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Anti-Mouse IgG2a Heavy Chain VHH Magarose Beads

Product Information

Catalog Number: KTSM1343

Volume: 500 μL (50% anti-mouse IgG2a heavy chain VHH conjugated magarose beads)

Storage: 12 months at 4-8°C (Do Not Freeze)

Product Property

Beads size: 30-100 μm (magarose beads)

Storage buffer: 1xPBS pH7.4, 25% glycerol, 0.02% NaN_3

Antibody: anti-mouse heavy chain VHH antibody (fused with 6xhis tag and Myc tag)

Binding capacity: 20 μL slurry bind 100 μg of mouse IgG2a antibody or mouse IgG2a Fc-tagged protein

Specificity: Selectively recognizes mouse IgG2a antibody heavy chain

Host: Alpaca

Class: Recombinant

Application

IP, Co-IP, CHIP, RIP, MS

Product Description

VHH (variable domain of heavy chain of heavy chain-only antibody) is 4 nm in length, 2.5 nm in width, and have a molecular weight of 12-15 kDa, only one tenth the size of conventional antibodies. VHH exhibits high specificity and affinity and outperforms conventional antibodies in many aspects, such as high stability, great specificity, and an easy and feasible development process.

Anti-mouse IgG2a heavy chain nanobody magarose beads are magarose beads covalently coupled with VHH antibodies, which have high specificity and affinity for mouse IgG2a antibody heavy chain. These beads can efficiently capture and separate mouse IgG2a antibody or mouse IgG2a Fc-tagged protein, along with associated proteins, from cell extracts of mammals, plants, bacteria, yeast, insects, and other organisms.

Product Advantage

IP/co-IP result analysis by SDS-PAGE will not be interfered by heavy or light chains.

Extraordinary performance under harsh buffer conditions.

Animal-free production.

Consistent and reproducible results.

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Suggested Buffer for IP

Buffer	Composition
Lysis buffer	50 mM Tris-HCl pH 7.5; 150 mM NaCl; 1% Triton-100; 1 mM EDTA
RIPA buffer	10 mM Tris-HCl pH 7.5; 150 mM NaCl; 0.5 mM EDTA; 0.1% SDS; 1% Triton X-100; 1% Deoxycholate
Dilution/Wash buffer	50 mM Tris-HCl pH 7.5; 150 mM NaCl; 1 mM EDTA
Loading buffer	120 mM Tris-HCl pH 6.8; 20% glycerol; 4% SDS; 0.04% Bromophenol blue; 10% β-mercaptoethanol
Glycine-elution buffer	200 mM glycine pH 2.5
Neutralization buffer	1 M Tris-HCl pH 10.4

NOTE: For other cell types such as yeast, plant, insect, bacteria, please use equivalent lysis buffer.

IP Protocol

- **Harvest Cells**

  For an immunoprecipitation (IP) experiment, it is recommended to use 10^6–10^7 mammalian cells expressing the target protein. Aspirate the growth medium and wash the cells twice with 2 mL of pre-chilled PBS buffer. Collect adherent cells using a cell scraper or trypsin digestion. Transfer the cells to a centrifuge tube and centrifuge at 1200 × g for 3–5 minutes. Discard the supernatant.

- **Sample Preparation**

  a) For cytoplasmic proteins, resuspend the cell pellet in 200 µL of pre-chilled lysis buffer with 1 mM PMSF and other appropriate protease inhibitors.

  For nuclear proteins, use RIPA buffer supplemented with 1 mg/mL DNase, 2.5 mM $MgCl_2$, 1 mM PMSF and other appropriate protease inhibitors.

  b) Place the tube on ice for 30–40 minutes, resuspending the cells every 10 minutes.

  c) Centrifuge at 12,000 × g for 10 minutes at 4°C. Transfer the supernatant to a new pre-chilled centrifuge tube and add 300 µL of dilution buffer (PBS buffer can be used as an alternative).

  d) Add 25 µL beads (50% slurry) and incubate on ice for 30 minutes. Centrifuge at 1,200 × g for 3 minutes, then transfer the supernatant to a new tube to remove proteins that bind non-specifically to the beads.

  e) Add 5 µg of mouse primary antibody (IgG2a isotype) to the supernatant and incubate at 4°C for 1 hour.

- **Binding**

  a) Add 25 µL beads (50% slurry) to the mixture of cell lysate supernatant and mouse primary antibody. Incubate at 4°C with gentle end-over-end rotation for 1 hour.

  b) Place the tube on a magnetic rack for 60 sec until the supernatant turns clear, remove supernatant.

- **Wash**

a) Resuspend the beads in 500 µL of dilution buffer or PBST buffer (0.05% Tween-20).

b) Place the tube on a magnetic rack for 60 sec until the supernatant turns clear, remove supernatant. Repeat this wash step 2 to 5 times.

Optional: Increase the NaCl concentration to 500 mM during the second wash step.

- **Detection**

a) Resuspend the beads in 30 µL of loading buffer.

b) Heat the beads at 95°C in a water bath for 10 minutes to dissociate the immunoprecipitated complexes from the beads.

c) Place the tube on a magnetic rack for 60 sec until the supernatant turns clear, and collect the supernatant for SDS-PAGE or Western blot analysis.

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This product is only for research use.