Protein A MagBeads MX
Cat No: L00672

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1. Product Description

1.1 Intended Use

GenScript Protein A MagBeads MX are ideal for small-scale antibody purification and immunoprecipitation of proteins, protein complexes or other antigens.

1.2 Principle

The sample containing antibody is added to the Protein A MagBeads MX. The antibody will bind to the beads during a short incubation. Then the bead-bound antibody can be eluted off from the beads using a magnetic separation rack, or used for immunoprecipitation (IP). A cross-linking procedure may be needed before IP to prevent co-elution of the primary antibody. Magnetic separation eliminates the changes of micro tubes, minimizes the loss of sample and removes excessive steps of traditional centrifugation method.

1.3 Description of Material

Material Supplied

GenScript Protein A MagBeads MX are super paramagnetic beads of average 50 µm in diameter, covalently coated with recombinant Protein A. The beads are supplied as 25% slurry in 20% ethanol. The Protein A MagBeads MX have a binding capacity of more than 30 mg human IgG per 1 ml settled beads.

Protein A, a bacterial cell wall protein isolated from *Staphylococcus aureus*, binds to mammalian IgGs, mainly through Fc regions. Native Protein A has five IgG binding domains and many unknown-function repeated sequences. Recombinant Protein A only contains five high-affinity IgG binding domains to reduce nonspecific binding.

Additional Material Required

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

Additional Buffers Required

Binding/Wash Buffer: 20 mM Na_2HPO_4, 0.15 M 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

2. Instruction For Use

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

2.1 Preparation of the MagBeads

1. Completely resuspend the beads by shaking or vortexing the vial.
2. Transfer 100 µl beads into a clean 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.

2.2 Separation of Target IgG

1. Resuspend the beads in 100 µl Binding/Wash Buffer.
2. Add the sample containing target IgG to the tube 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 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 more.
6. Proceed to elution of isolated IgG (Section 2.3).

2.3 Elution of Isolated IgG

1. Add 100 µl Elution Buffer to the tube and mix well. Incubate for five minutes at room temperature with occasional mixing.
2. Use the magnetic separation rack to collect the beads and transfer the supernatant that contains the eluted IgG into a clean tube.
3. Repeat Step 1 and 2 twice.
4. Add 10 µl of Neutralization Buffer to each 100 µl eluate to neutralize the pH. If needed, perform a buffer exchange by dialysis or desalting.

2.4 Immunoprecipitation

Bound IgG will be co-eluted along with the target when using elution methods. If the presence of IgG does not disturb the desired detection system, go directly to section 2.4.2 below. For applications where co-elution of the IgG is not desired, the primary IgG can be cross-linked to the Protein A MagBeads MX as described in section 2.4.1 below.

2.4.1 Cross-linking IgG to the Beads

1. Add 1 ml 0.2 M triethanolamine, pH 8.2 to the Protein A MagBeads MX with immobilized IgG. Wash twice using the magnetic separation rack with 0.2 M triethanolamine, pH 8.2 as the washing buffer.

2. Resuspend the beads in 1 ml of 20 mM dimetyl pimelimidate dihydrochloride (DMP) in 0.2 M triethanolamine, pH 8.2 (5.4 mg DMP/ml buffer). This cross-linking solution must be prepared freshly.
3. Incubate the beads with rotational mixing for 30 minutes at room temperature. Use the magnetic separation rack to collect the beads and discard the supernatant.
4. Resuspend the beads in 1 ml of 50 mM Tris, pH 7.5 to stop the reaction and incubate for 15 minutes at room temperature with rotational mixing.
5. Use the magnetic separation rack to collect the beads and discard the supernatant.
6. Wash the cross-linked beads three times with 1 ml PBS, pH7.4.

2.4.2 Binding Antigen to the IgG Cross-linked Beads

1. Add sample containing target antigen to the beads. For a 100 kD protein, use a volume containing approximate 25 µg target antigen/ml beads to assure an excess of antigen. If dilution of antigen is necessary, PBS or 0.1 M phosphate buffer (pH 7-8) can be used as dilution buffer.
2. Incubate with tilting and rotation for one hour at room temperature.
3. Place the tube on the magnetic separation rack for 2 minutes to collect the IgG-coated Beads-target complex at the tube wall. For viscous samples, double the time on the rack. Discard the supernatant.
4. Wash the beads 3 times using 1 ml PBS.

2.4.3 Elution of Target Protein

A. Denaturing elution

1. Place the tube from section 2.4.2 on the magnetic separation rack to collect the beads and discard the supernatant.
2. Add 100 µl 1×SDS Sample Buffer to the tube and mix well.
3. Heat the tube at 100°C for five minutes.
4. Use the magnetic separation rack to collect the beads and transfer the supernatant containing desired sample into a new tube.
5. Analyze the sample by SDS-PAGE followed by Western blot analysis.

B. Non-denaturing elution

1. Place the tube from section 2.4.2 on the magnetic separation rack to collect the beads and discard the supernatant.
2. Add 100 µl Elution Buffer to the tube and mix well. Incubate for five minutes at room temperature with occasional mixing.
3. Use the magnetic separation rack to collect the beads and transfer the supernatant into a new tube.
4. Repeat Step 2 and 3 twice.
5. Add 10 µl Neutralization Buffer to each 100 µl of eluate to neutralize the pH.

3. Troubleshooting

Review the information below to troubleshoot your experiments using the GenScript Protein A MagBeads MX.

Problem	Possible Cause	Solution
The beads are difficult to immobilize using the magnetic separation rack.	Too many beads are used.	Decrease the volume of MagBeads suspension.
A considerable amount of sample has been added, but very little specific antibody of interest is detected.	The antibody of interest is at very low concentration.	Use a serum-free medium for cell supernatant samples. Affinity-purify the antibody using its specific antigen coupled to an affinity supporting material.
The antibody of interest is purified, but it is degraded (as determined by loss of function in downstream assay).	The antibody is sensitive to low-pH elution buffer. The downstream application is sensitive to the neutralized elution buffer.	Try another elution reagent, such as 3.5 M $MgCl_2$, 10 mM phosphate, pH 7.2. Desalt or dialyze the eluted sample into a suitable buffer.
No antibody is detected in any eluate.	The antibody in the sample cannot bind to Protein A.	Try GenScript Protein G MagBeads or Protein A/G MagBeads.

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
L00274	Protein G MagBeads
L00277	Protein A/G MagBeads
L00295	Ni-Charged MagBeads
L00327	Glutathione MagBeads
L00275	Mouse Anti-His mAb MagBeads
L00336	Mouse Anti-GST mAb MagBeads

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