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Anti-HA VHH Agarose Beads

Product Information

Catalog Number: KTSM1305

Volume: 500 µL (20 times, 50% anti-HA VHH coated agarose beads)

Storage condition: 4°C for 12 months

Product Description

HA-tag (YPYDVPDYA) has been extensively used as a general epitope tag, which does not generally interfere with the bioactivity or the biodistribution of the recombinant protein. This tag facilitates the detection, isolation and purification of the protein of interest. VHH (variable domain of heavy chain of heavy chain-only antibodies) is 4 nm in length, 2.5 nm in width, and has a molecular weight of 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 easy and feasible development process. Anti-HA VHH agarose Beads are agarose beads covalently coupled with VHH antibodies acquiring high specificity and affinity for HA tag. These beads can efficiently capture and separate HA tagged proteins, alongside with the associated proteins from cell extracts of mammal, plant, bacteria, yeast, insect, and other organisms.

Advantages

· No heavy & light antibody chains

· Consistent and reproducible results

· Extraordinary binding, even under harsh conditions

· Short incubation time (1-3 hours)

· Animal-free production

Application

Immunoprecipitation (IP), co-immunoprecipitation (CoIP), chromatin immunoprecipitation (CHIP), RNA-binding protein immunoprecipitation (RIP), Mass Spectrometry (MS).

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Beads Property

· Beads size: ~45 μm (7.5% cross-linked agarose beads)

· Storage buffer: 1XPBS, 0.03% sodium azide, 50% glycerol

· Binding capacity: 10 μL slurry bind about 18 μg of HA tagged protein

· Specificity: Selectively recognizes HA fusion protein

Recommended Buffers

Buffer	Composition
Lysis buffer	50 mM Tris-HCl (pH 7.5); 150 mM NaCl; 1% Triton X-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
SDS loading buffer	120 mM Tris-HCl (pH 6.8); 20% glycerol; 4% SDS; 0.04% bromophenol blue; 10% β-mercaptoethanol
Glycine - elution buffer	0.2 M glycine (pH 2.0)
Neutralization buffer	1 M Tris (pH 10.4)

Note: For yeast, plant, insect, or bacteria cell extracts, use an equally effective amount of cytolysis buffer.

Working Procedures

Harvest Cells

For co-immunoprecipitation, 10^6 - 10^7 mammalian cells expressing HA fusion protein (about one 10 cm 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. Transferred to centrifuge 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 500 μL of pre-chilled lysis buffer;

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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 centrifuge tubes 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 prechilled new centrifuge tube with 300 µL dilution buffer (1X PBS), discard precipitation (if required, keep 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.

Beads Equilibration

a. Vortex Anti-HA VHH Agarose Beads, and transfer 25 µL beads suspension into a 1.5 mL centrifuge tube;
b. Add 500 µL prechilled dilution buffer [1x PBST (0.05% Tween-20)];
c. Centrifuge at 4°C and 1,200 g for 3 min, and remove the supernatant, and repeat step b) and c) twice.

Protein Binding

d. Add protein extracts (from **Cell Lysis** step) to the equilibrated Anti-HA VHH Agarose Beads, and keep the tube at 4°C and upside down for 1 - 3 hours;
e. Centrifuge at 4°C and 1,200 g for 3 min, remove supernatant.

Washing

a. Add 500 µL dilution buffer (or 1 mL 1x PBST) to resuspend the beads;
b. Centrifuge at 4°C and 1,200 g for 3 min, and remove the supernatant, and repeat step a) and b) 2 - 5 times.

Optional: Increase salt concentration to 500 mM in the second washing

Protein Elution Procedure 1

a. Add 100 µL SDS Loading Buffer to resuspend the beads and boil at 95°C for 10 min to denature and separate HA-fusion proteins from the beads;
b. Boil at 95°C for 10 min to separate beads from the immunoprecipitation complex;
c. Centrifuge at 4°C and 1,200g for 2 min, use the supernatant for SDS-PAGE analysis;

Protein Elution Procedure 2 (Alternative to Protein Elution Procedure 1)

d. Resuspend beads with 50 µL elution buffer (0.2 M glycine pH 2.0), incubate on ice for 30 sec;
e. Centrifuge at 4°C and 1,200 g for 2 min;
f. Add 5 µL neutralizing buffer (1M Tris-base pH 10.4) to elute protein binders.

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Elution with HA peptide elution buffer (alternative to Protein Elution Procedure 1 or 2)

a) Add 20~30 μL 0.2 mM HA peptide (YPYDVPDYA) to every 10 μL beads, resuspend Anti-HA Agarose Beads, incubate for 10 minutes in a mixed state, centrifuge at 1200 g for 3 minutes at 4°C;

b) Transfer the supernatant to a new centrifuge tube and repeat step 1 above for at least 3 times to increase the elution efficiency;

c) Column regeneration: Add 5~10 times column volume of 0.2 M, pH2.5 glycine, keep mixing and incubate for 3 min.;

d) Centrifuge at 1200 g for 3 minutes, discard the supernatant, and use 5~10 column volumes of PBS solution (pH = 7.4) to wash beads for 3 times.

FAQs

A. Question: Can I use an alternative Lysis Buffer with higher NaCl and NP40 concentration?

Answer: Yes, the nanobodies conjugated to Anti-HA VHH Agarose Beads are highly stable and resistant to harsh buffer conditions.

B. Question: Can I incubate Anti-HA VHH Agarose Beads with cell lysate at 4 ∞C overnight to increase binding?

Answer: Yes, despite that the binding efficiency of our Beads and HA tagged protein does not seem to increase very much over time, as our team has compared the results of 1 - 3 hr. and overnight co-incubation. Therefore, in most situations, 1 hr. incubation should be sufficient to achieve excellent results.

Disclaimer: Products are for life science research only. Not for use in diagnostic procedures unless otherwise indicated.