ABGENT CUSTOM SERVICES:

Immunoprecipitation Protocol

Step-by-step procedure:

1. Harvest cells with PBS-EDTA or Trypsin, and count cells.
2. Lyse the cells in prechilled RIPA buffer (1 ml/10^7cells) for 1 hr rocking at 4℃.
3. Centrifuge for 20 min at 14,000 g at 4℃.Transfer super natant to a new tube.
4. Prepare protein A/G agarose beads by washing twice with PBS and restoring to a 50% slurry bead suspension with RIPA buffer.
5. Pre-clear the cell lysate by adding 50 ml of bead slurry per ml of cell lysate and incubate at 4℃ rocking for 10 min. Centrifuge for 10 min at 10,000 g at 4℃. Transfer supernatant to a new tube.
6. Prepare IP reaction by pipetting 0.5 ml pre-cleared cell lysate (corresponding to 5 ×10^6 cells) into a new tube. Add 8-15 ug antibody per reaction and incubate rockin g for 3 hrs on ice. The optimal amount of antibody required to immunoprecipitate the antigen from a given cell lysate should be empirically tested.
7. Add 50 ml of 50% slurry beads and rock for 1 hr at 4℃.
8. Centrifuge sample at 10,000 g for 15 sec in microcentrifuge. Carefully discard the supernatant.
9. Wash beads twice with 1ml RIPA buffer（to remove non specifically associated proteins）and then 3 times with 1ml PBS to remove detergents.
10. Finally, resuspend beads in 60 ul Sample buffer, and boil at 95℃ for 5 min. Centrifuge sample at 10,000 g for 15 sec in microcentrifuge before loading on SDS-PAGE.

Reagents:

RIPA buffer:
50 mM Tris-Cl (pH 7.4), 150 mM NaCl, 1 mM EDTA, 1% Triton×100, 1% Na deoxycholate, 0.1% SDS, 1 mM PMSF, 1 ug/mL aprotinin, 1 ug/mL leupeptin.

PBS (pH7.4):
10 mM Na_2HPO_4, 1.8 mM KH_2PO_4, 50 mM NaCl, 2.7 mM KCl.

1 × Sample buffer:
65 mM Tris-Cl (pH8.0), 10% (v/v) glycerol, 2.3% (w/v) SDS, 0.01% bromophenol blue, 1% DTT.

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