

Omnimabs® Protein A Magnetic Beads

Packaging List

Name	Catalog Number	Package
Protein A Magnetic Beads	OM760024	1mL
Protein A Magnetic Beads	OM760024	5mL

Product Overview

Omnimabs® Protein A Magnetic Beads are 200nm nanoscale magnetic beads covalently conjugated with a large amount of recombinant Protein A. The ultra-large specific surface area provided by nanoscale beads offers more binding sites, requiring less bead usage and resulting in low non-specific adsorption. Recombinant Protein A has a higher affinity for binding mouse IgG2a, IgG2b, IgA, rabbit IgG, and human IgG1, IgG2, and IgG4. This product is widely used for immunoprecipitation (IP) or co-immunoprecipitation (Co-IP) of antigens in samples such as cell lysates, cell culture supernatants, serum, and ascites. Magnetic separation reduces IP/Co-IP time by 40%.

Product Features

Matrix	Silica-Based Magnetic Beads
Ligand	Recombinant Protein A
Particle Size	200nm
Bead Concentration	10mg/mL
Binding Capacity	≥0.9mg hIgG/mL beads
Applications	IP, Co-IP, ChIP, RIP, etc.
Shelf Life	Stable for 2 years at 2 – 8°C

Protocol

Note: Prepare buffers independently or purchase our IP Kit directly.

Adherent Cell Samples

1. Remove culture medium and wash cells twice with PBS.
2. Collect cells into a 1.5mL EP tube, add IP Lysis/Wash Buffer in proportion, include inhibitors (e.g., PMSF), mix, and incubate on ice for 5–20 minutes (mix occasionally).
3. Centrifuge at 12,000–16,000 x g for 10 minutes at 4°C, collect supernatant, and store on ice (or at -80°C long-term).

Recommended IP Lysis/Wash Buffer Volume for Culture Dishes:

Dish Size/Surface Area	Buffer Volume
100mm × 100mm	500 – 1000μL
100mm × 60mm	100 – 300μL
6-well plate	100 – 200μL

Suspended Cell Samples:

1. Centrifuge cells at 500–1000 x g for 10 minutes at 4°C, discard supernatant.
2. Wash cells once with PBS: resuspend cell pellet in PBS, centrifuge at 500–1000 x g for 5 minutes at 4°C, discard supernatant.
3. Resuspend cells in pre-chilled IP Lysis/Wash Buffer (500μL per 50mg cells). Add inhibitors (e.g., PMSF), mix, and incubate on ice for 5–20 minutes (mix occasionally).
4. Centrifuge at 12,000–16,000 x g for 10 minutes at 4°C, collect supernatant, and store on ice (or at -80°C long-term).

Serum Samples

Dilute serum with IP Lysis/Wash Buffer to a target protein concentration of 50–150μg/mL. Store on ice (or at -20°C long-term).

Immunocomplex Preparation

Note: Optimize sample volume and incubation time based on the antibody-antigen system.

For 2–10 μ g affinity-purified antibody (scale up as needed):

1. Combine cell lysate with 2–10 μ g IP antibody in a tube. Total protein per IP reaction: 500–1500 μ g.
2. Dilute antibody and sample to 300–500 μ L with IP Lysis/Wash Buffer.
3. Incubate at room temperature for 1–2 hours or at 4°C for 2–4 hours to form immunocomplexes.

Immunoprecipitation

Note: Vortex gently before use to ensure uniform bead distribution.

1. Add 20–50 μ L Omnimabs® Protein A Magnetic Beads to a 1.5mL tube.
2. Add 500 μ L pre-chilled PBS, mix gently.
3. Place tube on a magnetic stand to collect beads, discard supernatant.
4. Add 200–500 μ L IP Lysis/Wash Buffer, invert tube or vortex for 1 minute, collect beads magnetically, discard supernatant.
5. Add antigen/antibody mixture to beads, mix, and incubate at room temperature for 1–2 hours or at 4°C for 2–4 hours.
6. Collect beads magnetically, retain unbound supernatant for analysis.
7. Wash beads with 1000 μ L IP Lysis/Wash Buffer for 5–10 minutes, discard supernatant. Repeat twice.
8. **Denaturing Elution:** Add 80–100 μ L SDS-PAGE Sample Loading Buffer (1 \times), heat at 100°C for 10 minutes, separate beads magnetically, retain supernatant.

Note: For native elution:

Low pH Elution: Add 100 μ L Elution Buffer, incubate 5–10 minutes, neutralize with 20 μ L Neutralization Buffer per 100 μ L eluate.

Precautions

1. Read the manual thoroughly before use.
2. Avoid high-speed centrifugation, drying, or freezing beads to prevent aggregation.
3. Optimize buffers based on antibody-antigen compatibility.
4. Resuspend beads thoroughly before use and store in solution to prevent drying.
5. For research use only.

Troubleshooting

1. Antigen not immunoprecipitated

1: Insufficient antigen in the sample for detection

Suggestion: Verify the protein expression and/or lysis efficiency in the lysate through SDS - PAGE or Western blotting. Increase the sample volume if necessary.

2: Antibody fails to bind to the antigen

Suggestion: Select another specific antibody or an antibody that recognizes a different antigenic epitope.

3: Components in the IP Lysis/Wash Buffer interfere with the binding between the antigen and the antibody

Suggestion: Use other buffers for immunoprecipitation and rinsing (e.g., TBS containing 0.5% CHAPS).

2. Low protein yield

1: Protein degradation

Suggestion: Add protease inhibitors.

2: Insufficient amount of magnetic beads used

Suggestion: Increase the amount of magnetic beads for capturing immunocomplexes.

3: Insufficient amount of target protein in the sample

Suggestion: Increase the volume of the antigen sample.

3. Multiple non - specific bands

Specific proteins bind to the magnetic beads

Suggestion: Add 50 - 350 mM NaCl to the IP Lysis/Wash Buffer. Increase the intensity and frequency of elution.

4. Magnetic bead aggregation

Aggregation of magnetic beads in the low - pH Elution Buffer is a normal phenomenon and does not affect the normal use of the beads.

- **Suggestion:** Neutralize the buffer with IP Lysis/Wash Buffer, then resuspend the magnetic beads by shaking in a Tris buffer (pH 7.5) containing 0.1% (v/v) Tween - 20, and treat them in an ultrasonic water bath. This can restore the beads to a uniform state, and these treatments do not affect the antibody - binding efficiency of the beads. You can also add a non - ionic detergent (such as Tween - 20 or Triton X - 100) at a final concentration of 0.1% (v/v) to effectively prevent bead aggregation.
- **Note:** Ultrasonic treatment can also cause the antibodies captured by the magnetic beads in the sample solution to fall off. Therefore, this method should not be used between sample addition and elution.