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I. DESCRIPTION

Protein G MagBeads (GenScript, L00274) is a suspension of magnetic particles with the size of approximately 20-75 μm that are covalently coupled with Protein G. The suspension is supplied in 2 ml of 1XPBS containing 20% ethanol with 0.5 ml of MagBeads (bed volume). BioMag Protein G is suitable for binding and purification of immunoglobulins. It can be used for immunoprecipitation (IP), it can also be used for the removal of Fc fragments during Fab fragment preparation. Quick and convenient separations are accomplished magnetically.

Protein G, a bacterial cell wall protein isolated from group G *Streptococci*, binds to mammalian IgGs mainly through Fc regions. Native Protein G has 3 IgG binding domains and also sites for albumin and cell-surface binding. Albumin and cell-surface binding domains have been eliminated from recombinant Protein G to reduce nonspecific binding. 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.

II. KEY FEATURES

- Quick and convenient separation accomplished by magnetic force
- High capacity: 0.5 mL Protein G MagBeads (bed volume) can bind more than 5 mg of goat IgG
- Low nonspecific binding

III. Characteristics of Protein G MagBeads

Ligand	Recombinant protein G produced in <i>E. coli</i>
Number of IgG binding sites per ligand	3
M. W. of ligand	Approximately 22 kDa
pI of ligand	4.69
Conc. Of ligand	Approximately 2 mg protein G/ml beads
Volume of beads (bed volume)	0.5 ml
Static binding capacity	> 10 mg goat IgG/ml beads (bed volume)
Total binding capacity	> 5 mg goat IgG
Stability	14 days at 37°C or 1 year at 4°C
Material	Bio-Magnetic agarose, 4% cross-linked
Particle size	20-75 μm
Storage	At +4 to +8°C



IV. Immunoglobulin Purification Procedure

A. Additional Materials Required

Binding buffer: 20 mM Na₂HPO₄, 0.15 M NaCl, pH 7.0.

Elution buffer: 0.1 M glycine, pH 2 - 3.

Neutralization buffer: 1 M Tris; pH 7.5.

1.5 ml microcentrifuge tubes.

Magnet for a 1.5 ml microcentrifuge tube.

B. Antibody Purification Procedure

Note: Shake beads vigorously or vortex before use

1. Place 100 μ l of the MagBeads into a 1.5 ml microcentrifuge tube.
2. Add 1 ml of binding buffer to the tube and invert tube several times to mix. Use the Magnet to separate the beads from the buffer. Once the supernatant becomes clear, remove and discard the supernatant. Repeat this step three more times.
3. Resuspend the beads in 100 μ l of binding buffer.
4. Add 50 μ l serum or cell culture supernatant to the tube and gently invert tube to mix. Incubate tube at room temperature with mixing for 1 hour.
5. Magnetically separate the beads. Once the supernatant becomes clear, remove the supernatant.
6. Add 1 ml of binding buffer to the tube, mix well, magnetically separate the beads and remove the supernatant. Repeat the wash step three times.
7. Add 100 μ l of elution buffer to the tube, mix well and incubate for 5 minutes at room temperature with occasional mixing. Magnetically separate the beads. Once the supernatant becomes clear, remove and save the supernatant, which contains the eluted antibody. Repeat this elute three times to recover the IgG as completely as possible.
8. To neutralize the elute, add 2.5 μ l of neutralization buffer for each 50 μ l of eluate. If desired, perform a buffer exchange by dialysis or desalting.

V. Application Example

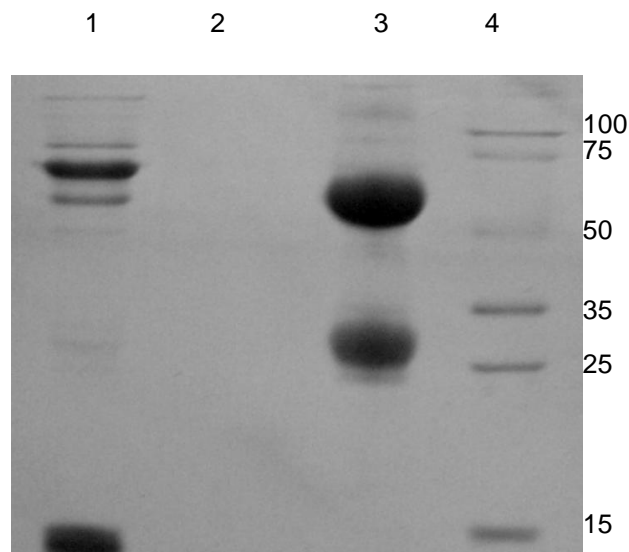


Fig 1. SDS-PAGE of binding capacity analysis of Protein G MagBeads (GenScript, L00274)

1. Flow

2. Wash

3. Elute

4. Molecular standard



VI. Troubleshooting

Problem	Possible Cause	Solution
The magnetic particles are hard to be immobilized by magnet.	The quantity of Protein G MagBeads is too large to be immobilized by magnet.	Decrease the volume of MagBeads used.
Antibody of interest purified, but it is degraded (as determined by lack of function in downstream assay).	Antibody is sensitive to low-pH elution buffer.	Try other elution reagent. For example, 2.0 M Trichloroacetic acid-NaOH, pH 7.0-7.3.
	Downstream application is sensitive to neutralized elution buffer.	Desalt or dialyze eluted sample into suitable buffer.
No antibody detected in any elution fraction.	Sample containing antibody species or subclass that binds to Protein G weakly.	Try Protein A MagBeads.

VII. ORDERING INFORMATION

Protein G MagBeads, Cat. No. L00274

For Research Use Only.

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