Anti-mCherry Nanobody Affinity Agarose

Cat. No: EA-IP-005N **Size: 2 mL**

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

Performance metrics

Scope of application	Immuno (co) precipitation of mCherry tag fusion protein. The mCherry tag can be located at the N-terminal and C-terminal of the protein, such as the N-terminal mCherry fusion protein (mCherry-Protein) and the C-terminal mCherry fusion protein (Protein-mCherry).
Antibody properties	Anti-mCherry Nanobody.
Gel properties	Agarose gel granules, average size 100~200 μm.
Binding capacity	1mL Sepharose 4B agarose granules are covalently coupled with 6mg Anti-mCherry Nanobody. 1mL affinity gel can precipitate at least 1.2mg mCherry fusion protein
Components	1mL Anti-mCherry affinity gel, stored in 1mL PBS containing preservative and 50% glycerol.

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 gel in the form of gel suspension. The content of affinity gel in gel suspension is 50%. Before use, gently re-suspend the gel suspension, and then use it as required.
4. 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 with 1xPBS 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 mCherry Tagged Protein by Immuno (co) precipitation Method

1) Gently re-suspend Anti-mCherry affinity gel, mix it evenly, and aspirate 40 μL gel suspension (containing about 20 μL affinity gel) into the centrifuge tube with the pipette (cut off the tip head). Wash the affinity gel with 5 times the gel volume (approximately 100μL) of centrifuge at 5000 rpm for 30 sec, discard the supernatant, and repeat the procedure three times.

2) Add 50-200 μL eukaryotic cell lysate containing target protein and incubate for 2h in a shaker at room temperature or overnight at 4°C.

3) Wash the affinity gel with 10 times the gel volume (approximately 200μL) of 1x PBS, centrifuge at 5000 rpm for 30 sec, discard the

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supernatant, and repeat the procedure three times.

4) Wash the affinity gel with 5 times the gel volume (approximately 100μL) of 1x PBST pre-cooled to 4 °C and remove the non-specific binding protein, centrifuge at 5000 rpm for 30 sec, discard the supernatant.
5) Add 20 μL 1x PBS and 5μL 5x loading buffer, boil for 5 min, cool to room temperature and centrifuge.
6) Take the supernatant for SDS-PAGE test and for subsequent Western Blotting detection。

Background

mCherry, a red fluorescent protein derived from sea anemone (Discosoma sp.), is composed of 266 amino acids. Because of its color and light stability of monomer molecules, it performs better than other fluorescent protein tags. mCherry is often used as eukaryotic protein recombinant expression marker. Anti-mCherry nanobody affinity gel is made by covalently coupling high-quality mCherry nanobody with agarose gel. As the nanobody 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, and can be used for immuno (co) precipitation of mCherry tagged fusion protein.

Storage

-20°C for 12 months.