Protein A/G Immunoprecipitation Kit

DESCRIPTION

Protein A/G immunoprecipitation magnetic bead kit series products are coated with Protein A/G high-density directional coating on the surface of superparamagnetic microspheres. The amount of magnetic beads used is small, the non-specific binding rate is low, and immunoprecipitation experiments can be carried out conveniently and efficiently. . The ability of immunoprecipitation magnetic beads to bind Human IgG per milliliter can reach more than 300μg, and only 50μg magnetic beads can be detected in a single precipitation reaction. The large specific surface area provided by the nano-scale magnetic beads greatly shortens the equilibrium time required for antibody and antigen adsorption. The antibody adsorption process can be completed within 10 minutes, and the antigen precipitation operation can be completed within 30 minutes. The short operation time avoids the hydrolysis of the target protein caused by long-term operation, and ensures the activity of the target protein and the integrity of the protein complex. The immunoprecipitation magnetic bead kit is equipped with an optimized prefabricated buffer, which provides the best reaction conditions for immunoprecipitation experiments and improves the stability of immunoprecipitation experiments. It can be widely used in the immunoprecipitation of antigens in cell lysates, cell secretion supernatants, serum, ascites and other samples.

PRODUCT INFORMATION

Immunoprecipitation Magnetic Beads Protein A/G for IP	1 mL
IP Binding Buffer	30 mL
Phosphate Buffered Saline	20 mL

PBS (10×, please dilute to 1× before use)	
Washing Buffer IP Washing Buffer	20 mL
IP Elution Buffer	0.5 mL
Neutralization Buffer IP Neutralization Buffer	0.5 mL
Magnetic Separator Stand	0.2 mL
2/15	
Storage conditions	Store at 2~8℃

Note: The ability of magnetic beads to bind Human IgG is: Protein A: 0.4~0.5 mg/mL; Protein A/G: 0.5~0.6 mg/mL

OPERATING PROCEDURES

1. Antigen Sample Preparation

1.1 Serum sample processing: The target protein has a high abundance. Dilute the serum sample with binding buffer to a final concentration of the target protein of 10-100 μg/mL, and keep it on ice for future use (or store it at -20°C for a long time).

1.2 Treatment of suspended cell samples: collect cells by centrifugation (4°C, 500 g, 10 min), discard the supernatant, weigh, and wash twice with 1× PBS at a rate of 50 μL per mg of cells; 5~10 cells per mg of cells Add binding buffer and protease inhibitors in the ratio of μL, mix well and place on ice for 10 min; collect supernatant by centrifugation (4°C, 14000 g, 10 min), put it on ice for later use (or place it on ice for 10 min). -20℃ long-term storage).

1.3 Treatment of adherent cell samples: remove the medium and wash twice with 1×PBS at a ratio of 150 μL per 1.0×105 cells; scrape off the cells with a cell scraper, collect them into 1.5 mL EP tubes, and press 1.0× per 1.0× Add 20~30 μL of binding buffer to 105 cells, and add protease inhibitors at the same time, mix well and place on ice for 10 min;

collect the supernatant by centrifugation (4°C, 14000 g, 10 min) and place on ice Reserve for later use (or store at -20°C for a long time).

1.4 Treatment of Escherichia coli samples: Collect Escherichia coli by centrifugation (4°C, 12000 g, 2 min), discard the supernatant, weigh, and wash twice with 1×PBS at a ratio of 10 mL per gram (wet weight) of bacterial cells; press Add binding buffer at a ratio of 5-10 mL per gram (wet weight) of bacterial cells, and at the same time add protease inhibitors, resuspend the bacterial cells, lyse the cells by ultrasonic, and collect the supernatant by centrifugation (4°C, 17000 g, 10 min).

2. Magnetic Bead Pretreatment

Vortex the immunoprecipitated magnetic beads for 1 min to fully resuspend the magnetic beads; take 25-50 μL of the magnetic bead suspension into a 1.5 mL EP tube. Add 200 μL of binding buffer to wash, perform magnetic separation, discard the supernatant, remove the EP tube from the magnetic separator, and repeat the washing once. Finally, add 200 μL of binding buffer to resuspend the magnetic beads for use.

3. Antibody Binding Reaction

3.1 Preparation of antibody working solution: Dilute the antibody sample with binding buffer to prepare a final concentration of 5-50 μg/mL antibody working solution, and keep it on ice for later use.

3.2 Antibody adsorption: Magnetically separate the magnetic bead suspension pretreated in step 2, discard the supernatant; add 200 μL of antibody working solution, quickly resuspend, and place it in an inversion mixer at room temperature or manually flip the EP tube gently. Magnetic separation was performed after 15 min, and the supernatant was collected and placed on ice for subsequent detection.

3.3 Washing: add 200 μL of binding buffer to the EP tube for washing, gently pipette with a pipette to disperse the magnetic bead-antibody complex evenly, and then perform magnetic separation, discard the supernatant, and remove the EP from the magnetic separator. Tube. Repeat the above washing operation once.

4. Antigen Precipitation

4.1 Antigen adsorption: Add 200 μL of the antigen sample prepared in step 1, and gently

pipette with a pipette to evenly disperse the antigen and the magnetic bead-antibody complex. Place the inverting mixer at room temperature or gently invert the EP tube by hand for 10 min to fully bind the antigen to the antibody. If the binding force is weak, it can be reacted at room temperature for 1 h or at 4 °C overnight.

4.2 Washing and transfer: Magnetic separation of the magnetic beads-antibody-antigen complexes that have completed antigen adsorption was carried out, and the supernatant was collected and placed on ice for subsequent detection. Add 200 μL of washing buffer to the EP tube to wash, gently pipette with a pipette to disperse the magnetic bead-antibody-antigen complexes evenly, then perform magnetic separation, discard the supernatant; remove the EP tube from the magnetic separator , repeat the wash two more times. Finally add 200 μL of wash buffer, pipette the magnetic bead-antibody-antigen complex suspension to a new 1.5 mL EP tube, perform magnetic separation, and discard the supernatant.

5. Antigen Elution

5.1 Denaturing elution method: The samples eluted by this method are suitable for SDS-PAGE detection. Remove the EP tube from the magnetic separator, add 25 μL of 1× SDS-PAGE Loading Buffer to it, mix well, and heat at 95°C for 5 min. Magnetic separation was then performed and the supernatant was collected for SDS-PAGE.

5.2 Non-denaturing elution method: The samples eluted by this method maintain the original biological activity and can be used for later functional analysis. Remove the EP tube from the magnetic separator, add 20 μL of elution buffer to it, mix well, and incubate at room temperature for 10 min. Magnetic separation was then performed, the supernatant was collected into a new EP tube, and 1.0 μL of neutralization buffer was immediately added to adjust the pH of the eluted product to neutrality for later functional analysis.

Notice

1. Before performing immunoprecipitation, please read this instruction manual carefully.

2. The magnetic beads should be fully shaken before use.

3. Magnetic beads should be kept in storage solution to prevent drying.

4. Do not freeze or centrifuge magnetic beads to avoid irreversible aggregation.

5. 10×PBS should be diluted under sterile conditions. Once the solution is found to be contaminated, please stop using the solution.

6. To ensure the best experimental results, please select antibodies with strong specificity for immunoprecipitation.

7. The operator can use the antibody binding reaction step and the supernatant collected in the antigen binding reaction step to detect the binding of antibodies, antigens and magnetic beads according to actual needs.

8. For IP experiments, the binding affinity of different types of antibodies to antigens is different, and the binding of antibodies to antigens will also be affected by the binding buffer and washing buffer. Therefore, if the operator uses the The buffer system cannot obtain good experimental results, and you can screen and prepare buffers for experiments.

9. The recombinant protein Protein A/G coated on the surface of the magnetic beads has extremely low protein shedding under extreme conditions (such as low pH, heat treatment), but it is still not recommended for operators to use it for target proteins with a molecular weight of about 130 kD. Immunoprecipitation experiments.

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