Immunoprecipitation Protocol

1. Lyse Cells

Prepare Lysis Buffer (50ml): 10% Glycerol, 50mM Tris pH 7.7, 150 mM NaCl, 0.5% NP40, 1X DTT, 1X Protease Inhibitors, 0.1 μM Okadaic Acid or Microcysteine. Keep everything on ice.

a. Add appropriate volume of lysis buffer to cell pellet
b. Use 22 gauge needle and syringe to lyse cells
c. Add sample of lysate to SDS for loading control
d. If volume <1.5 ml, spin top speed in cold room centrifuge for 30 minutes.

2. Prepare Beads

Depending upon antibody and beads wanted, wash 5 μl of solid beads 1X in lysis buffer. Pre-coupled antibodies work better for western blotting after IP.

3. Add supernatant from centrifugation to beads (remember all controls) and incubate beads with supernatant in cold room, rotating, for 2 hours.

4. Wash beads 4X with lysis buffer. Careful not to lose beads during washes.

5. Prepare SDS-Page gel for western blotting, or proceed to other assays.