Procedure of Immunoprecipitation Assay with Magnetic Beads

Note: This is a general protocol. It may need to be optimized for your specific purposes.

A. Materials and Reagents

1. 1X Phosphate Buffered Saline (PBS): Dissolve 8g NaCl, 0.2g KCl, 1.15g Na_2HPO_4 and 0.2g KH_2PO_4 in 800mL distilled water (dH_2O). Adjust the pH to 7.4 with HCl and the volume to 1 liter. Store at room temperature.
2. 1X RIPA buffer: 2mM Tris HCL, 150mM NaCl, 1% NP40, 1mM EDTA, adjusted to pH 7.0
3. 4X SDS sample buffer
4. Magnetic stand(s)

B. Immunoprecipitation procedure

1. Mix the magnetic beads with conjugated antibody well in the original bottle.
2. Apply 20uL magnetic beads to 1.5mL Eppendorf tube.
3. Apply the tube with magnetic beads to the magnetic stand. Let it stand for 30-60 seconds. The beads will be adhesive to the side of the tube.
4. Vacuum out the reagent at the bottom of the tube by avoiding touching the magnetic beads.
5. Apply 1mL RIPA buffer and mix well with the magnetic beads.
6. Apply the tube with magnetic beads to the magnetic stand. Let it stand for 30-60 seconds. Remove the RIPA buffer from the tube.
7. Repeat step 5 and 6 for 3 times to clean the magnetic beads.
8. Apply the protein mixture solution (cell lysates containing target protein).
9. Mix well with the magnetic beads and incubate at 4^{o}C overnight.

10. On the 2nd day, apply the tube with magnetic beads to the magnetic stand. Let it stand for 30-60 seconds. Vacuum out the buffer and wash the beads with RIPA buffer for 3 times as previously mentioned in steps 5 and 6.
11. Apply 20uL 4X SDS sample buffer to the magnetic beads after washing.
12. Boil the beads at 95^{o}C for 10min.
13. Spin the magnetic beads down by centrifuging for 3-4 minutes at 1,500 rpm; alternatively, apply the tube to the magnetic stand to allow the magnetic beads to be adhesive to the wall of the tube.
14. Load the supernatant to the SDS-PAGE gel and perform Western blot assays with appropriate antibody.