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# Protein G MagBeads MX Cat No: L00673

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# 1. Product Description

#### 1.1 Designed Use

GenScript Protein G MagBeads MX are ideal for small-scale antibody purification.

#### 1.2 Principle

The sample containing antibody is added to the Protein G MagBeads MX. The antibody will bind to beads during a short incubation. Then the bead-bound antibody may be eluted off the beads by using a magnetic separation rack. Magnetic separation eliminates the need for multiple tubes, minimizes the loss of sample and removes tedious steps of conventional centrifugation method.

# 1.3 Description of Material Material Supplied

GenScript Protein G MagBeads MX are superparamagnetic beads of average  $45\pm 5~\mu m$  in diameter, covalently coated with recombinant Protein G. The beads are supplied as 25% slurry in 20% ethanol. The Protein G MagBeads MX have a binding capacity of more than 25 mg Human IgG per 1 ml settled beads (e.g. 4 ml 25% slurry).

Protein G is a genetically engineered protein (MW≈22 kDa) that combines the IgG binding sites of both Protein A and Protein G. 6×His-tag was attached to its N-terminal to facilitate the purification. Protein G has greater affinity than protein A for most mammalian IgGs, especially for certain subclasses including human IgG3, mouse IgG1 and rat IgG2a. Unlike protein A, protein G does not bind to human IgM, IgD and IgA.

#### **Additional Material Required**

Magnetic separation rack (L00722 and L00723)
Mixing/Rotation Device
Test tubes and pipettes
Buffers and solutions (see below)



#### **Additional Buffers Needed**

Binding/Wash Buffer: 20 mM Na<sub>2</sub>HPO<sub>4</sub>, 0.15M NaCl, pH 7.0

Elution Buffer: 0.1 M glycine, pH 2-3

Neutralization Buffer: 1 M Tris-HCl, pH 8.5

Storage Buffer: 1 x PBS, pH 7.4, containing 20% ethanol

#### 2. Instructions For Use

The protocol uses 100 µl Protein G MagBeads MX, but this may be scaled up or down as required.

#### 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 at tube wall. Remove and discard the supernatant.
- 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 your 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 desired, 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 low pH. If needed, perform a buffer exchange by dialysis or desalting.

#### 2.4 Post-treatment of magnetic beads

- 1. Add 1 ml Elution 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. 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.
- 3. Add storage buffer to resuspend the magnetic beads and store at 2~8℃.



# 3. Troubleshooting

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

Problem	Possible Cause	Solution
The beads are hard 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 <sub>2</sub> , 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 can't bind to Protein G.	Try GenScript Protein A MagBeads MX or Protein A/G MagBeads.

# 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°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 and the latest revision of the package insert/instructions for use is available on <a href="https://www.genscript.com/product/documents">https://www.genscript.com/product/documents</a>.

#### 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 <a href="https://www.genscript.com/product/documents">https://www.genscript.com/product/documents</a>.



4.4 Related MagBeads Products

Cat. No.	Product Name	
L00273	Protein A MagBeads	
L00672	Protein A MagBeads MX	
L00695	AmMag™ Protein A Magnetic Beads	
L00274	Protein G MagBeads	
L00277	Protein A/G MagBeads	
L00894	Protein A/G Magbeads MX	
L00295	Ni-Charged MagBeads	
L00776	AmMag <sup>™</sup> Ni Magnetic beads	
L00895	Glutathione MagBeads	
L01013	AmMag™ SA Plus System	

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