

AmMag™ Protein A Magnetic Beads

Cat No: L00695

Version 09232019

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I Product Description

1. Intended Use

GenScript AmMag™ Protein A Magnetic Beads are developed and optimized for antibody purification for high throughput and high volume applications.

2. Principle

The sample containing antibody is added to the AmMag™ Protein A Magnetic Beads and incubated on a shaker overnight or 2 hours (depending on the concentration of samples) for the antibody to bind to the beads. The antibody bound to the beads can be eluted off by using acidic elution facilitated by a magnetic separation device. Magnetic separation eliminates the need for centrifugation, minimizes the loss of sample and makes the process more user friendly.

3. Description of Material

Material Supplied

GenScript AmMag™ Protein A Magnetic Beads are super paramagnetic beads with diameter of 45-100 µm, covalently coated with alkaline tolerant protein A. The beads are supplied as 25% slurry in 20% ethanol. The AmMag™ protein A Magnetic Beads have a binding capacity of 40mg human IgG per 1 ml settled beads. They can be reused for up to 40 cycles. A total of 20% reduction in binding capacity is observed after 40 cycles.

Protein A, a bacterial cell wall protein isolated from *Staphylococcus aureus*, binds to mammalian IgG's, mainly through the Fc regions. The alkaline tolerant protein A not only keeps the specific binding capacity to Fc region of immunoglobulin molecules, but also tolerates alkaline conditions and can withstand rigorous cleaning procedures (See section II.4 CIP for details).

Additional Material Required

Rotor/Rocker (if the proteins are already secreted) or orbital shaker (if the beads are added when the proteins are secreting)

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 (optional), pH 7.0

Elution Buffer: 1. 0.1 M glycine, pH 2-3

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2. 0.1M NaAc-HAc, pH3.6

3. 0.05M citrate, pH3.5

Note: Please use one of the above mentioned buffers for elution.

Neutralization Buffer: 1 M Tris, pH 8.5

Regeneration Buffer: 0.1M NaOH

II Protocol

The protocol uses 100 μ l AmMag™ Protein A Magnetic Beads for purification of 1 ml human IgG sample. Depending upon your sample volume and antibody concentration, additional optimization may be required to determine the volume of magnetic beads optimal for purification.

Sample concentration(hIgG)	Working capacity(mg/ml)			
	RT/2hour	37° C/overnight	4 ° C/2hour	4 ° C/overnight
0.01mg/ml	2.5	2.5	2.5	2.5
0.02mg/ml	5	5	5	5
0.1mg/ml	20	30	15	20
0.5mg/ml	35	60	30	50
1mg/ml	45	70	40	60
3mg/ml	70	100	60	85

1. Preparation of the MagBeads

1. Completely resuspend the beads by shaking the vial.
2. Transfer 400 μ l slurry (100 μ l settled beads in 400 μ l 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.
(For customers need endotoxin control, please proceed with the following steps.)
5. Add 1 ml 0.1M NaOH to the tube with magnetic beads and incubate for 15min. Then use the magnetic separation rack to collect the beads and discard the supernatant, repeat this step twice.
6. 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. Separation of target IgG

1. Add the sample containing target IgG into the tube and gently invert the tube to mix.
2. Incubate the tube at room temperature with mixing (on a rocker or rotator) for 1~4hours or overnight.
Note: If the protein of interest is unstable and prone to degradation 100mM of PMSF may be added to prevent protein degradation.
3. Use the magnetic separation rack to collect the beads and discard the supernatant. If necessary, keep the supernatant for analysis.
4. 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.

3. Elution of Isolated IgG

1. Add 500 μ l 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.

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4. Add Neutralization Buffer to each 500 µl eluate to neutralize the pH. If needed, perform a buffer exchange by dialysis or desalting.

4. CIP

CIP (clean in place) method is performed after each cycle, by incubating with 10 CV of 0.1M NaOH for 15 min. After the magnetic beads are adsorbed, discard the supernatant and add another 10 CV of 0.1M NaOH for further treatment for 15 min. Then rinse the magnetic beads with PBS and store in 20% ethanol.

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. Bind the samples to the beads overnight at 4°C. 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 0.1M Sodium acetate, pH3.6 Add protease inhibitor to the binding, washing and elution buffers. 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

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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	Protein A Magnetic Beads MX
L00673	Protein G Magnetic Beads MX
L00274	Protein G Magnetic Beads
L00277	Protein A/G Magnetic Beads
L00295	Ni-Charged Magnetic Beads
L00776	AmMag™ Ni Magnetic beads
L00424	Streptavidin Magnetic Beads
L00327	Glutathione Magnetic Beads
L00275	Mouse Anti-His mAb Magnetic Beads
L00336	Mouse Anti-GST mAb Magnetic Beads
L00722	AmMag™ MR-mini Magnetic Rack
L00723	AmMag™ MR Magnetic Rack

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