Mouse Anti-Trx mAb MagBeads Cat. No. L00337

Technical Manual No. TM0276 **Version 04202010**

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I. DESCRIPTION

Mouse Anti-Trx mAb MagBeads (Cat. No. L00337, 2 ml 25% slurry) are average 40µm in size, superparamagnetic beads covalently coated with Mouse Anti-Thioredoxin (TRX) Monoclonal Antibody (GenScript, A00164). The beads are supplied as 25% slurry in phosphate buffered saline (PBS, pH 7.4) containing 20% ethanol. The Mouse Anti-Trx mAb MagBeads display high specificity for Trx and Trx-fusion proteins, therefore becoming the ideal tool for immunoprecipitation and quick purification of Trx-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-Trx mAb
Number of IgG binding sites per ligand	2
Molecular weight of ligand	150 kDa
Binding capacity	300 µg Trx-fusion protein/ settled beads
Material	Bio-magnetic agarose
Particle size	20-75 µm
Storage	2°C to 8°C
Shelf life	12 months at 2-8 ℃

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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 Trx-fusion proteins

1) Place at least 10-20 µl 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 Trx-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 µl 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. Neutralize the elution immediately with 15 µl of neutralization buffer.
9) 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 µl of PBS, pH 7.4 containing 0.1% BSA, and 0.02% sodium azide at 4℃.

2. Immunoprecipitation

1) Place 10-20 µl 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 Trx-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 µl of 1X SDS loading buffer, heat the tube at 100℃ 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.

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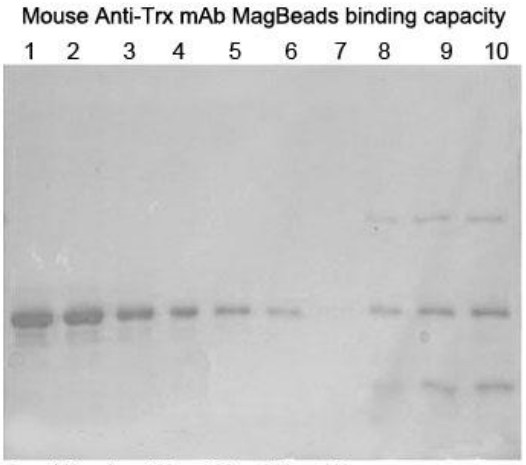
V. EXAMPLES

Figure 1: Binding Capacity of Mouse Anti-Trx MagBeads
Lane 1-7: Serially diluted Trx-fusion protein standards were loaded into lanes 1-7, respectively for quantitation purposes. Lane 8-10: 3 µl, 6 µl, and 9µl, respectively, of Mouse Anti-Trx mAb MagBeads were used to purify (or immunoprecipitate) Trx-fusion protein. The gel was stained using Coomassie Blue. It is here shown that 3µl of the MagBeads can pull out about 300 ng of the Trx-fusion protein.



Lane 1-7: Proportional diluted Trx-fusion protein
Lane 8-10: 3µl, 6µl, 9µl Mouse Anti-Trx mAb MagBeads (GenScript, L00337)

VI. TROUBLESHOOTING

Problem	Possible Cause	Solution
No target protein is recovered.	The protein of interest is present in the sample at a very low concentration.	Increase the size of the sample.
	The number of MagBeads is too small.	Increase the amount of MagBeads suspension added.
	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

Mouse Anti-Trx mAb MagBeads, Cat. No. L00337

For Research Use Only.

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