Product Information

Immunoprecipitation Protocol for Alpaca Anti-Tag (GFP, HA, mCherry, MYC) Single Domain Antibody, Magnetic Agarose Beads

SAB5900002, SAB5900004, SAB5900006, SAB5900008

Immunoprecipitation Protocol

Harvest Cells

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

1. Gently remove cell growth medium by aspiration.
2. Wash cells twice with 1 mL pre-chilled PBS.
3. Collect the adherent cells using cell scraper or tryptic digestion.
4. Transfer to Eppendorf tubes, and centrifuge at 1200 × *g* for 3-5 minutes.
5. Discard the supernatant, resuspend cells with prechilled 1X PBS.
6. Repeat cell washing twice.

Cell Lysis

1. 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).
2. Place the tube with cells on ice for 30-40 minutes, and resuspend cells every 10 minutes.
3. Centrifuge at 4 °C and 12,000 × *g* for 10 minutes, transfer the supernatant into a pre-chilled new Eppendorf® tube with 300 µL dilution buffer (1X PBS), 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

1. Resuspend and transfer 25 µL beads suspension into a 1.5 mL Eppendorf® tube.
2. Add 500 µL pre-chilled dilution buffer or 1X PBST (0.05% Tween® 20).
3. Place the tube on a magnetic rack for 60 seconds until the supernatant turns clear, remove supernatant and repeat step 2 and 3 twice.

Protein Binding

1. Add cell lysate to the equilibrated beads.
2. Incubation with rotating at 4 °C for 1-3 hours.
3. Place the tube on a magnetic rack for 60 seconds until the supernatant turns clear, and remove supernatant.

Washing

1. Add 500 µL Dilution buffer or 1X PBST and resuspend the magnetic agarose beads.
2. Place the tube on a magnetic rack for 60 seconds until the supernatant turns clear, remove supernatant, and repeat step 1 and 2 for 2-5 times.

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

Detection

1. Remove the remaining supernatant.
2. Add 30 µL Loading Buffer and resuspend the beads.
3. Incubate at 95 °C for 10 minutes to denature and separate protein from the beads.
4. Separate the magnetic beads on a magnetic rack, collect the supernatant for SDS-PAGE.

Alternative Elution Procedure Following Washing Step

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

Suggested Buffers for IP

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
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

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SAB5900002 Rev 11/25