Immunoprecipitation Protocol

1. Resuspend Protein A or G sepharose bead slurry by gently vortexing the storage bottle. Quickly add 50 µl of 50% bead slurry per 0.5-1 mg of total protein to the microfuge tube containing lysate.
2. Incubate on a rotary mixer for 30 minutes at 4°C.
3. Centrifuge at 1000 rpm for 3 minutes at 4°C and transfer the supernatant to a fresh tube.
4. Add an appropriate amount (1-4 µg) of primary antibody to the whole (or pre-cleared) lysate. Optimal antibody concentration should be determined by titration. Set up a negative control experiment with control IgG (corresponding to the primary antibody source). Gently rock the incubations at 4°C for 2-4 hours or overnight.
5. Add 50 µl of Protein A or G sepharose bead slurry to capture the immunocomplex. Gently rock the mixture at 4°C for 1-4 hours.
6. Centrifuge the IP mixture at 1000 rpm for 30 seconds at 4°C and discard the supernatant.
7. Wash the beads 3-4 times with 1 ml of RIPA lysis buffer or 1X PBS with 0.2% Tween 20 (less stringent), centrifuge and discard the supernatant while following the instructions in step 6.
8. Elute the pellet twice with 40 µl of 150 mM glycine-HCl (pH 1.5-2.5) elution buffer containing 500 mM NaCl. Pool elutions and neutralize by adding 10 µl of alkali neutralization buffer (1.7M NaOH) to a final dilution of 1X.
9. Add 25 µl of 5X SDS sample buffer to the elutions. Heat at 95°C for 5 min.
10. Centrifuge at 10,000X g (approximately 9700 rpm for rotors of a 9.5 cm radius) for 3 minutes. Load supernatants onto a SDS-PAGE gel, alternatively, transfer the supernatant carefully to a fresh, well-labeled microfuge tube and store at -80°C for later use.
11. Separate IPs by SDS-PAGE and transfer proteins to PVDF membrane. Probe with appropriate antibodies.