# Protein A/G Magnetic Beads

**PB101**

Version 24.1

## Product Description

Protein A/G Magnetic Beads are formed by covalently binding polymer magnetic microspheres with high purity recombinant Protein A/G. The mean diameter is 1 μm. Recombinant Protein A/G includes five Fc binding domains from Protein A and two Fc binding domains from Protein G, with stronger binding ability than Protein A or Protein G. This product has a larger specific surface area and more antibody binding sites, which can greatly shorten the antibody adsorption time and reduce non-specific binding.

## Information

| | |
|---|---|
| **Composition:** Polymer magnetic microspheres | **Ligand:** Recombinant Protein A/G |
| **Mean Diameter:** 1 μm | **Concentration:** 10 mg/ml |
| **Binding Capacity:** 0.5 mg/ml | **Storage Buffer:** PBS, 0.01% Tween 20, 0.005% ProClin 300 |

## Components

| Components | PB101-01 | PB101-02 |
|---|---|---|
| Protein A/G Magnetic Beads | 1 ml | 5 × 1 ml |

## Storage

Store at 2 ~ 8°C. Adjust the shipping method according to the destination.

## Applications

It is applicable for IP, CoIP, ChIP, immunoprecipitation and antibody purification under non-reducing conditions.

## Notes

For research use only. Not for use in diagnostic procedures.

1. Please do not dry or freeze the magnetic beads, and avoid placing it on the magnetic rack for a long time, as this can cause the beads to aggregate.
2. Mix the magnetic beads thoroughly by vortexing or gently inverting the tube; do not centrifuge them at high speeds.
3. When aspirating magnetic beads, select low adsorption pipette tips to prevent loss caused by the beads sticking to the tip.
4. If the IP effect is unsatisfactory, the binding efficiency can be improved by increasing the incubation time of antibodies with magnetic beads (0.5 - 2 h) and optimizing the binding system.
5. Wear a lab coat and disposable gloves for protection.

## Experiment Process

▲ Buffer concentration should be determined based on specific experiments. It is recommended to conduct a pilot experiment to determine the optimal concentration.

Binding Buffer: 50 mM Tris, 150 mM NaCl, 1 mM EDTA, 1% NP-40, pH 7.4.

Wash Buffer: Binding buffer or PBST (1 × PBS, 0.5% Tween 20, pH 7.4).

Elution Buffer: 0.1 M Glycine, pH 2.0.

Neutralization Buffer: 1.5 M Tris-HCl, pH 8.5.

### Step 1: Preparation of the Antigen Sample

▲ Optimal processing methods vary in different samples.

1. Adherent cells: Carefully remove culture medium and wash the cells twice with PBS. Collect cells in a 1.5 ml microcentrifug tube, add 20 - 30 µl binding buffer with 1 × protease inhibitor per 1 × $10^5$ cells. Incubate on ice for 10 - 20 min with periodic mixing. Centrifuge at 4°C, 12,000 rpm (13,800 × g) for 5 min, collect the supernatant and store it on ice for immediate use or at -80°C for long-term storage.
2. Suspension cell: Collect cell pellets, discard the supernatant and weigh the pellet. Wash twice with PBS. Add 50 µl binding buffer with 1 × protease inhibitor per mg cells pellets. Incubate on ice for 10 - 20 min with periodic mixing. Centrifuge at 4°C, 12,000 rpm (13,800 × g) for 5 min, collect the supernatant and store it on ice for immediate use or at -80°C for long-term storage.
3. Serum sample: Dilute the serum sample with binding buffer to a final protein concentration of 10 - 100 µg/ml, and store it on ice for immediate use or at -80°C for long-term storage.

### Step 2: Preparation of the Immune Complex

▲ The amount of sample needed and the incubation time are dependent upon each specific antibody-antigen system and the experimental system can be optimized according to the actual situation.

The following protocol is for 2 - 10 µg of affinity-purified antibody and can be scaled up as needed.

1. Mix 500 - 1000 µg cell lysate sample with 2 - 10 µg of antibody in a microcentrifuge tube.
2. Dilute antibody/cell lysis solution to 500 µl with the binding buffer.
3. Rotate and incubate for 30 min at room temperature or overnight at 4°C to form the immune complexes.

### Step 3: Preparation of Magnetic Bead

1. Mix the magnetic beads thoroughly by vortex or turning upside down. Transfer 30 - 50 µl of magnetic beads into a 1.5 ml microcentrifug tube. Add 500 µl of binding buffer to resuspend. Briefly centrifuge and then place the tube on a magnetic rack for 30 sec. Carefully discard the supernatant and repeat the washing step once.
2. Add 100 µl binding buffer to resuspend magnetic beads for later use.

### Step 4: Immunoprecipitation

1. Add 500 µl antigen sample/antibody mixture (Step 2) to the 1.5 ml microcentrifug tube containing magnetic beads and incubat at room temperature on a vertical rotator for 30 min.
2. Briefly centrifuge, then place the tube on a magnetic rack for 30 sec to allow the magnetic beads to adsorb. Carefully discard the supernatant.
3. Remove the tube from the magnetic rack and add 500 µl of wash buffer to resuspend the magnetic beads. Briefly centrifuge and place the tube on the magnetic rack for 30 sec to allow complete bead adsorption. Carefully discard the supernatant. Repeat the washing step three times.

### Step 5: Antigen elution

▲ Selection of a suitable antigen elution method.

1. Denaturation: The method is applicable for SDS-PAGE detection.

   Place the tube on the magnetic rack and let it stand for 30 sec until the magnetic beads are fully adsorbed. Carefully discard the supernatant and add 25 - 50 µl of 1 × SDS-PAGE loading buffer to resuspend the magnetic beads. Mix by vortexing for 30 sec and heat the samples at 95ºC in a heating block for 5 min. Brief centrifugation and place the centrifuge tube on the magnetic rack and let it stand for 30 sec. Transfer the supernatant to a new tube for further SDS-PAGE analysis.

2. Non-denaturation: The eluted samples from this method maintain their original biological activity and can be used for subsequent functional analysis.

   Place the tube on the magnetic rack and let it stand for 30 sec until the magnetic beads are fully adsorbed. Carefully discard the supernatant and add 25 - 50 µl elution buffer to resuspend the magnetic beads. Incubate at room temperature for 5 - 10 min. Briefly centrifuging and place the tube on the magnetic rack for 30 sec. Transfer the supernatant to a new tube and add 1/10 volume of neutralization buffer (adjust the eluted sample to neutral pH) for immediate use or storage at -80°C for long-term .