Mouse IgG Affinity Agarose

Cat. No: EA-IP-100M **Size: 1 mL**

Note: *Do not centrifuge and use after mixing gently.*

Performance metrics

Scope of application	When applied in antibody related experiments such as immunoprecipitation (IP), the control mouse derived IgG immunomagnetic beads can exclude non-specific binding of IgG itself to specific target proteins or other specific biomolecules.
Antibody properties	Mouse-derived IgG.
Magnetic beads properties	Magnetic beads, average particle size 3μm.
Loading capacity	1mL magnetic bead suspension, containing about 20mg magnetic beads, covalently conjugated to about 1mg mouse IgG antibody.
Components	Store in preservative-containing PBS.

Matters Needing Attention

1. This product is only for scientific research by professionals and may not be used for clinical diagnosis or treatment.
2. For your safety and health, please wear a lab coat and disposable gloves.
3. This product provides affinity magnetic beads in the form of suspension. Gently re-suspend the magnetic bead suspension before use, and then use it as needed.
4. This product needs to maintain a pH of 6 to 8, and avoid high-speed centrifugation.
5. Do not centrifuge, freeze or dry the magnetic beads, do not sonicate the magnetic beads, and do not allow acid treatment of the magnetic beads for more than 10 minutes.
6. The relevant reagents used must be prepared by the laboratory.

Method of Application

1. Sample Preparation of Target Proteins

1) Sample processing serum and recombinant proteins

Collect serum or culture medium supernatant and detect the target protein concentration. If the target protein concentration is high, it is recommended to dilute it with 1×PBS to a final protein concentration of 10~100μg/mL for subsequent experiments.

2) Sample processing of target protein for intracellular expression

a. Blow off in case of adherent cells or take suspension cells from the cell culture flask and transfer them to a centrifuge tube, centrifuge at 1000 rpm for 5 min, and discard the supernatant.

b. Re-suspend cells in 1× PBS pre-cooled at 4 °C, centrifuge at 1,000 rpm for 3 min, and discard the supernatant. Repeat once.

c. Add the corresponding volume of cell lysis buffer according to the amount of cells, and place on ice for 10~20 min after repeated pipetting

Note: Generally, 1mL of cell lysate can process about 0.5~1×10^7 cells. To avoid degradation of the target protein, you can add a protease inhibitor.

d. Use a sonicator to treat the cell lysate until the cell lysate is transparent and no longer viscous. After placing on ice for 30 minutes, centrifuge at 12,000 rpm and 4°C for 10 minutes. Take the supernatant for subsequent experiments.

2. Column Installation and Incubation

1) Preparation of Mouse IgG immunomagnetic beads

a. Gently re-suspend the Mouse IgG immunomagnetic beads, mix evenly, and take 50 μL of the magnetic bead suspension (containing approximately 12.5 μL of magnetic beads) into a centrifuge tube.

b. Add 500 μL of 1×PBS to gently re-suspend and wash magnetic beads, let stand on the magnetic stand for 10 seconds, discard the supernatant, and repeat the above steps twice.

Note: For multiple samples, the magnetic beads can be re-suspended and divided into several reaction tubes for separate reactions.

2) Binding of target protein to Mouse IgG immunomagnetic beads

a. Incubation: Add 500 μL of the prepared sample to the washed magnetic beads, and incubate on a shaker at room temperature for 2 hours. It can also be incubated at 4°C overnight or longer.

b. Washing: After incubation, perform magnetic separation and discard the supernatant. Add 500 μL 1×PBST, mix gently, wash the magnetic beads, magnetically separate, and discard the supernatant. Repeat 3 times.

3) Target protein elution

Taking Anti-DYKDDDDK immunomagnetic beads (product number: EA-IP-001M) as an example, when using a certain elution method for Anti-DYKDDDDK immunomagnetic beads, Mouse IgG immunomagnetic beads serve as a negative control and require the same elution method.

Denaturing elution method

This method is only suitable for SDS-PAGE detection.

a. Add 20 μL 1×PBS and 5 μL 5× loading buffer, boil the sample for 5 minutes, cool it down room temperature and centrifuge.

b. Take the supernatant and run the SDS-PAGE in preparation for subsequent Western Blot detection.

Acid elution method

Acidic elution method has low cost, short operation time, generally does not cause protein denaturation, and facilitates subsequent analysis and detection of proteins.

a. Add pre-cooled acid eluent pH 3.0, 0.5 mL or 20 times the volume of magnetic beads, to the above precipitation, suspend the magnetic beads, and incubate at room temperature for 5 minutes.

Note: Acidic environment will shorten the service life of immunomagnetic beads. The contact time between magnetic beads and acidic eluent should be shortened as much as possible. It is recommended not to exceed 10 minutes.

b. After the incubation, magnetically separate, transfer the supernatant to a new centrifuge tube, and immediately add 1/10 volume of pH 8.0 neutralizing solution and mix well.

c. Use immediately or store protein at -80℃.

Background

Mouse IgG immunomagnetic beads are made of high-quality normal mouse IgG covalently conjugated to magnetic beads. They are usually used as a control for mouse-derived antibody magnetic beads in antibody-related experiments such as immunoprecipitation, co-immunoprecipitation, and chromatin immunoprecipitation. IgG magnetic beads. When used as control magnetic beads, non-specific binding between IgG itself and specific target proteins or other specific biomolecules can be eliminated.

Storage

4°C for 12 months.

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