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I. DESCRIPTION

Mouse Anti-GST mAb MagBeads (GenScript, L00336, 1 ml of 50% slurry) is a suspension of BioMag particles of approximately 20-75 µm in size, covalently coated with THE™ Anti-GST Monoclonal Antibody (Mouse) (GenScript, A00865). The beads is supplied as a suspension of 500 µl beads/ml in phosphate buffered saline (PBS, pH 7.4) containing 0.1% bovine serum albumin (BSA) and 0.02% sodium azide (NaN₃).

GenScript Mouse Anti-GST mAb MagBeads display both high specificity and high affinity for GST. As such, It is an ideal tool for projects, such as immunoprecipitation (IP) experiments, that require the quick purification of GST or GST-fusion proteins.

II. KEY FEATURES

- Quick and convenient separation accomplished by magnetic means
- Superior sensitivity
- Low levels of nonspecific binding

III. CHARACTERISTICS

Ligand	Mouse Anti-GST mAb
Number of IgG binding sites per ligand	2
Molecular weight of ligand	150 kDa
Static binding capacity	50-100 µg GST protein
Stability	4°C for six months
Material	Bio-magnetic agarose
Particle size	20-75 µm
Storage	4°C to 8°C



IV. GENERAL PROTOCOL

A. Additional materials required

Washing buffer: PBS, pH 7.4
Elution buffer: 0.1 M glycine, pH 2.5
Neutralization buffer: 1 M Tris, pH 9.0
2.0 ml microcentrifuge tubes
Magnet for a 1.5-2.0 ml microcentrifuge tube

B. Procedure

NOTE: Shake or vortex beads vigorously before use

1. Purifying GST protein or GST-fusion protein

- 1) Place at least 10-20 μ l of MagBeads suspension in a 2.0 ml microcentrifuge tube.
- 2) Add 1 ml of washing buffer and invert the tube several times to mix. Use the magnet to separate the beads. Once the supernatant becomes clear, remove and discard it. Repeat this step three more times.
- 3) Resuspend the beads with GST protein or GST-fusion protein containing the sample. Gently invert tube several times to mix. Incubate tube at room temperature on a shaker for one hour.
- 4) Magnetically separate the beads. Once the supernatant becomes clear, remove it.
- 5) Wash the beads with washing buffer to prevent nonspecific binding. Magnetically separate the beads. Once the supernatant becomes clear, remove and discard it. Repeat this wash step three more times.
- 6) Add 30 μ l of Elution buffer to MagBeads. Mix well by tapping and rotating the tube for two minutes.
- 7) Magnetically separate the beads. Once the supernatant becomes clear, transfer it to a clean tube.
- 8) Repeat this elution step to elute any remaining target protein. Transfer the supernatant to another clean tube.
- 9) Neutralize the elution immediately with 15 μ l of Neutralization buffer.
- 10) Wash the MagBeads two times with 1 ml of washing buffer so that the beads can be reused in the future. Store the MagBeads in 100 μ l of PBS, pH 7.4 containing 0.1% BSA and 0.02% sodium azide at 4°C.

2. Immunoprecipitation

- 1) Place 10-20 μ l of the MagBeads in a 2.0 ml microcentrifuge tube.
- 2) Add 1 ml of washing buffer and invert the tube several times to mix. Use the magnet to separate the beads. Once the supernatant becomes clear, remove and discard it. Repeat this step three more times.
- 3) Resuspend the beads with GST protein or GST-fusion protein containing the sample. Gently invert tube several times to mix. Incubate tube at room temperature on a shaker for one hour.
- 4) Magnetically separate the beads. Once the supernatant becomes clear, remove it.
- 5) Wash the beads with washing buffer to prevent nonspecific binding. Magnetically separate the beads. Once the supernatant becomes clear, remove and discard it. Repeat this wash step three more times.
- 6) Add 20 μ l of 1X SDS loading buffer, heat the tube to 100°C for five minutes.
- 7) Magnetically separate the beads. Transfer the supernatant to a new tube.
- 8) Analyze the sample by SDS-PAGE or western blot analysis.



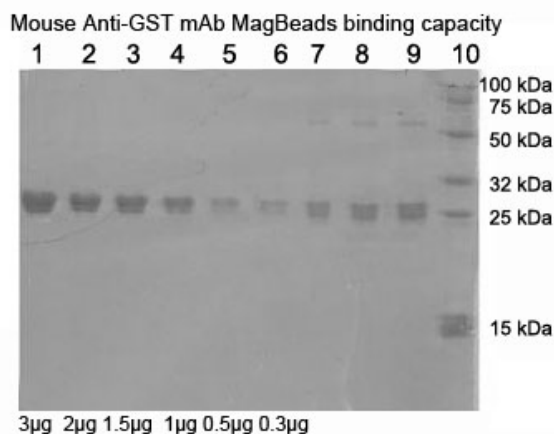
V. EXAMPLES

Figure: Anti-GST mAb MagBeads binding capacity

Lane 1-6: Serially diluted GST-fusion protein standards were loaded into lanes 1-6, respectively, for quantitation purposes.

Lane 7-9: 3 μ l, 5 μ l and 7 μ l, respectively, of Anti-GST mAb MagBeads were used to purify (or immunoprecipitate) GST-fusion protein.

The gel was stained using Coomassie Blue. It was shown that 5 μ l of the MagBeads can pull out about 1 μ g of the GST-fusion protein.



Lane 1-6: Proportional diluted GST

Lane 7-9: 3 μ l, 5 μ l, 7 μ l Mouse Anti-GST mAb MagBeads (GenScript, L00336)

Lane 10: EasyWestern Protein Standard (GenScript, MM0908)

VI. TROUBLESHOOTING

Problem	Possible Cause	Solution
No target protein is recovered.	The protein of interest is at a very low concentration in the sample.	Increase the size of the sample.
	The amount of MagBeads suspension is too small.	Increase the amount of MagBeads suspension.
	The binding time is too short.	Increase the binding time.
There are non-specific proteins.	The wash solution is not stringent enough or the volume used is too small.	Increase the concentration of NaCl in the washing buffer.
		Add 0.01% Tween-20 or Triton X-100 to the washing buffer.

VII. ORDERING INFORMATION

GenScript Anti-GST mAb MagBeads, Cat. No. L00336

For Research Use Only.

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