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Anti-HA VHH Agarose Beads

Product Information

Catalog Number: KTSM1306

Volume: 500 µL (50% anti-HA VHH conjugated agarose beads)

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

Product Property

Beads size: 45-165 µm (4% cross-linked agarose beads)

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

Antibody: anti-HA (YPYDVPDYA) VHH antibody (fused with 6xhis tag and Myc tag)

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

Specificity: Selectively recognizes HA tag fusion protein

Host: Alpaca

Class: Recombinant

Application

IP, Co-IP

Product Description

HA 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. HA 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-HA VHH agarose beads are agarose beads covalently coupled with VHH antibodies, which have high specificity and affinity for HA tag. These beads can efficiently capture and separate HA-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 HA-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) Centrifuge at 4°C, 1,200 × g for 3 min, remove the 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) Centrifuge at 4°C, 1,200 × g for 3 min, remove the supernatant.

- **Washing**

a) Add 500 µL Dilution buffer or 1xPBST and resuspend the agarose beads;

b) Centrifuge at 4°C, 1,200 × g for 3 min, 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) Centrifuge at 4°C, 1,200 × g for 2 min, keep the supernatant for SDS-PAGE or western blot.

- **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) Centrifuge at 4°C, 1,200 × g for 2 min, transfer the supernatant to a new Eppendorf 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.