Anti-GFP Nanobody Immunomagnetic Beads

Cat. No: EA-IP-004MN **Size: 1 mL**

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

Performance metrics

Scope of application	Immuno (co) precipitation of GFP tag fusion protein. The GFP tag can be located at the N-terminal and C-terminal of the protein, such as the N-terminal GFP fusion protein (GFP-Protein) and the C-terminal GFP fusion protein (Protein-GFP).
Antibody properties	Anti-GFP Nanobody.
Magnetic beads properties	Agarose coated superparamagnetic beads with an average particle size of 3 μm.
Binding capacity	0.5mL nano magnetic beads are covalently coupled with 2mg Anti-GFP mouse nanobody. 0.5mL Anti-GFP immunomagnetic beads can precipitate at least 0.6mg GFP fusion protein.
Components	0.25mL Anti-GFP immunomagnetic beads stored in 0.75mL PBS containing preservatives.

Matters Needing Attention

1. This product is only used for scientific research by professionals, and shall not be used for clinical diagnosis or treatment.
2. For your safety and health, please wear lab clothes and disposable gloves.
3. This product provides affinity magnetic beads in the form of suspension. The content of magnetic beads in the suspension is 25%. Before use, gently re-suspend the magnetic bead suspension, and then use it as required.
4. Do not centrifuge, freeze or dry the magnetic beads do not use sonication for the magnetic beads, and do not allow the acid treatment time of the magnetic beads to exceed 10min.
5. When mixing the magnetic beads, please use the pipette to gently blow, use soft vortex, turn upside down, shaking table mixing and other methods.
6. Related reagents for supporting use shall be prepared by the laboratory itself.

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 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 GFP Tagged Protein by Immuno (co) precipitation Method

1) Gently re-suspend the Anti-GFP magnetic beads, take 40 μL magnetic beads suspension (including 10 μL magnetic beads), put it

in the centrifuge tube, add 500 μL 1x PBS, fully suspended the suspension and place on the magnetic rack for magnetic separation. After the magnetic rack is left standing for 10 seconds, 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 10 seconds, transfer the supernatant to a new centrifuge tube for standby (the supernatant can be used to detect whether there is residual GFP tag protein). Add 500 μL 1xPBS, mix gently, clean the magnetic beads, perform magnetic separation, and discard the supernatant. Repeat twice.
4) Add 20 μL 1x PBS and 5μL 5x loading buffer, boil for 5 min, cool to room temperature and centrifuge.
5) Take the supernatant for SDS-PAGE test and for subsequent Western Blotting detection.

Background

GFP, a green fluorescent protein, is derived from *Aequorea victoria* multi tube luminous jellyfish and is composed of about 238 amino acids. It can stimulate green fluorescence from blue light to ultraviolet light. Anti-GFP nanobody immunomagnetic beads are made by covalently coupling high-quality GFP nanobody with magnetic beads，As the nano antibody only contains the variable region of antibody molecules, there will be no signal interference from the heavy and light chains of the antibody during immuno (co) precipitation. It has the characteristics of high binding capacity of protein, high specificity and stability, fast and convenient operation, and can be used for immuno (co) precipitation of GFP tagged fusion proteins.

Storage

4°C for 12 months.