Protein A Magnetic Beads
Cat No: L00273

Version 01302020

I Product Description

1.1 Intended Use

GenScript Protein A MagBeads 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. The antibody will bind to beads during a short incubation. Then the beads-bound antibody can be eluted off from the beads using a magnetic separation rack. 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 are super paramagnetic beads of average 40 µm in diameter, covalently coated with recombinant Protein A. The beads are supplied as 25% slurry in phosphate buffered saline (PBS), pH 7.4, containing 20% ethanol. The Protein A MagBeads have a binding capacity of more than 10 mg Rabbit IgG per 1 ml settled beads (e.g. 4 ml 25% slurry).

Protein A, a bacterial cell wall protein isolated from *Staphylococcus aureus*, binds to mammalian IgGs, mainly through Fc region. 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.

Cat. No. L00273. Size 4 ml.

Additional Material Required

Mixing/Rotation Device
Magnetic Separation Rack (L00722 for AmMag™ MR-mini and L00723 for AmMag™ MR)
Test tubes and pipettes
Buffers and solutions (see below)

Additional Buffers Required

Binding/Wash Buffer: 1×PBS, 0.1% Tween 20, pH 7.0
Elution Buffer: 1. 0.1 M glycine, pH 2-3
Neutralization Buffer: 1 M Tris, pH 8.5

1xSDS Sample Buffer: 62.5 mM Tris-HCl (pH 6.8 at 25 °C), w/v SDS, 10% glycerol, 50 mM DTT, 0.01% w/v bromophenol blue

II Instructions For Use

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

2.1 Preparation of the MagBeads

1. Completely resuspend the beads by shaking the vial.
2. Transfer 400ul slurry (100 µl settled beads in 400µl slurry) 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 ul Binding/Wash buffer.
2. Add the sample containing target IgG into the tube and gently invert the tube to mix.
3. Incubate the tube at room temperature with mixing (on a shaker or rotator) for 1~4 hours or overnight.
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 more times.
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.

III Troubleshooting

Review the information below to troubleshoot your experiments using the GenScript AmMag™ Protein A Magnetic beads.

Problem	Possible Cause	Solution
The beads are difficult to immobilize using the magnetic separation rack.	Too many beads are used.	Decrease the volume of magnetic beads used.

A considerable amount of sample has been added, but very little specific antibody of interest is detected.	The antibody of interest is at a 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.

IV General Information

1. Storage and Stability

This product is stable until the expiration date of 2 years, when stored unopened at 2-8°C. **Do NOT freeze the product**. Keep the magnetic beads 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.

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.

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. Related MagBeads Products

Cat. No.	Product Name
L00695	AmMag™ Protein A Magnetic Beads
L00672-4	Protein A MagBeads MX
L00673-4	Protein G MagBeads MX
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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