Immunoprecipitation

1) To a microcentrifuge tube, add 1-5µg of antibody, 400µl of H2O, 100µl total lysate (200-500µg total protein), and 500µl of 2x immunoprecipitation buffer.

2) Vortex and incubate at 4ºC for one hour. (If monoclonal antibodies are used, add 5µg rabbit anti-mouse IgG antibody, vortex, and continue the incubation for an additional 30 minutes at 4ºC).

3) Add 10µl 50% Protein A:Agarose (BD Biosciences #610437). Vortex and incubate with agitation for 30 minutes at 4ºC.

4) Centrifuge agarose solution for 4 minutes (16,000 x g, 4ºC) and remove supernatant.

5) Wash with 1x immunoprecipitation buffer by centrifuging 4 minutes (16,000 x g, 4ºC). Repeat wash twice.

6) Resuspend the pellet in 30µl of 2X SDS sample buffer.

7) Boil for 5 minutes and centrifuge (16,000 x g, 4ºC) for 5 minutes.

8) Load the supernatant onto an SDS-PAGE gel and electrophorese.

9) Transfer to PVDF membrane and probe with appropriate antibodies (Western blot).

2x IP buffer:	**1x IP buffer:**
2% Triton X-100	1% Triton X-100
300mM NaCl	150mM NaCl
20mM Tris pH 7.4	10mM Tris pH 7.4
2mM EDTA	1mM EDTA
2mM EGTA pH 8.0	1mM EGTA pH 8.0
0.4mM sodium ortho-vanadate	0.2mM sodium ortho-vanadate
0.4mM PMSF	0.2mM PMSF
1.0% NP-40	0.5% NP-40

2x SDS sample buffer:
250mM Tris pH 6.8
4% SDS
10% glycerol
0.006%bromophenol blue
2% ß-mercaptoethanol