

Affinity Gel-conjugated Rabbit anti GFP-Tag mAb

Catalog No.: AE119

Basic Information

Regent: Anti-GFP rabbit mAb
Affinity Gel, 50% slurry

Size: 0.1 mL, 0.5 mL, 1.0mL, 5.0 mL
bead diameter: 45-165 µm

Buffer: PBS with 0.05% proclin300,
0.05% BSA, 50% glycerol, pH7.3.

Binding capacity: 20µl-40µl Affinity
Gel for 100µg-300µg extracts of whole cells.

Applications: IP

Reactivity : Species Independent

Category: Tag antibody

Conjugation: Agarose Beads

Background

Green fluorescent proteins are widely used in protein localization and protein dynamics analysis. In biochemical analysis methods such as mass spectrometry and enzyme activity measurement, these GFP fusion proteins and their interaction factors can be quickly and efficiently separated by the action of Anti GFP Agarose Beads.

Anti GFP Agarose Beads are covalently coupled to agarose beads by green fluorescent protein nanobody. Anti GFP Agarose Beads are used to capture fluorescent protein containing fusion proteins and closely interacting proteins from cell extracts of various organisms such as animals, plants, bacteria, yeast, insects, and so on.

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Product Information

Source	Isotype	Purification
Rabbit	IgG	Affinity purification

Shelf life: 12 months

Transportation: Ice bag transportation

Storage: Store at -20°C. Avoid freeze / thaw cycles.

Procedures

Note: It is recommended the entire technical bulletin be read before use.

1. Experimental reagents

1.1 Affinity Gel-conjugated Rabbit anti GFP-Tag mAb (AE119)

1.2 Cell lysis buffer for IP (without inhibitors) (RM00022)

1.3 Protease inhibitor cocktail(RM029)

1.4 1× PBS (RM00012)

1.5 5× SDS-PAGE loading buffer (RM00001) (Dilute with deionized water to 1x when using)

1.6 Elution solution: 0.1-0.2M glycine, pH: 2.5-3.1

1.7 Neutralization solution: 1M Tris-base, pH:10.4

1.8 Trypsin-EDTA solution

2. Sample Preparation

2.1 Adherent cell culture:

- a. Dilute the cell lysis buffer for IP to 1x at room temperature, and add 1x or 2x protease inhibitor cocktail as needed.
- b. Remove the culture medium from the cells and wash once with 1x PBS, NS, or serum-free medium.
- c. Lift the cells with trypsin-EDTA solution at room temperature until 90-95% of the cells are detached from the culture surface. Typically, 2 mL of trypsin-EDTA solution covers a 10 cm culture dish.
- d. Stop the trypsinization with an equal volume of serum-containing culture medium to the added trypsin-EDTA solution.
- e. Transfer the cells to a microcentrifuge tube or conical tube.
- f. Centrifuge the cells at 3500x g for 5 minutes and discard the supernatant.
- g. Add cell lysis buffer to the cells, using at least a 10-fold excess volume compared to the cell pellet volume, roughly 1mL of lysis buffer per 10⁷ cells.
- h. Gently pipette to ensure a complete suspension of cells in the lysis buffer, and allow lysis to continue rotate at 20 rpm for 5 - 20 minutes on ice.
- i. Sonicate at low temperature for 1 minute.
- j. Centrifuge the lysate at 14,000x g for 10 minutes at 4°C.
- k. Collect the supernatant to a new 1.5 mL microtube.

2.2 Suspension cell culture

2.2 Suspension cell culture

- a. Dilute the cell lysis buffer for IP to 1x at room temperature, and add 1x or 2x protease inhibitor cocktail as needed.
- b. Transfer the cells to a microcentrifuge tube or conical tube.

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- c. Centrifuge the cells at 300x g for 5 minutes and discard the supernatant.
- d. Add cell lysis buffer to the cells, using at least a 10-fold excess volume compared to the cell pellet volume, roughly 1mL of lysis buffer per 10^7 cells.
- h. Gently pipette to ensure a complete suspension of cells in the lysis buffer, and allow lysis to continue rotate at 20 rpm for 5 - 20 minutes on ice.
- i. Sonicate at low temperature for 1 minute.
- j. Centrifuge the lysate at 14,000x g for 10 minutes at 4°C.
- k. Collect the supernatant to a new 1.5 mL microtube.

2.3 Tissue sample

- a. Dilute the cell lysis buffer for IP to 1x at room temperature, and add 1x or 2x protease inhibitor cocktail as needed.
- b. Transfer the tissue to a microcentrifuge tube or conical tube. and cut it into small pieces.
- c. Take tissue that has been frozen for more than 30 minutes in liquid nitrogen or an ultra-low temperature freezer, quickly grind it with liquid nitrogen. Limit the grinding time within 1-2 minutes to avoid protein degradation.
- d. Transfer the tissue to a microcentrifuge tube or conical tube. Add 1mL Cell lysis buffer for IP (already added with Protease Inhibitor Cocktail) per 100-200mg tissue.

- c. Gently pipette to ensure a complete suspension of cells in the lysis buffer, and allow lysis to continue rotate at 20 rpm for 5 - 20 minutes on ice.

[Alternatively,

- b. Add 1mL Cell lysis buffer for IP (already added with Protease Inhibitor Cocktail) per 100-200mg tissue.
- c. Homogenize with a glass homogenizer or tissue grinder at low temperature until fully lysed, limit the process within 1-2 minutes to avoid protein degradation.]
- d. Sonicate at low temperature for 2 minutes.
- e. Centrifuge the tube at $14,000 \times g$, at 4°C for 10 minutes
- f. Collect the supernatant to a new 1.5 mL microtube.

3. Beads Pre-treatment:

- 3.1 Invert or vortex the Affinity Gel-conjugated Rabbit anti GFP-Tag mAb (AE119) to mix well (no separation in the solution).
- 3.2 Transfer 30-40µL the Affinity Gel-conjugated Rabbit anti GFP-Tag mAb (AE119) to a new EP tube.
- 3.3 Add 500µL of pre-cooled Cell lysis buffer for IP. Use 1mL-pipette to gently mix 10 times at a steady speed.
- 3.4 Place the tube in a magnetic separation rack for 2 minutes, discard the supernatant.
- 3.5 Repeat washing step twice for a total of 3 washes.

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4. Binding protein:

4.1 Add the antigen-containing sample (usually 300 μ L, total protein amount 200-500 μ g or purified protein amount 20 μ g) to the pre-treated Affinity Gel-conjugated Rabbit anti GFP-Tag mAb (AE119) , mix well and incubate at 4°C with gentle agitation for 2 hours or overnight.

4.2 Centrifuge the tube at 4°C, at 1200 \times g for 2 minutes, discard the supernatant.

4.3 Add 500 μ L pre-cooled Cell lysis buffer for IP (already added with Protease Inhibitor Cocktail), use 1mL-pipette to gently mix 10 times at a steady speed.

4.4 Centrifuge the tube at 4°C, at 1200 \times g for 2 minutes, discard the supernatant.

4.5 Repeat washing step 3 times for a total of 4 washes.

5. Antigen Elution:

5.1 Denaturing Elution:

This method is suitable for SDS-PAGE detection analysis.

a. After removing the supernatant from Agarose Beads, add 35 μ L of 1X SDS-PAGE Loading Buffer, mix well, and heat at 95°C for 10 minutes.

b. Centrifuge the tube at 1200 \times g for 2 minutes, collect the supernatant for SDS-PAGE detection.

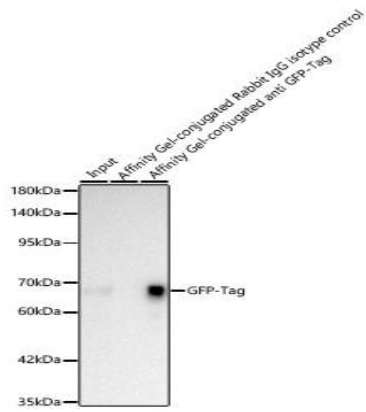
5.2 Non-denaturing Elution:

a. After removing the supernatant from Agarose Beads, add 50 μ L elution buffer, mix well, and incubate at room temperature for 5 minutes.

b. Centrifuge the tube at 1200 \times g for 2 minutes, collect the supernatant in a new EP tube.

c. Add neutralization buffer to adjust the pH to 7.0-8.0. This sample can be used for subsequent functional analysis.

Validation Data



Immunoprecipitation of GFP-Tag in 150 μ g extracts from 293F cells transfected with GFP-Tag using 20 μ l Affinity Gel-conjugated Rabbit anti GFP-Tag mAb (AE119). Western blot analysis was performed using HRP-conjugated Mouse anti GFP-Tag mAb (AE030) at 1:5000 dilution.