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Anti-SUMO VHH Magarose Beads

Product Information

Catalog Number: KTSM2502

Volume: 500 µL (50% anti-SUMO 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% sodium azide.

Antibody: anti-SUMO VHH antibody (fused with 6xhis tag)

Binding capacity: 10 µL slurry bind 15-20 µg of SUMO-tagged protein.

Specificity: Selectively recognizes SUMO tag fusion protein.

Host: Alpaca

Class: Recombinant

Application

IP, Co-IP

Product Description

SUMO tag is an epitope tag designed for fusion proteins, which usually does not affect the activity of conjugated proteins and is widely used for protein purification. SUMO tag is also widely used in various immunoassays for target protein detection.

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-SUMO VHH magarose beads are magarose beads covalently coupled with VHH antibodies, which have high specificity and affinity for SUMO. These beads can efficiently capture and separate SUMO-tagged proteins, 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

IP Protocol

- **Harvest Cells**

For immunoprecipitation, 10^6 - 10^7 mammalian cells expressing SUMO tag fusion protein (about one 10 cm cell culture dish) are required.

a) Gently remove cell growth medium by aspiration;
b) Wash cells twice with 1 mL pre-chilled PBS;
c) Collect the adherent cells using cell scraper or tryptic digestion;
d) Transfer to Eppendorf tubes, and centrifuge at 1,200 × g for 3 - 5 min;
e) Discard the supernatant, resuspend cells with prechilled 1xPBS;
f) Repeat cell washing twice.

- **Cell Lysis**

a) For cytoplasmic proteins, resuspend cells with 200 µL of pre-chilled lysis buffer;

Note: Make sure protease inhibitors and 1 mM PMSF are added.

For nuclear proteins: add 1 mg/mL DNase and 2.5 mM $MgCl_2$ to RIPA buffer (with protease inhibitor and 1mM PMSF).

b) Place the tube with cells on ice for 30 - 40 min, and resuspend cells every 10 min;
c) Centrifuge at 4°C and 12,000 × g for 10 min, transfer the supernatant into a pre-chilled new Eppendorf tube with 300 µL dilution buffer (1xPBS), discard precipitation (if required, aliquot 50 µL lysate for further analysis);

Note: the cell lysate collected at this point should be stored at -80°C.

Optional: Add 1mM PMSF and protease inhibitor.

- **Equilibration**

a) Resuspend and transfer 25 µL beads suspension into a 1.5 mL Eppendorf tube;
b) Add 500 µL pre-chilled dilution buffer or 1x PBST (0.05% Tween-20);
c) Place the tube on a magnetic rack for 60 sec until the supernatant turns clear, remove supernatant and repeat step b) and c) twice.

- **Protein Binding**

a) Add cell lysate to the equilibrated beads;
b) Incubation with rotating at 4°C for 1 -3 hour;
c) Place the tube on a magnetic rack for 60 sec until the supernatant turns clear, and remove supernatant.

- **Washing**

a) Add 500 µL Dilution buffer or 1xPBST and resuspend the magarose beads;
b) Place the tube on a magnetic rack for 60 sec until the supernatant turns clear, remove supernatant, and repeat step a) and b) for 2 - 5 times.

Optional: Increase NaCl concentration to 500 mM in the second washing step.

- **Detection**

a) Remove the remaining supernatant;
b) Add 30 µL Loading Buffer and resuspend the beads;
c) Incubate at 95°C for 10 min to denature and separate protein from the beads;
d) Separate the magnetic beads on a magnetic rack, collect the supernatant for SDS-PAGE.

- **Alternative Elution Procedure（following Washing step）**

a) Remove the remaining supernatant;
b) Add 50 µL 200 mM glycine pH 2.5 and resuspend the beads, incubate on ice for 30-60 sec;
c) Separate the magnetic beads on a magnetic rack, and transfer the supernatant to a new tube;
d) Add 5 µL neutralizing buffer (1M Tris-base pH 10.4) to neutralize the eluate fractions;
e) Repeat this step at least once to increase elution efficiency.

This product is only for research use.