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Agarose beads-conjugated anti-mCherry VHH Single Domain antibody

Catalog No.: AE073

Basic Information

Regent: Anti- mCherry VHH Agarose beads, 50% slurry

Size: 0.5 mL, 1.0mL

bead diameter: 45 μm

Buffer: PBS with 0.02%NaN3,25% glycerol,pH7.3.

Binding capacity: 1.5-2.0 mg of mCherry fusion protein per 1 mL slurry

Applications : IP,CoIP,ChIP

Reactivity : Species Independent

Category: Tag antibody

Conjugation: Agarose Beads

Background

Protein tags are peptide sequences genetically grafted onto a recombinant protein. Often these tags are removable by chemical agents or by enzymatic means, such as proteolysis or intein splicing. Tags are attached to proteins for various purposes.Epitope tags are short peptide sequences which are chosen because high-affinity antibodies can be reliably produced in many different species. These are usually derived from viral genes, which explain their high immunoreactivity. Epitope tags include V5-tag, Myc-tag, HA-tag and NEtag. These tags are particularly useful for western blotting, immunofluorescence and immunoprecipitation experiments, although they also find use in antibody purification.

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Product Information

Source	Isotype	Purification
Alpaca	VHH	Affinity purification

Shelf life: 12 months

Transportation: Ice bag transportation

Storage: Store at 4 ℃. Avoid freeze / thaw cycles.

Procedures

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

1. Experimental reagents

1.1 Agarose beads-conjugated anti-mCherry VHH Single Domain antibody (AE073)

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 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.

2.3 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. Beads Pre-treatment:

3.1 Invert or vortex the Agarose beads-conjugated anti-mCherry VHH Single Domain antibody (AE073) to mix well (no separation in the solution).

3.2 Transfer 30-40μL the Agarose beads-conjugated anti-mCherry VHH Single Domain antibody (AE073) 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 Centrifuge the tube at 4°C, at 1200 × g for 2 minutes, discard the supernatant.

3.5 Repeat washing step twice for a total of 3 washes.

4.Binding protein:

4.1Add the antigen-containing sample (usually 300μL, total protein amount 200-500μg or purified protein amount 20μg) to the pre-treated

Agarose beads-conjugated anti-mCherry VHH Single Domain antibody (AE073) , mix well and incubate at 4°C with gentle agitation for 2 hours or overnight.

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4.2 Centrifuge the tube at 4°C, at 1200 × g 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.

4.4 Centrifuge the tube at 4°C, at 1200 × 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 35μL of 1X SDS-PAGE Loading Buffer, mix well, and heat at 95℃ for 10 minutes.

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

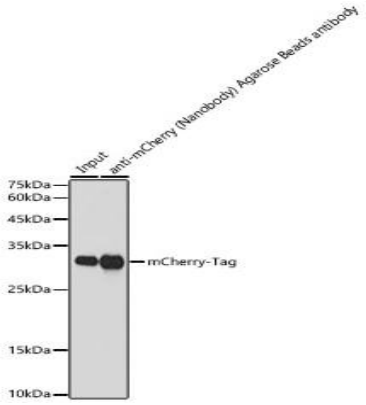
5.2 Non-denaturing Elution:

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

b. Centrifuge the tube at 1200 × 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 analysis of 300 μg extracts from 293T cells transfected with mCherry-tag, using 30μl Agarose beads Anti-mCherry VHH Single Domain antibody (AE073). Western blot analysis was performed using Anti-mCherry-tag antibody (AE171) at 1:5000 dilution.