

Anti-GFP Nanobody Affinity Agarose

Cat. No: EA-IP-004N

Size: 2 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 or 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.
Gel properties	Agarose gel granules, average size 100~200 μm.
Binding capacity	1mL Sepharose 4B agarose granules are covalently coupled with 6mg Anti-GFP Nanobody. 1mL affinity gel can precipitate at least 1.2mg GFP fusion protein.
Components	1mL Anti-GFP 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 1x PBS pre-cooled to 4 °C, centrifuge at 1000rpm for 3min, and discard the supernatant. Repeat.
- 3) Add the corresponding volume of lysate according to the amount of cells, and place it on the ice for 10-20 min after repeated blowing.

Note: Generally, 1mL of lysis buffer can process about 0.5–1 x10⁷ cells. To avoid degradation of 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 Anti-GFP 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) 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, to wash the affinity gel 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

Anti-MYC (EQKLISEEDL) affinity agarose is made by covalent coupling high-quality MYC monoclonal antibody with agarose gel. It has the characteristics of high binding capacity of protein, high specificity and stability, and repeatability. It can be used for affinity purification and immuno (co) precipitation of MYC tagged fusion proteins.

Storage

-20°C for 12 months.