

Affinity Gel-conjugated Rabbit anti DDDDK-Tag mAb

Catalog No.: AE121

Basic Information

Regent: Anti-DDDDK rabbit mAb

Affinity Gel, 50% slurry

Size: 0.1 mL, 0.5 mL, 1.0mL, 5.0 mL

bead diameter: 45-165 μ m

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

Binding capacity: 0.4 mg of FLAG
fusion protein per 1 slurry.

Applications: IP

Reactivity : Species Independent

Category: Tag antibody

Conjugation: Agarose Beads

Background

FLAG-tag, or FLAG octapeptide, or FLAG epitope, is a polypeptide protein tag that can be

added to a protein using recombinant DNA technology, having the sequence motif

DYKDDDDK. It has been used for studying proteins in living cells and for protein purification

by affinity chromatography. It has been used to separate recombinant, overexpressed

protein from wild-type protein expressed by the host organism. It can also be used in the

isolation of protein complexes with multiple subunits, because its mild purification procedure

tends not to disrupt such complexes. It has been used to obtain proteins of sufficient purity

and quality to carry out 3D structure determination by x-ray crystallography. A FLAG-tag can

be used in many different assays that require recognition by an antibody. If there is no

antibody against a given protein, adding a FLAG-tag to a protein allows the protein to be

studied with an antibody against the FLAG sequence. Examples are cellular localization

studies by immunofluorescence or detection by SDS PAGE protein electrophoresis and

Western blotting.

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Product Information

Source
Rabbit

Isotype
IgG

Purification
Affinity purification

Shelf life: 12 months

Transportation: Ice bag transportation

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

Validation Data

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

1. Experimental reagents

1.1 Affinity Gel-conjugated Rabbit anti DDDDK-Tag mAb (AE121)

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.

Procedures

- 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 DDDDK-Tag mAb (AE121) to mix well (no separation in the solution).
- 3.2 Transfer 30-40µL the Affinity Gel-conjugated Rabbit anti DDDDK-Tag mAb (AE121) 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.

Procedures

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 DDDDK-Tag mAb (AE121) , 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.

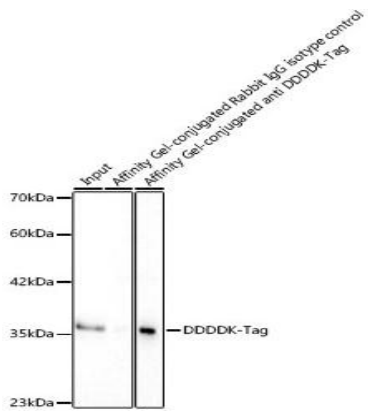
5.2 Non-denaturing Elution:

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 DDDDK-Tag in 150 μ g extracts from 293F cells transfected with DDDDK-Tag using 20 μ l Affinity Gel-conjugated Rabbit anti DDDDK-Tag mAb (AE121). Western blot analysis was performed using HRP-conjugated Mouse anti DDDDK-Tag mAb(AE024) at 1:5000 dilution.