Anti-HA Nanobody Immunomagnetic Beads

Cat. No: EA-IP-002MN **Size: 1 mL**

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

Performance metrics

Scope of application	Immunoprecipitation (IP), Co-immunoprecipitation (Co-IP), Chromatin Immunoprecipitation (ChIP), and RNA-Binding Protein Immunoprecipitation (RIP) of HA-tagged fusion proteins. The HA tag can be located at the N-terminal and C-terminal, or the middle of the protein, such as the N-terminal HA fusion protein (HA-Protein) and the C-terminal HA fusion protein (Protein-HA).
Antibody properties	Anti-HA Nanobody.
Magnetic beads properties	Magnetic agarose beads with an average particle size of 2 μm.
Binding capacity	1 mL Anti-HA immunomagnetic beads can precipitate at least 1.5 mg HA fusion protein.
Storage buffer	20 mM PBS, 5% BSA.

Matters Needing Attention

1. This product is intended for research use only by professionals and should not be used for clinical diagnosis or treatment.
2. For your safety and health, please wear a lab coat and disposable gloves when handling the product.
3. This product provides affinity magnetic beads in suspension form. Before use, gently mix the bead suspension by repeated inversion or mild vortexing.
4. Do not wash the magnetic beads with PBS, as this will result in the beads not being attracted by the magnetic rack.
5. Before use, add sufficient protease inhibitors (PMSF) to the lysis buffer. For RIP experiments, also add sufficient RNase inhibitors. Mix well and keep on ice. Prepare and use immediately.Reagents for use in conjunction with this product need to be prepared by the laboratory.

Method of Application

1. Preparation of cell lysate

1) Collecting cells

Blow the suspended cells and semi-adherent cells off the cell culture flask and transfer them into a centrifuge tube, centrifuge at 1000rpm for 5min, and discard the supernatant.

Gently scrape the adherent cells off the bottle wall with a cell scraper, transfer them into a centrifuge tube together with the culture medium, centrifuge at 1000rpm for 5min, and discard the supernatant.

2) Re-suspend the cells with 1x PBS pre-cooled to 4 °C, centrifuge at 1000rpm for 3min, and discard the supernatant. Repeat.

3) Add the corresponding volume of cell lysate according to the amount of cells, and place it on the ice for 10-20 min after repeated blowing.

Note: Generally, 1mL of cell lysis buffer can process about 0.5–1 x10^7 cells. To avoid degradation of that target protein, you may add protease inhibitor.

4) Treat cell lysate with ultrasonic crusher until cell lysate is clear and no longer viscous. After 30 min on ice, centrifuge at 12000 rpm for 10 min at 4 °C. Take out the supernatant and freeze at −80 °C.

5) If the target protein is secreted and expressed, the above treatment is not required, the supernatant of the medium can be directly collected and the following steps can be performed after concentration.

2. Detection of HA Tagged Protein by Immuno (co) precipitation Method

1) Gently re-suspend the Anti-HA magnetic beads, take 20~30 μL magnetic beads suspension, put it in the centrifuge tube, add 500 μL 1x PBST, fully suspended the suspension and place on the magnetic rack for magnetic separation. After the magnetic rack is

left standing for 1 min, discard the supernatant. Repeat this washing step twice.

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

2) Add 50-200 μL eukaryotic cell lysate containing target protein, gently re-suspend magnetic beads, and incubate in a shaking table at room temperature for 2h or at 4°C overnight.

3) After standing on the magnetic rack for 1 min, transfer the supernatant to a new centrifuge tube for later use (the supernatant can be used to detect whether HA-tag protein remains). Add 500 μL 1x PBST, mix gently, clean the magnetic beads, perform magnetic separation, and discard the supernatant. Repeat twice.

3. **Elution of the target protein**

1) Denaturing elution method

a. Add 20 μL of 1×PBS and 5 μL of 5×loading buffer to the magnetic beads, mix thoroughly, and heat at 95°C for 5 minutes.

b. Separate using a magnetic stand, and collect the supernatant into a new centrifuge tube. Store at -20°C or proceed directly to SDS-PAGE and Western Blot analysis.

2) Acid elution method

a. Add 50–100 μL of acidic elution buffer to the magnetic beads, gently mix for 20 seconds, and incubate at room temperature for 10 minutes.

Note: The acidic environment will shorten the lifespan of the immunomagnetic beads. The contact time between the beads and the acidic elution buffer should be minimized, and it is recommended not to exceed 10 minutes.

b. Separate the magnetic beads, collect the supernatant into a new centrifuge tube, and immediately add 1/10 of the total volume of 10× PBST Buffer to neutralize the eluate, adjusting the pH of the eluted product to neutral. The sample can then be used for subsequent functional analysis.

Background

Anti-HA (YPYDVPDYA) Nanobody Magnetic Beads are manufactured by covalently coupling high-quality HA nanobodies to magnetic beads. These beads are characterized by high capacity, high specificity, stability, and reusability. They can be used for affinity purification and immunoprecipitation (IP), co-immunoprecipitation (Co-IP), chromatin immunoprecipitation (ChIP), and RNA-binding protein immunoprecipitation (RIP) of HA-tagged fusion proteins.

Storage

4°C for 12 months.

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