Immunoprecipitation Protocol

Reagents Needed:
Immunoprecipitation (IP) lysis buffer
Protease Inhibitors (Calbiochem Cat#539131)
Primary Antibodies made in Rabbit
Normal IgG, negative control (Rabbit IgG- Bethyl Cat. No. P120-101)
Protein A Sepharose Beads (Amersham Cat# 17-0780-01)
Cell Lysate
Sample Buffer

IP lysis Buffer
12.5 ml 1M NaCl (250mM)
2.5 ml 1M Tris (50mM)
500ul 0.5M EDTA (5mM)
2.5ml 10% NP-40
32 ml dH20

Protein A Beads
Resuspend 400 mg of Protein A beads in 10 ml of distilled H2O. Mix well to resuspend. Spin at 250 rpm for 5 minutes. Wash 3X in 10 ml IP Lysis buffer. Resuspend to 10 ml with IP lysis buffer for a 20% solution. Use 100 mcl per IP reaction.

4X Sample Buffer (Store at 4 C)
Glycerol - 4.0 g
Tris Base - 0.68 g
Tris HCL - 0.67 g
LDS - 0.80 g
EDTA - 6 mg
Brilliant Blue G250 - 2.5 mg
Phenol red - 2.5 mg

1X Sample Buffer
4X sample buffer - 150 µl
1M DTT - 60 µl
Distilled water - 390 µl
Make **fresh** for each use.

Procedure:

1. Place 500 µl of the pooled cell lysate (1-3 mg/ml) into a 1.5 ml micro-centrifuge tube.
2. To this tube add 2 to 10 µg of the primary antibody (If using neat sera or an IgG fraction such as Protein-A purified antibody, larger amounts are likely to be required. For best results, optimal amounts of antibody should be empirically defined.)
3. To a negative control reaction, add an equivalent amount of normal rabbit IgG.

4. Add 100 µl of a 20% Protein A suspension.(Amersham Biosciences, Cat# 17-0780-01) to the mixture of antibody and cell lysate. Rotate the immunoprecipitation reactions (end-to-end) for 3 hours at room temperature or overnight at 4 C.
5. Centrifuge (200 x g; 5 minutes) to pellet the complex.
6. Remove the supernatant and add 500 µl cold cell lysis buffer. Centrifuge (200 x g; 5 minutes).
7. Repeat wash step 6 twice more. After each centrifugation remove as much of the supernatant as possible.
8. After removing the supernatant from the third wash, add 40 µl of freshly prepared 1X sample buffer to each tube and heat at 90 C for 5 minutes.
9. Continue with electrophoresis and immunoblotting as described under western blotting protocol. Load 8 to 16 µl (20 to 40% of the IP reaction) to a polyacrylamide gel.

Note: For optimal results, complete reduction of the sample is required. We recommend the use of 0.1 M DTT in SDS-PAGE sample buffer and immediately heating samples, loading and running gels