Mouse Anti-His mAb MagBeads Cat. No. L00275

Technical Manual No. TM0250 **Version 07072010**

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1. Product Description

1.1 Designed Use

Mouse Anti-His mAb MagBeads, with high specificity and affinity to polyhistidine tag, is ideal for quick and efficient purification of recombinant polyhistidine tagged proteins and pull-down of polyhistidine-tagged protein-target protein complex.

1.2 Principle

Add the sample containing the polyhistidine-tagged protein to the Mouse Anti-His mAb MagBeads and allow the protein bind to the MagBeads during a short incubation. Then the beads bound polyhistidine-tagged protein can be eluted off the beads, or used directly for pull-down of target protein. Magnetic separation eliminates the need for multiple tubes, minimizes the loss of sample and removes tedious steps of the conventional centrifugation method.

1.3 Description of Material

Material Supplied

GenScript Mouse Anti-His mAb MagBeads are superparamagnetic beads of average 40 µm in diameter, covalently coated with THE™ His Tag Antibody, mAb, Mouse (GenScript, A00186). The beads are supplied as 25% slurry in phosphate buffered saline (PBS), pH 7.4, containing 20% ethanol. The Mouse Anti-His mAb MagBeads have a binding capacity of more than 300 µg polyhistidine-tagged protein (27kDa)/ml settled beads (e.g. 4 ml 25% slurry).

Cat. No. L00275: 2 ml.

Additional Material Required

Magnetic separation rack (GenScript Cat. No. M00140)
Mixing/Rotation Device
Test tubes and pipettes
Buffers and solutions (see below)

Additional Buffers Needed

Binding/Wash Buffer: 20 mM Na_2HPO_4, 0.15M NaCl, pH 7.0
Elution Buffer: 0.1 M glycine, pH 2-3
Neutralization Buffer: 1 M Tris, pH 8.5
1×SDS Sample Buffer: 62.5 mM Tris-HCl (pH 6.8 at 25°C), 2% w/v SDS, 10% glycerol, 50 mM DTT, 0.01% w/v bromophenol blue

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2. Instruction For Use

The protocol uses 100 µl Mouse Anti-His mAb MagBeads, but this may be scaled up or down as required.

2.1 Preparation of Cell Lysate

Many different ways may be used for preparing a cell lysate containing expressed polyhistidine-tagged proteins. We recommend the use of Easy BacLysis Protein Extraction Solution (GenScript Cat. No. L00230, L00240) to prepare a cell lysate from *E.coli.* The other alternative lysis strategies such as French Press and sonication also can be applied 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 tube at tube wall. 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.

2.3 Separation of polyhistidine-tagged protein

1. Resuspend the beads in 100 µl Binding/Wash Buffer.
2. Add sample containing polyhistidine-tagged protein to the beads and gently invert the tube to mix.
3. Incubate the tube 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 desired, keep the supernatant for SDS-PAGE 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 isolated polyhistidine-tagged protein (Section 2.3).

2.4 Elution of polyhistidine-tagged protein

1. Add 100 µl Elution Buffer to the tube. Mix well and incubate for five minutes at room temperature with occasional mixing.
2. Use the magnetic separation rack to collect the beads and transfer the supernatant containing the eluted polyhistidine-tagged protein into a clean tube
3. Repeat Step 1 and 2 more twice to recover as much protein as possible.
4. Add 10 µl Neutralization Buffer for each 100 µl eluate to neutralize the low pH. If needed, perform a buffer exchange by dialysis or desalting.

2.5 Pull-down of target protein

1. Resuspend the beads with immobilized polyhistidine-tagged protein from section 2.3 in 100 µl Binding/Wash Buffer.
2. Transfer 20 - 50 µl of the beads into a new tube and invert the tube several times to mix.
3. Add your sample containing target protein such as cell culture supernatant or cell lysate to the tube. Gently invert the tube several times to mix.
4. Incubate at 4 ℃ with mixing on a shaker for 1 hour.
5. Use the magnetic separation rack to collect the beads and discard the supernatant.
6. Add 500 µl 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 three times.
7. Add 20 µl of 1×SDS Sample Buffer to the tube and heat the tube at 100℃ for 5 min.
8. Use the magnetic separation rack to collect the beads and transfer the supernatant to a new tube.
9. Analyze the sample by SDS-PAGE followed by Western blot.

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3. Troubleshooting

Review the information below to troubleshoot your experiments using the GenScript Mouse Anti-His mAb MagBeads.

Problem	Possible Cause	Solution
Sample is too viscous	The sample contains high concentration of host nucleic acid	Continue sonication until the viscosity is reduced, and/or add DNase I to 5 μg/ml, Mg^{2+} to 1 mM, and incubate on ice for 10 – 15 minutes.
The yield of the purified target protein is low or undetectable.	The protein of interest is at a very low concentration in the sample.	Increase the amount of your sample.
	The amount of MagBeads is too small.	Increase the amount of MagBeads slurry.
	The binding time is not long enough.	Increase the binding time.
	Target antigen may loss activity.	Analyze samples taken before and after induction of expression with anti-His antibody by Western blotting.
Multiple non-specific bands observed in the eluted sample	Not enough Binding/Wash Buffer was used.	Increase the volume of Binding/Wash Buffer.
	Nonspecific hydrophobic or other interaction.	Increase the concentration of NaCl in the Binding/Wash Buffer.
		Add 0.01% Tween-20 or Triton X-100 to the Binding/Wash Buffer.
	The target protein degraded	Add protease inhibitors to Binding/Wash Buffer.

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4. General Information

4.1 Storage and Stability

This product is stable until the expiration date stated on the label, when stored unopened at 2–8℃. **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 vapour. 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
L00327	Glutathione MagBeads
L00336	Mouse Anti-GST mAb MagBeads
L00337	Mouse Anti-Trx mAb MagBeads
L00328	Goat Anti-Rabbit IgG MagBeads
L00332	Donkey Anti-Goat IgG MagBeads

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