

Magnetic beads-conjugated anti-mCherry VHH

Single Domain antibody

Catalog No.: AE094 **1 Publications**

Basic Information

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

Size: 0.5 mL, 1.0 mL

bead diameter: 30-100 μm

Buffer: 0.03% NaN₃, 20% ethanol

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

Applications : IP, CoIP, RIP, CHIP

Reactivity : Species Independent

Category: Tag antibody

Conjugation: Magnetic Beads

Background

mCherry is a monomeric, fluorescent, photostable red dye that was isolated from and further engineered from the Discosoma sea anemone. Its peak fluorescent excitation is at 587 nm and emission 610 nm. Fluorescent protein mCherry is frequently used as reporter and fusion tag. VHH or Nanobody is the next generation monoclonal antibody, which is a Single domain antibody reserving only one single antigen recognizing domain from camelid heavy chain antibody. With size of only ~ 15 kDa, Nanobody display numerous advantages over conventional antibody and its derivatives, such as higher tissue penetration capability, more accessible to hidden epitopes, higher stability, solubility and binding affinity. Anti-mCherry Nanobody Beads are magnetic agarose beads covalently coupled with VHH antibodies/ nanobodies acquiring high specificity and affinity for mCherry. These nanobeads can efficiently capture and separate mCherry and mCherry tagged proteins from cell crudes or other samples

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Product Information

Source
Alpaca

Isotype
VHH

Purification
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 anti-mCherry VHH Single Domain antibody (AE094).

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

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Procedures

- c. Centrifuge the cells at 300x g for 5 minutes and discard the supernatant.
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- 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. Magnetic Bead Pre-treatment:

- 3.1 Invert or vortex the Magnetic beads-conjugated anti-mCherry VHH Single Domain antibody (AE094) to mix well (no separation in the solution).
- 3.2 Transfer 30-40 μ L the Magnetic beads-conjugated anti-mCherry VHH Single Domain antibody (AE094) to a new EP tube.
- 3.3 Add 500 μ L 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

Magnetic beads-conjugated anti-mCherry VHH Single Domain antibody (AE094) , 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 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 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 35 μ L 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 μ L 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.]

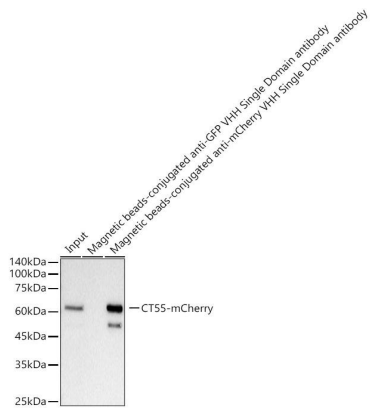
(2) Non-denaturing Elution:

a. After removing the supernatant from magnetic beads, add 50 μ L 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 of CT55-mCherry from 300 μ g extracts of 293T cells transfected with a CT55 expression vector containing a single C-terminal mCherry-Tag was performed using 30 μ l of Magnetic beads-conjugated anti-mCherry VHH Single Domain antibody (AE094). Magnetic beads-conjugated anti-GFP VHH Single Domain antibody (AE079) was used to precipitate the Control sample. IP samples were eluted with 1X Laemmli Buffer. The Input lane represents 10 % of the total input. Western blot analysis of immunoprecipitates was conducted using Mouse anti mCherry-Tag mAb (AE002) at a dilution of 1:2000.