Anti-His（HHHHHH） Immunogenetic Beads

Cat. No: EA-IP-008M **Size: 1 mL**

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

Performance metrics

Scope of application	Applied to the immunoprecipitation of His-tagged fusion proteins or their protein complexes. The His tag can be located at the N-terminus, C-terminus or in the middle of the protein, such as N-terminal His fusion protein (His-Protein), C-terminal His fusion protein (Protein-His) and Met-modified N-terminal His fusion protein (Met-His- Protein).
Binding properties	His-Tag Mouse mAb：Mouse IgG.
Magnetic beads properties	Agarose coated superparamagnetic beads with an average particle size of 3 μm.
Binding capacity	1mL magnetic bead suspension, containing 20mg magnetic beads, covalently conjugated to approximately 1 mg Anti-His mouse monoclonal antibody. 1mL of Anti-His immunomagnetic beads can precipitate 1~2 mg of His fusion protein.
Components	0.25mL Anti-His immunomagnetic beads, stored in 0.75mL PBS containing preservatives.

Matters Needing Attention

1. This product is limited to scientific research by professionals and cannot be used for clinical diagnosis or treatment.
2. For your safety and health, please wear laboratory clothes and disposable gloves for operation.
3. This product is in the form of gel suspension, and the content of affinity gel is 50%. Before use, gently re-suspend the gel suspension, and then use it as required.
4. It is best to prepare and use the IP-WB sample on site to avoid affecting the experimental results.
5. Do not dry the gel, do not sonicate the gel, and do not allow the acid treatment of gel to exceed 10 minutes.
6. The amount of gel mentioned in the method is the demonstration amount prepared in small quantities, and the specific amount should be adjusted according to the actual situation.

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 lysate 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) Anti-His Immunomagnetic beads preparation

a. Gently re-suspend the Anti-His immunomagnetic beads, mix evenly, and take 40 μL of the magnetic bead suspension (containing approximately 10 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 Anti-His 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

This instruction manual provides the following two target protein elution schemes. Please choose different target protein elution methods according to the needs of later detection.

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. Process and store proteins according to subsequent experimental needs.

Background

Anti-His immunomagnetic beads are covalently conjugated to high-quality His-tagged antibodies and magnetic beads, which can specifically bind to His-tagged proteins in the sample, and can be used for immunoprecipitation, immuno(co)precipitation, and other experiments of His-tagged fusion proteins or protein complexes.

Storage

4°C for 12 months.

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