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Anti-mCherry VHH Magarose Beads

Product Information

Catalog Number: KTSM1337

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

Storage condition: 4°C for 12 months

Product Description

Fluorescent protein mCherry exhibits bright green fluorescence when exposed to ultraviolet light and frequently used as reporter and fusion tag. 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-mCherry VHH Magarose Beads are magarose beads covalently coupled with VHH antibodies acquiring high specificity and affinity for mCherry. These beads can efficiently capture and separate mCherry and mCherry 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 hr.)

· 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: ~30-100 μm (magnetic agarose beads)

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

Binding capacity: 10 μL slurry binds about 18 μg of recombinant mCherry.

Specificity: Selectively recognizes mCherry, mRFP, mKate2, mRFPruby, tagRFP, mPlum, and many more RFP derivatives.

Recommended Buffers

Buffer	Composition
Lysis buffer	50 mM Tris-HCl (pH 7.5); 150mM 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 equally effective amount of cytolysis buffer.

Working Procedures

Harvest Cells

For co-immunoprecipitation, 10^6 - 10^7 mammalian cells expressing mCherry 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.

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 1 mM PMSF)

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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 1 mM PMSF and protease inhibitor.

Beads Equilibration

a. Vortex Anti-mCherry Magnetic Nanobeads, and transfer 25 µL beads suspension into a 1.5 mL centrifuge tube;
b. Add 500 µL dilution buffer [1x PBST (0.05% Tween-20)], gently invert the tube multiple times for 60 sec;
c. Place the tube on a magnetic rack for 60 sec, until the supernatant turns clear, and repeat step b) and c) three times.

Protein Binding

a. Add equilibrated magnetic beads to the protein extracts (from **Cell Lysis** step).
b. Keep the tube turning on a rotating shaker for 1 - 3 hr. at 4°C.

Washing

a. Set the tube on a magnetic rack until the sample turns clear, and remove the supernatant (if required, keep 50 µL lysate for further analysis).
b. Add 500 µL dilution buffer (or 1x PBST) to resuspend the beads.
c. Separate the magnetic beads on a magnetic rack, then remove the supernatant.
d. Repeat step b) and c) at least 4 times.

Protein Elution

a. Remove the supernatant;
b. Add 30 µL 1X SDS Loading Buffer to resuspend the magnetic beads
c. At 95°C, boil for 10 min to separate magnetic beads from the immunoprecipitation complex.
d. Obtain the supernatant for SDS-PAGE analysis.

Alternative Elution (alternative to **Protein Elution)**

a. Remove the supernatant;
b. Resuspend the beads with 50 - 70 µL elution buffer (0.2 M glycine, pH 2.5), and further mixing on a shaker for 5-10 min at 4°C;
c. Separate beads on a magnetic rack, and transfer the supernatant into a new centrifuge tube;

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d. Add 25 - 35 µL neutralizing buffer (1 M Tris, pH 10.4) to the magnetic beads, and repeat step b) and c) to increase elution efficiency.

FAQs

A. Question: Can I use Anti-mCherry VHH Agarose Beads to pulldown RFP- or GFP-fusion proteins and their partners?

Answer: Anti-mCherry VHH Agarose Beads can pulldown RFP-fusion proteins and their partners, but do not cross-react with GFP-fusion proteins and their partners. Anti-mCherry VHH Agarose Beads selectively recognizes mCherry, mRFP, mKate2, mRFPruby, tagRFP, mPlum, and many more RFP derivative and do not bind to GFP or EGFP derivatives.

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

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

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

Answer: Yes, despite that the binding efficiency of our Beads and mCherry 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.