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Magnetic Beads-conjugated Mouse anti DDDDK-Tag mAb

Catalog No.: AE037 3 Publications

Basic Information

Regent: Anti-DDDDK mouse mAb magnetic beads, 50% slurry

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

bead diameter: 30-150 μm

Buffer: PBS with 0.05% proclin300,pH7.3

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

Applications: IP

Reactivity: Species Independent

Category: Tag antibody

Conjugation: Magnetic 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	Isotype	Purification
Mouse	IgG1	Affinity purification

Shelf life: 12 months

Transportation: Ice bag transportation

Storage: Store at 4°C. Avoid freeze / thaw cycles.

Procedures

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

1. Experimental reagents

1.1 Magnetic Beads-conjugated Mouse anti DDDDK-Tag mAb (AE037)

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 300x 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 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.

2.2 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 × g, at 4°C for 10 minutes

f. Collect the supernatant to a new 1.5 mL microtube.

3. Magnetic Bead Pre-treatment:

3.1 Invert or vortex the Magnetic Beads-conjugated Mouse anti DDDDK-Tag mAb (AE037) to mix well (no separation in the solution).

3.2 Transfer 30-40μL the Magnetic Beads-conjugated Mouse anti DDDDK-Tag mAb (AE037) 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.

4. Binding protein:

4.1 Add the antigen-containing sample (usually 300μL, total protein amount 200-500μg or purified protein amount 20μg) to the pre-treated Magnetic Beads-conjugated Mouse anti DDDDK-Tag mAb (AE037) , mix well and incubate at 4°C with gentle agitation for 2 hours or overnight.

4.2 Place the tube in magnetic separation rack for 2 minutes, discard the supernatant.

4.3 Add 500μL 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.

Procedures

4.4 Place the tube in magnetic separation rack for 2 minutes, discard the supernatant.

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

5. Antigen Elution:

(1) Denaturing Elution:

This method is suitable for SDS-PAGE detection analysis.

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

b. Place the tube in magnetic separation rack. Collect the supernatant for SDS-PAGE detection.

[Alternatively,

a. Remove the tube from magnetic separation rack, add 35 μL of 1X non-reduced SDS-PAGE loading buffer, mix well, let it stand at room temperature for 10 minutes. Place the tube back in magnetic separation rack, collect the supernatant.

b. Add 10X DTT, heat at 95°C for 10 minutes, and perform SDS-PAGE detection.]

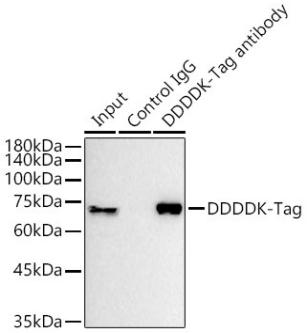
(2) Non-denaturing Elution:

a. After removing the supernatant from magnetic beads, add 50μL elution buffer, mix well, and incubate at room temperature for 5 minutes.

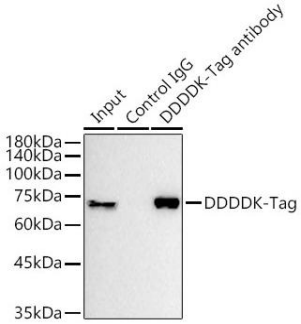
b. Place the tube in magnetic separation rack for 2 minutes, collect the supernatant into a new EP tube.

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 analysis of 300 ug extract cell lysate from 293T cells transfected with GSK3B expression vector containing a C-terminal DDDDK-Tag (1x) with 30uL Magnetic Beads-conjugated Mouse anti DDDDK-tag mAb antibody （AE037). Magnetic Beads-conjugated mouse IgG isotype control pAb (AC044N) was used as a negative control. Western blot was performed from the immunoprecipitate using Rabbit anti DDDDK-Tag mAb antibody (AE092) at 1: 10000 dilution.



Immunoprecipitation analysis of 600ug extract cell lysate from 293T transfected with SERPINB1 expression vector containing a N-terminal DDDDK-Tag (1x) with 30ul Magnetic Beads-conjugated Mouse anti DDDDK-Tag antibody (AE037). Magnetic Beads-conjugated mouse IgG isotype control pAb (AC044N) was used as a negative control. Western blot was performed from the immunoprecipitate using Rabbit anti DDDDK-Tag mAb antibody (AE092) at 1: 5000 dilution.