Tel: 240-252-7368(USA)
Fax: 240-252-7376(USA)
Email: techsupport@elabscience.com
Website: www.elabscience.com

Anti-GFP Affinity Agarose

Cat. No: EA-IP-004

Size: 2 mL

Background Information

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. GFP is often used as a marker for recombinant expression of eukaryotic proteins. Anti-GFP affinity gel, which is made by covalent coupling of high-quality GFP mouse monoclonal antibody with red enhanced Sepharose 4B agarose gel, has the characteristics of high binding capacity of protein, high specificity and stability, and can be used for immuno (co) precipitation of GFP tagged fusion proteins.

Performance Index

Scope of application	Immune (co) precipitation of GFP tag fusion protein. GFP tag can be located at the N-terminal, C-terminal, such as N-terminal GFP fusion protein (GFP–Protein), C-terminal GFP fusion protein (Protein-GFP).
Antibody properties	Mouse monoclonal antibody, IgG1 subtype.
Gel Properties	Agarose gel granules, average size 50 µm.
Binding capacity	1mL Sepharose 4B agarose granules are covalently coupled with 800ug Anti-GFP mouse monoclonal antibody. 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.
Storage	-20 ℃ for 12 months.

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 ℃, centrifuge at 1000rpm for 3min, and discard the

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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 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 ℃. 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 Proteins 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 of 1xPBS (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 ℃.

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 supernatant, and repeat the procedure three times.

4) Wash the affinity gel with 5 times the gel volume of PBST prewashing solution (about 100μL) precooled to 4 ℃ to remove non-specific binding proteins. Centrifuge at 5000rpm for 30sec and 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.