Protein A/G Immunoprecipitation Kit

Catalog No.: BRC0016

Product Description

EnkiLife Protein A/G Immunoprecipitation Kit utilizes high-purity Protein A/G fusion protein that is covalently and densely immobilized on the surface of superparamagnetic microspheres. The kit offers high antibody binding capacity, low non-specific binding, and simple, rapid operation. It is suitable for efficient immunoprecipitation of antigens from cell lysates, serum, ascites, and other samples. Each milliliter of immunoprecipitation magnetic beads has been tested to bind ≥300 μg of human IgG. A single precipitation reaction requires only 25 μL of beads, with antibody adsorption completed within 15 minutes and antigen precipitation within 30 minutes, significantly shortening experimental time while preserving protein activity.

This kit includes optimized, ready-to-use buffers that provide optimal reaction conditions for immunoprecipitation experiments, enhancing experimental consistency and reliability.

Kit Components

No.	Reagent Name	Size	Note
Reagent 1	Protein A/G Immunoprecipitation Magnetic Beads	1 mL	2 μm, binding capacity 0.5–0.6 mg/mL
Reagent 2	IP Binding Buffer	30 mL	Ready-to-use
Reagent 3	IP Washing Buffer	20 mL	Ready-to-use
Reagent 4	IP Elution Buffer	0.5 mL	Low pH
Reagent 5	IP Neutralization Buffer	0.2 mL	For neutralizing eluate
Reagent 6	10× PBS	20 mL	Dilute before use
Other	Magnetic Separator	Optional	Supplied by user

Storage Conditions and Shelf Life

Storage Conditions: Store at 2–8°C. DO NOT FREEZE.
Shelf Life: 2 years

Protocol

1. Sample Preparation

Select appropriate lysis and processing methods based on sample type (cells, serum, bacterial culture, etc.) to ensure target protein release.

Serum Sample Processing: If target protein abundance is high, dilute serum samples with IP Binding Buffer (Reagent 2) to a final target protein concentration of 10–100 μg/mL. Keep on ice (or store at –20°C for long-term storage).

Suspension Cell Sample Processing: Collect cells by centrifugation (4°C, 500 g, 10 min). Weigh after discarding supernatant. Wash twice with 1× PBS (Reagent 6) at 50 µL per mg of cells. Add IP Binding Buffer (Reagent 2) at 5–10 µL per mg of cells along with protease inhibitors (e.g., PMSF at a final concentration of 1 mM). Mix and incubate on ice for 10 min. Centrifuge to collect supernatant (4°C, 14,000 g, 10 min). Keep on ice (or store at –20°C for long-term storage).
Adherent Cell Sample Processing: Remove culture medium. Wash twice with 1× PBS (Reagent 6) at 150 µL per 1.0×10^5 cells. Scrape cells with a cell scraper, collect into a 1.5 mL microcentrifuge tube. Add IP Binding Buffer (Reagent 2) at 20–30 µL per 1.0×10^5 cells along with protease inhibitors (e.g., PMSF at a final concentration of 1 mM). Mix and incubate on ice for 10 min. Centrifuge to collect supernatant (4°C, 14,000 g, 10 min). Keep on ice (or store at –20°C for long-term storage).
E. coli Sample Processing: Collect E. coli by centrifugation (4°C, 12,000 g, 2 min). Weigh after discarding supernatant. Wash twice with 1× PBS (Reagent 6) at 10 mL per gram (wet weight) of bacteria. Resuspend pellet in IP Binding Buffer (Reagent 2) at 5–10 mL per gram (wet weight) of bacteria along with protease inhibitors (e.g., PMSF at a final concentration of 1 mM). Sonicate to lyse cells. Centrifuge to collect supernatant (4°C, 17,000 g, 10 min).

2. Magnetic Bead Pretreatment

Vortex the immunoprecipitation magnetic beads for 1 min before use to ensure complete resuspension. When ready to use, resuspend the vortexed beads and transfer 25–50 µL of beads. Wash twice with 200 µL of IP Binding Buffer (Reagent 2) (magnetic separation), then resuspend in 200 µL of IP Binding Buffer for later use.

3. Antibody Binding

Antibody Dilution: Dilute the antibody with IP Binding Buffer (Reagent 2) to a final concentration of 5–50 µg/mL as the working antibody solution.
Antibody Adsorption: Magnetically separate the pretreated beads, then add 200 µL of antibody working solution. Resuspend promptly and place the tube on a rotator for gentle end-over-end mixing at room temperature. After 15 min, perform magnetic separation and collect the supernatant on ice for subsequent detection.
Washing: Add 200 µL of IP Binding Buffer (Reagent 2) to the tube. Gently pipette to evenly disperse the bead-antibody complexes. Perform magnetic separation and discard the supernatant. Remove the tube from the magnetic separator. Repeat the washing step once.

4. Antigen Precipitation

Antigen Binding: Add 200 µL of antigen sample prepared in Step 1. Gently pipette to evenly disperse the antigen with the bead-antibody complexes. Place the tube on a rotator for gentle end-over-end mixing at room temperature for 10 min to allow full antigen-antibody binding. If binding is weak, incubate at room temperature for 1 h or overnight at 4°C.
Washing and Transfer: Magnetically separate the bead-antibody-antigen complexes and collect the supernatant on ice for subsequent detection. Add 200 µL of IP Washing Buffer (Reagent 3) to wash. Gently pipette to evenly disperse the bead-antibody-antigen complexes. Perform magnetic separation and discard the supernatant. Remove the tube from the magnetic separator. Repeat the wash two more times. Finally, add 200 µL of IP Washing Buffer (Reagent 3), transfer the bead-antibody-antigen suspension to a new 1.5 mL microcentrifuge tube using a pipette, perform magnetic separation, and discard the supernatant.
(Note: Be sure to transfer the beads to a new tube before antigen elution to avoid co-eluting non-specifically adsorbed proteins from the tube wall.)

5. Antigen Elution

Denaturing Elution: Add SDS-PAGE loading buffer, heat at 95°C for 5 minutes, then perform magnetic separation (or centrifuge at 13,000 g for 10 min at room temperature). Collect the supernatant for SDS-PAGE analysis.

Non-Denaturing Elution: Add 20 µL of IP Elution Buffer (Reagent 4), mix well, and incubate at room temperature for 10 min. Perform magnetic separation (or centrifuge at 13,000 g for 10 min at room temperature). Transfer the supernatant to a new microcentrifuge tube and immediately add 1.0 µL of IP Neutralization Buffer (Reagent 5) to adjust the pH of the eluate to neutral for downstream functional analysis.

Warnings and Precautions

1. Thoroughly resuspend magnetic beads before use. Avoid drying or freezing.
2. Use low-retention microcentrifuge tubes to reduce non-specific adsorption.
3. If bead aggregation occurs, sonicate in a water bath for 2 minutes to restore dispersion.
4. For research use only. Not for diagnostic or therapeutic use.

Frequently Asked Questions

Q1: How can I improve antibody-bead binding efficiency?

A1: Antibody-bead binding efficiency depends on the species and isotype of the antibody. Please confirm antibody compatibility with the Protein A/G ligand binding efficiency. If the antibody isotype has low affinity for Protein A/G, you can improve binding efficiency by: (1) increasing incubation time (30–120 min), (2) raising the binding buffer pH (8–9), and (3) reducing ionic strength (25–100 mM NaCl).

Q2: How can I improve specificity in immunoprecipitation reactions?

A2: Pre-incubate the antibody with the sample to form antibody-antigen complexes, then capture the complexes with Protein A/G magnetic beads. This method enhances antibody-antigen binding efficiency and reduces bead-sample contact time, thereby improving specificity of the precipitated product. This approach is also recommended for protein/nucleic acid co-precipitation or chromatin immunoprecipitation.

Q3: How can I avoid bead aggregation during storage or use?

A3: Store beads at 2–8°C. Avoid irreversible aggregation caused by contamination or drying during use. Aggregation in low-pH elution buffer is normal and does not affect bead performance. Adding a final concentration of 0.1% (V/V) non-ionic detergent (e.g., NP-40, Tween-20, or Triton X-100) to Binding and Elution buffers can effectively prevent bead aggregation. Beads processed with low-pH elution can be washed to neutral pH with binding buffer, then resuspended in Tris buffer (pH 7.5) containing 0.1% (V/V) Tween-20 with sonication for 2 min to restore uniform dispersion. These treatments do not affect antibody binding efficiency.

Q4: How can I prevent beads from sticking to tube walls?

A4: Use low-retention consumables for magnetic bead operations. Additionally, adding 0.01%–0.1% (V/V) non-ionic detergent (e.g., NP-40, Tween-20, or Triton X-100) to the buffer can effectively reduce bead adhesion to consumables.

Q5: What if beads clump during use?

A5: Bead clumping during use is generally difficult to disperse by vortexing and can lead to uneven distribution. This occurs when beads are left in the magnetic field for too long, causing them to bind firmly together. Dispersion can be restored by sonicating in a water bath for 2 minutes. Note that

sonication may also cause antibody detachment from beads captured in the sample solution, so this method should not be used after sample addition and before elution.