Product Data Sheet

Product Name: Protein G Magnetic Beads

Cat. No.: GK30001

Product Introduction:

GLPBIO Protein G Magnetic Beads are commonly used for IgG antibody enrichment and immunoprecipitation. The Protein G Magnetic Beads employ nanosurface biotechnology to covalently link Protein G to the surface of ultra-paramagnetic magnetic beads with a diameter of 2 μm. These beads offer numerous antibody binding sites, making them easy and efficient to use. With a large specific surface area, GLPBIO Protein G Magnetic Beads significantly reduce the time required for antibody adsorption and antigen binding. The beads exhibit high affinity for the Fc region of IgG antibodies from various species, making them suitable for purifying antibodies from serum, cell lysates, cell secretion supernatants, and ascites. Additionally, these beads display low non-specific binding.

Product Characteristics

Item	Features
Bead Concentration	10 mg/mL
IgG Binding Capacity	0.5 mg/mL
Application Range	IP, Co-IP, CHIP
Applicable Antibody Species	Broad-spectrum antibody species

Operating Instructions

Recommended Buffer (provided by user)	
Binding/Washing Buffer	PBST: 1×PBS + 0.5% Tween-20, pH 7.4
Elution Buffer	0.15 M Glycine, pH 2.5-3.1

1. Preparation of the antigen sample (please choose the appropriate treatment method based on the type of sample).

Samples	Sample Processing
Serum	If the target protein is abundant, it is recommended to dilute the serum sample so that the final concentration of the target protein is between 10-100 μg/mL. Keep on ice for immediate use (or store at -20°C for long-term preservation).
Suspended Cells	Centrifuge to collect the cells (at 4°C, 500g for 10 mins). Discard the supernatant and weigh the pellet. Wash twice with 1×PBS (pH 7.4) using 50 μL per milligram of cells. Add cell lysis buffer at a ratio of 5-10 μL per milligram of cells, and also add protease inhibitors. Mix well and incubate on ice for 10 minutes. Centrifuge (at 4°C, 14,000 g for 10 mins). Collect the supernatant and keep on ice for immediate use (or store at -20°C for long-term preservation).
Adherent Cells	Remove the culture medium. Wash twice with 1×PBS (pH 7.4) using 150 μL per 1×10^5 cells. Use a cell scraper to detach the cells and collect them in a 1.5 mL EP tube. Add cell lysis buffer at a ratio of 20-30 μL per 1×10^5 cells, and also add protease inhibitors. Mix well and incubate in an ice-water bath for 10 minutes. Centrifuge (at 4°C, 14,000 g for 10 mins). Collect the supernatant and keep on ice for immediate use (or store at -20°C for long-term preservation).

Tel: (909) 407-4943 Fax: (626) 353-8530 E-mail: tech@glpbio.com
Address: 10292 Central Ave. #205, Montclair, CA, USA

Product Data Sheet

E. coli (Escherichia coli)	Centrifuge the E. coli (at 4°C, 12,000 g for 2 mins). Discard the supernatant and weigh the pellet. Wash twice with 1×PBS (pH 7.4) using 10 mL per gram (wet weight) of bacterial cells. Add cell lysis buffer at a ratio of 5-10 mL per gram (wet weight) of bacterial cells, and also add protease inhibitors. Resuspend the bacterial cells, and use ultrasonication to lyse them. Centrifuge (at 4°C, 17,000 g for 10 mins). Collect the supernatant and keep on ice for immediate use (or store at -20°C for long-term preservation).

2. Pre-treatment of Magnetic Beads

Resuspend the magnetic beads thoroughly. Take 25-50 µL of the beads and place them in a 1.5 mL EP tube. Add 400 µL of binding/washing buffer and mix well. Place the tube on a magnetic stand to allow for magnetic separation, then discard the supernatant. Repeat the above washing step two more times.

3. Antibody and Magnetic Bead Binding

(1) Antibody Pre-treatment: Dilute the antibody using the binding/washing buffer to a final concentration of 5-50 µg/mL.

(2) Antibody-Bead Binding: Add the 400 µL of diluted antibody to the magnetic beads prepared in step 2. Mix well and incubate on a rocker mixer (at room temperature for 30 mins; at 4°C for 2 hours). Use magnetic separation and transfer the supernatant to a new EP tube for subsequent assays.
(3) Washing: Add 400 µL of binding/washing buffer and resuspend the beads thoroughly. Use magnetic separation and discard the supernatant. Repeat the washing process 4 times. Note: During the binding process, magnetic beads might clump together or appear flaky. This is a normal phenomenon and will not affect the experimental results.

4. Binding of Antigen to Antibody-Magnetic Bead Complex

(1) Binding of Antigen to Antibody-Bead Complex: Add 400 µL of the antigen sample prepared in step 1, mix thoroughly, and incubate on a rocker mixer (at room temperature for 30 mins; at 4°C for 2 hours). Use magnetic separation and discard the supernatant.

(2) Washing: Resuspend the beads thoroughly using 400 µL of the binding/washing buffer. Use magnetic separation and discard the supernatant. Repeat the washing process 4 times.

5. Antigen Elution

Two elution methods are provided in this instruction. The operator can choose different antigen elution methods based on subsequent detection needs.

a. Denaturing Elution Method: The sample eluted by this method is suitable for SDS-PAGE detection.

Steps: Separate the beads and discard the supernatant. Add 25-50 µL of 1×SDS-PAGE Loading Buffer to the beads and mix well. Heat at 95°C for 5 mins. Separate the beads, collect the supernatant, and proceed with SDS-PAGE detection.

b. Non-denaturing Elution Method: The eluted sample retains its original biological activity, suitable for subsequent functional analysis.

Steps: Separate the beads and discard the supernatant. Add 25-50 µL of the elution buffer to the beads and incubate at room temperature for 10 mins. Separate the beads, collect the supernatant into a new EP tube, and immediately add 1/10th of the total volume of neutralizing buffer (0.1 M NaOH) to adjust the pH of the eluted product to neutral. The sample is then ready for subsequent functional analysis.

Note: The antigen eluted in this step is an antigen-antibody complex. If the operator wishes to elute the target antigen separately, it is recommended to use a cross-linking agent and follow the relevant experimental instructions.

Tel: (909) 407-4943 Fax: (626) 353-8530 E-mail: tech@glpbio.com
Address: 10292 Central Ave. #205, Montclair, CA, USA

Product Data Sheet

Storage

4°C	2 years

Precautions

1. The pH of this product is 6-8; do not freeze.

2. This product should avoid centrifugation, drying, or freezing. Prolonged exposure to a magnetic field may cause bead aggregation. Handle gently after antibody binding to avoid antibody detachment.

3. To minimize protein degradation, it is recommended to use protease inhibitor cocktails.

4. Before use, consult the appendix to confirm the affinity between the antibody subtype and Protein G. If the affinity is poor, it can be improved by increasing the incubation time of the antibody with the beads (30-120 mins), raising the pH of the binding buffer (8-9), or reducing the ionic strength (25-100 mM NaCl).

5. To improve the specificity of the beads in immunoprecipitation, you can incubate the antibody with the sample first to form the antibody-antigen complex, then use Protein G beads to capture the complex.

6. Bead aggregation in low pH elution buffer is normal. Adding 0.1% (V/V) non-ionic detergent (e.g., NP-40, Tween-20, or Triton X-100) to the binding/wash buffer and elution buffer can effectively prevent bead aggregation. Beads that have been eluted at low pH can be washed to neutrality with binding/wash buffer, then resuspended with 0.1% (V/V) Tween-20 in Tris Buffer (pH 7.5), and sonicated for 2 minutes to return to a uniform state without affecting the bead's antibody-binding efficiency.

7. Sonication can cause the detachment of antibodies captured by the beads in the sample solution, so this method should not be used to resuspend the beads after antibody capture.

8. This product is for research purposes only by qualified professionals and should not be used for clinical diagnosis, treatment, food, or drugs.

9. For your safety and health, please wear a lab coat and disposable gloves when handling.

Tel: (909) 407-4943 Fax: (626) 353-8530 E-mail: tech@glpbio.com
Address: 10292 Central Ave. #205, Montclair, CA, USA