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I. DESCRIPTION

Protein A/G MagBeads (GenScript, L00277) are BioMag particles of approximately 20-75 μm in size that are covalently coated with Protein A/G. The product is supplied as suspension of 0.5 ml of MagBeads (bed volume) in 2 ml of 1 \times PBS containing 20% ethanol. BioMag Protein A/G is an excellent purification tool for most immunoglobulins. Protein A/G binds to all human IgG subclasses and also binds somewhat to IgA, IgE, IgM and, to a lesser extent, IgD. Therefore, it has a much broader binding range than either Protein A or Protein G individually. Unlike non-recombinant Protein G, Protein A/G does not bind serum albumin because the gene sequence coding for the albumin-binding site has been eliminated. Protein A/G is effective for mouse monoclonal antibody purification from IgG subclasses because Protein A/G binds all mouse IgG subclasses but does not bind murine IgA, IgM or serum albumin. It is ready to use after a gentle shake.

Protein A/G is a genetically engineered protein (MW \approx 43 kDa) that combines the IgG binding sites of both Protein A and Protein G. 6 \times His-tag was attached to its N-terminal to facilitate the purification. The secreted Protein A/G contains four Fc-binding domains from Protein A and two from Protein G, making it a more universal tool to bind and purify immunoglobulins.

II. KEY FEATURES

- ◆ Quick and convenient separation accomplished by magnetic force
- ◆ High capacity: The product can bind more than 5 mg of porcine or goat IgG
- ◆ Low nonspecific binding

III. CHARACTERISTICS

Ligand	Recombinant protein A/G produced in <i>E. coli</i>
Number of IgG binding sites per ligand	4 of Protein A and 2 of Protein G
M. W. of ligand	Approximately 43 kDa
pI of ligand	4.48
Conc. Of ligand	Approximately 2 mg protein A/G/ml beads
Volume of beads (bed volume)	0.5 ml
Static binding capacity	> 10 mg goat or porcine IgG/ml beads (bed volume)
Total binding capacity	> 5 mg goat or porcine 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. GENERAL PROTOCOL

A. Additional materials required

Binding buffer: Na₂HPO₄ 20 mM, NaCl 0.15 M, pH 7.0
Elution buffer: 0.1 M glycine, pH 2-3
Neutralization buffer: 1 M Tris; pH 7.5-9
1.5 ml microcentrifuge tubes
Magnet for a 1.5 ml microcentrifuge tube

B. Procedure

NOTE: Shake the bottle gently before use

1. Place 100 µl of the BioMag Beads 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. Once the supernatant becomes clear, remove and discard it. Repeat this step three more times.
3. Resuspend the beads in 100 µl of binding buffer.
4. Add 50 µl of serum or cell culture supernatant to the tube and gently invert tube to mix. Incubate tube at room temperature on a shaker for one hour or longer.
5. Magnetically separate the beads. Once the supernatant becomes clear, remove and discard the supernatant.
6. Add 1000 µl of binding buffer to the tube, mix well, magnetically separate the beads and discard the supernatant. Repeat this wash step three more times.
7. Add 100 µl of elution buffer to the tube. Mix well and incubate for five minutes at room temperature with occasional mixing. Magnetically separate the beads. Once the supernatant becomes clear, remove and save it. It contains the eluted antibody. Repeat this elution three more times to recover as much of the IgG as possible.
8. Neutralize the elute immediately with 5.0 µl of neutralization buffer. If necessary, perform a buffer exchange by dialysis or desalting.

V. EXAMPLES

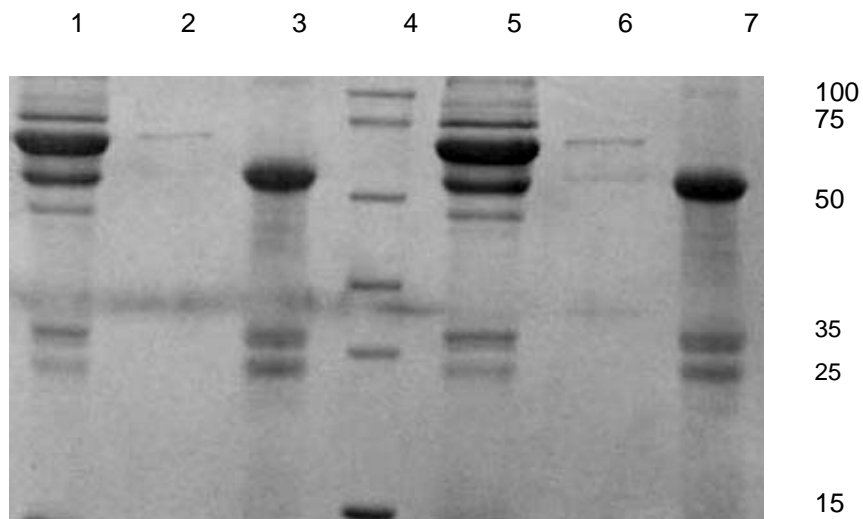


Fig 1. SDS-PAGE of binding capacity detection of Protein A/G MagBeads (GenScript, L00277)

- | | |
|---------------------------------|-----------------------|
| 1. Flowthrough of porcine serum | 5. Flow of goat serum |
| 2. Wash | 6. Wash |
| 3. Elute of porcine IgG | 7. Elute of goat IgG |
| 4. Molecular standard | |



VI. TROUBLESHOOTING

Problem	Possible Cause	Solution
The magnetic particles are hard to be immobilized with the magnet.	There are too much MagBeads for the magnet to properly immobilize them all.	Decrease the amount of MagBeads.
A considerable amount of antibody has been purified, but no specific antibody of interest is detected.	The antibody of interest is at very low concentration.	Use a serum-free medium for cell culture.
		Affinity-purify the antibody using its specific antigen coupled to an affinity support.
The antibody of interest is purified, but it is degraded (as determined by lack of function in downstream assay).	The antibody is sensitive to low-pH elution buffer.	Neutralize the elute immediately or use other gentle elution reagent.
	The downstream application is sensitive to the neutralized elution buffer.	Desalt or dialyze the elute using a suitable buffer.
No antibody is detected in any elution fraction.	The antibody may not bind to Protein A/G.	Try other purification method.

VII. ORDERING INFORMATION

GenScript Protein A/G MagBeads, Cat. No. L00277

For Research Use Only.

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