Ver. HB231025

rProtein A/G MagBeads (IP Grade)

Product description

Protein A/G MagBeads use "nano-surface biotechnology" (S-TEC) to target Protein A/G onto the surface of superparamagnetic polymer microspheres with high density. It has higher antibody binding ability and very low non-specific adsorption rate of protein. One-step purification can isolate the antibody with purity >90% from the serum sample, which is simple and effective to use. Natural Protein A is a cell wall surface protein found in Staphylococcus aureus. Natural Protein G is a cell surface protein isolated from the genus G or C Streptococcus. Both have similar functions and bind most mammalian IgG by interacting primarily with the Fc region of immunoglobulin (Ig), but differ in their binding specificity. Protein A/G MagBeads covalently conjugate both protein A and protein G, providing A wider binding range and higher utility than either protein A or protein G alone. At the same time, this product uses genetically modified proteins A and G, which not only maintain their Ig affinity properties, but also remove the non-major binding domain of the natural protein itself to reduce non-specific binding. This product has a wide range of application, can be used in cell lysis fluid, cell secretory fluid supernatant, serum, animal ascites and other immune antigen samples.

Components

Components No.	Name	36417ES03	36417ES08
36417	rProtein A/G MagBeads (IP Grade)	1 mL	5 mL

Specifications

Ligand	rProtein A/G
Binding Capacity	＞50 μg Rabbit IgG /mg
Particle size	1 μm
Concentration	10 mg/mL
Storage Buffer	PBS，0.01% Tween-20, 0.02% NaN_3
Application	rProtein Purification, Immunoprecipitation

Shipping and Storage

The products are shipped with ice pack. It can be stably stored at 2~8°C for 2 year.

Instructions

1. Buffer preparation

Note: The concentration of working liquid should be determined according to the specific experiment. It is recommended to conduct preliminary experiment to find out the best experimental concentration.

Lysis buffer	0.15 M NaCl, 50 mM Tris-HCl, 1% NP-40, pH7.4
Balance/bind/wash buffer	0.15 M NaCl, 20 mM Na_2HPO_4, pH 7.0
Crosslinked fluid	0.2 M Triethanolamine, pH 8.2
Neutralizing buffer	1 M Tris-HCl，pH 8.5

Elution buffer	0.1 M Glycine, pH 3.0
Stop Buffer	50 mM Tris, pH 7.5

2. Antigen sample preparation

This instruction manual provides the following four sample treatment methods. It is recommended that you choose the appropriate way to pretreat the antigen samples from different sources, so that the antigen to be detected is released into the sample solution.

1)Treatment of serum sample: If the target protein abundance is high, it is recommended to dilute the serum sample with binding buffer until the final target protein concentration is 10-100 μg/mL, and place it on ice for reserve (or store it at -20°C for long-term storage).

2)Treatment of suspended cell samples: Cells were centrifugally collected (4°C, 1000g, 5min),and flick with your fingers to spring the cells apart. According to the proportion of 150-250 μL lysis buffer/well of 6-well plate is added into lysis buffer containing protease inhibitor(PMSF should be added to the lysis buffer a few minutes before use so that the final concentration of PMSF is 1 mM), mixed and placed on ice for 10min; Centrifuge the supernatant (4°C, 14000g, 10min) and put it on ice for use (or store it at -20°C for a long time).

3)Adherent cell sample treatment: The medium was removed and washed twice with 1×PBS at a ratio of 150 μL per 1.0×10^5 cells. The cells were scraped off with a cell scraper and collected into a 1.5 mL EP tube. According to the proportion of 150-250 μL lysis buffer/well of 6-well plate is added into lysis buffer containing protease inhibitor (PMSF should be added to the lysis buffer a few minutes before use so that the final concentration of PMSF is 1 mM), mixed and placed on ice for 10min; Centrifuge the supernatant (4°C, 14000g, 10min) and put it on ice for use (or store it at -20°C for a long time).

4)Escherichia coli sample treatment: The E.coli were collected by centrifugation (4°C, 12000g, 2min), the supernatant was discarded and weighed. The bacteria were washed twice with 1×PBS at the rate of 10mL per gram (wet weight) of bacteria. The binding buffer was added at a ratio of 5-10 mL per gram (wet weight) of bacteria, at the same time, protease inhibitor was added, bacteria was suspended, cells were lysed by ultrasound, and supernatant was collected by centrifugation (4°C, 17000g, 10min).

3. Magnetic bead pretreatment

1) The magnetic bead vortex was oscillated for 1 min to make it fully suspended;

2) 50 μL magnetic bead suspension was placed in a 1.5 mL EP tube. Place on the magnetic separator, after the solution becomes clear, use the pipette to absorb and discard the protective liquid.

3) 200 μL binding buffer was added for washing, magnetic separation was performed, and the supernatant was aspirated and discarded for 1 repeat.

4. Antibody adsorption

1) add target antibody solution(5-25 μg total antibody), and mix thoroughly. When the volume is less than 500 μL, fill with a balance buffer

2) Incubate at room temperature for 10 min and mix evenly with oscillations or swirls.

3) Place the EP tube on the magnetic separator. After all the magnetic beads are absorbed, the supernatant is absorbed and discarded. It can be kept for further testing if necessary.

4) Add 500 μL of wash buffer, mix evenly, put on the magnetic separator, wait for all magnetic beads adsorption, absorb and discard the supernatant. Repeat at least 3 times.

5. Antibody crosslinking (optional)

1) If the antibody and target antigen complex need to be eluted together, please ignore this step and proceed directly 6. The following steps can be followed for any volume of 50 μL-1 mL magnetic beads without additional cross-linked liquid volume.

2) Add 1 mL of crosslinking liquid, suspend it in oscillation, and place it on a magnetic separator for about 1 min. After the solution becomes clear, the supernatant is absorbed and discarded. This operation is repeated twice.

3) 1 mL of cross-linking solution containing 20 mM DMP (dimetylpimelimidate dihydrochloride) is added, and the reagent needs to be used for distribution. Oscillating suspension, placed in the flip mixer at room temperature or hand gently flip the centrifugal tube to make full contact between the solution and the magnetic beads, about 30 min later, placed on the magnetic separator, about 1 min, after the solution became clear, suction and discard the supernatant.

4) Suspend magnetic beads with 1 mL Stop Buffer to terminate the crosslinking reaction. Place the mixer at room temperature or gently turn the centrifuge tube manually to make the solution fully contact with the magnetic beads. After about 15 min, place it on the magnetic separator for about 1 min. After the solution becomes clear, the supernatant is absorbed and discarded.

5) Add 1 mL balance buffer, mix it upside-down, and place it on a magnetic separator for about 1min. After the solution becomes clear, suck and discard the supernatant. Repeat two more times.

6. Antigen-binding reaction

1) Add the sample containing the antigen (usually 100-1000 μL) and gently blow it with a pipette to evenly disperse the antigen and the magnetic bead-antibody complex.

2) Place the rotating mixer at room temperature or turn the centrifuge tube gently by hand for 10min, so that the antigen and antibody are fully combined. If the binding force is weak, the reaction can be conducted at room temperature for 1 h or overnight at 4°C.

3) Magnetic separation of the above magnetic bead-antibody-antigen complex completed antigen adsorption was performed, and the supernatant was collected for subsequent detection.

4) Add 1 mL of wash buffer into the centrifuge tube, and gently blow with a pipette to make the magnetic beads-antibody-antigen complex evenly dispersed, and then carry out magnetic separation and discard the supernatant; Remove the centrifuge tube from the magnetic separator and repeat washing twice.

7. Antigen elution

A. Denatured elution

Samples eluted by this method are suitable for SDS-PAGE detection.

1) Remove the centrifuge tube from the magnetic separator, add 25 μL 1×SDS-PAGE Loading Buffer into it, mix evenly, and heat at 95°C for 5 min.

2) Placed on the magnetic separator for magnetic separation, and collected the supernatant for SDS-PAGE detection.

B. Non-denaturing elution

1) 300μL eluent was added into the magnetic bead-antibody-antigen complex, mixed evenly, and incubated at room temperature for 10 min.

2) Place on the magnetic separator for magnetic separation and collect the eluent into the new EP tube.

3) Repeat steps 1) and 2), collect the eluent, mix with the eluent in 2), and add the neutralizing solution to neutralize to PH 7.0-8.0.

Notes

1. For your safety and health, please wear lab coats and disposable gloves for operation.
2. For research use only.