THE™ Anti-His mAb MagBeads Cat. No. L00275

Technical Manual No. TM0250 **Version 03032009**

I. DESCRIPTION

THE™ Anti-His mAb MagBeads (GenScript, L00275) is a suspension of BioMag particles of approximately 1.5 μm in size which is covalently coated with THE™ Anti-His Monoclonal Antibody (GenScript, A00186). The suspension is supplied in phosphate buffered saline (pH 7.4) with stabilizer and sodium azide added. After shaking vigorously or vortexing, BioMag Anti-His mAb is ready to use.

THE™ Anti-His mAb MagBeads, with high specificity and affinity for poly-histidine tag, is an ideal tool for quick purification of recombinant polyhistidine-tagged proteins and enrichment of polyhistidine-tagged protein or protein complex in immunoprecipitation (IP) experiment.

The beads are supplied as a suspension of 250 μl beads/ml in 2ml phosphate buffered saline (PBS, pH 7.4) containing 20% ethanol.

II. KEY FEATURES

- Quick and convenient separation accomplished by magnetic means
- Superior sensitivity: coupled with the most sensitive Anti-His mAb on market
- Low nonspecific binding
- Can be used for quick purification of polyhistidine-tagged proteins
- Can be used for immunoprecipitation

III. CHARACTERISTICS

Ligand	THE™ Anti-His mAb
Number of IgG binding sites per ligand	2
Molecular weight of ligand	150kDa
Static binding capacity	1 – 2 µg polyhistidine-tagged proteins (35 kDa)
Stability	4°C for 6 months
Material	Bio-magnetic agorose
Particle size	1.5 µm
Storage	at +4 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
1.5 ml microcentrifuge tubes
Magnet for a 1.5 ml microcentrifuge tube

B. Procedure

NOTE: Shake beads vigorously before use

1. Purifying recombinant polyhistidine-tagged proteins

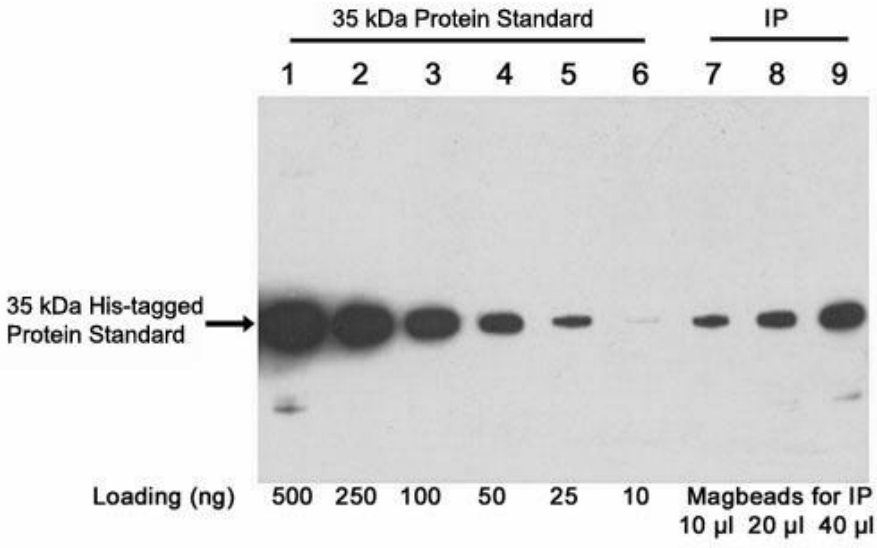
1) Place 50 - 100 µl (or more) of Magbeads into a 1.5 ml microcentrifuge tube.
2) Add 1 ml of washing buffer and invert the tube several times to mix. Use the magnet to separate the beads. Once the supernatant becomes clear, remove and discard it. Repeat this step three more times.
3) Resuspend the beads with target protein containing sample such as cell culture supernatant or cell lysate. Gently invert tube several times to mix. Incubate tube at 4 ℃ on a shaker for 1 hour.
4) Magnetically separate the beads. Once the supernatant becomes clear, remove and discard the supernatant.
5) Wash the beads with washing buffer to remove nonspecific binding. Magnetically separate the beads, once the supernatant becomes clear, remove and discard the supernatant. 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 2 minutes.
7) Magnetically separate the beads. Once the supernatant becomes clear, transfer the supernatant containing purified target protein 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 µl of Neutralization Buffer.
10) Wash the Magbeads two times with 1 ml of washing buffer so 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 20 - 50 µl of the MagBeads into a 1.5 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 target protein containing sample such as cell culture supernatant or cell lysate. Gently invert tube several times to mix. Incubate tube at 4 ℃ on a shaker for 1 hour.
4) Magnetically separate the beads. Once the supernatant becomes clear, remove and discard the supernatant.
5) Wash the beads with washing buffer to remove nonspecific binding. Magnetically separate the beads, once the supernatant becomes clear, remove and discard the supernatant. Repeat this wash step three more times.
6) Add 20 µl of 1X SDS loading buffer, heat the tube at 100℃ for 5 min.
7) Magnetically separate the beads. Transfer the supernatant to a new tube.
8) Analyze the sample by SDS-PAGE and western blot.

V. EXAMPLES

10 µl, 20 µl and 40 µl of THE™ Anti-His mAb MagBeads were used to purify (or immunoprecipitate) a 35 kDa polyhistidine-tagged protein from *E. coli* cell lysate, respectively. A serially diluted protein standard and immunoprecipitated products were analyzed by using One-Step IP-Western Kit (Mouse) (GenScript, L00232) and THE™ Anti-His mAb MagBeads (GenScript, A00186).



It was shown that 10 µl (0.05 mg) of the MagBeads can pull out about 25 ng of the 35 kDa polyhistidine-tagged protein.

VI. TROUBLESHOOTING

Problem	Possible Cause	Solution
No target protein is recovered	The protein of interest is at very low concentration in the sample.	Increase the amount of sample.
	The amount of Magbeads is too little.	Increase the amount of Magbeads.
	Binding time is too short.	Increase the binding time.
There is non-specific proteins	Washing solution is not stringent enough or washing solution volume is too small.	Increase the concentration of NaCl in the washing buffer.
		Add 0.01% Tween-20 or Triton X-100 into washing buffer.

VII. ORDERING INFORMATION

GenScript THE™ Anti-His mAb MagBeads, Cat. No. L00275

For Research Use Only.

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