at room temperature (or at a lower temperature if the protein is unstable at room temperature) with mixing (on a shaker or rotator) for 30 – 60 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 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 elusion protein (Section 2.4).

2.4 Elution of GST Fusion Protein

1. Add 100 µl 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.

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
	Insufficient target protein present in cell lysate	Increase amount of cell lysate
	The target protein degraded.	Add appropriate protease inhibitors such as PMSF to the cell lysate and Binding/Wash buffer.
	The target protein does not contain active 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 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 $MgSO_4$, 50 mM Tris-HCl) to your cell lysate an incubate at 37 ºC for 10 minutes prior to the purification.
	Nonspecific protein 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.

		The salt concentration in the Binding/Wash solution can also be optimized to reduce non-specific binding.

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 the product**. Keep the MagBeads in liquid suspension during storage and all handling steps. Drying will cause loss of binding capacity and result in reduced performance. Resuspend the beads well before use. Be careful to avoid bacterial/fungal contamination.

4.2 Technical Support

Please contact GenScript for further technical information (see contact details). Certificate of Analysis/Compliance is available upon request. The latest revision of the package insert/instructions for use is available on www.genscript.com.

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 http://www.genscript.com.

4.4 Related MagBeads Products

Cat. No.	Product Name
L00273	Protein A MagBeads
L00274	Protein G MagBeads
L00277	Protein A/G MagBeads
L00295	Ni-Charged MagBeads
L00275	Mouse Anti-His mAb MagBeads
L00336	Mouse Anti-GST mAb MagBeads

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