Immunoprecipitation and Immunoblotting:

Day 1

1. Prepare lysis buffer and keep it in ice
    - Lysis Buffer (Non-strigent example):

MQ water	13.65 ml
2M Tris/Hcl (ph7.5)	375 ul
5M NaCl	375 ul
Triton X-100	150 ul
0.1% SDS	150 ul
0.5M NaF	150 ul

100 mM Na3VO4	150 ul
100 mM PMSF	150 ul
10 mg/ml Leupeptin	15 ul
Total	15 ml

1. 3X wash cells with ice-cold PBS
2. l for each 10 cm2 area confluentμlyse the cells with lysis buffer, 200 cells, scrape the cell debris using the blunt end of the pipette tips, collect the cell lysis and kept in ice.
3. centrifuge top speed in 4oC micro-centrifuger for 10 min
4. transfer supernatant to new tubes
5. measure the protein concentration
    - 1:4 mixture of protein reagent: water
    - Take out 5 ul out from 1 ug/ul BSA and cell lysis samples, read the absorbance by photospectrometer and calculate the protein concentration of lysis samples.
6. Take out 400 ug of protein from each sample, add in 2 ug of antibody, rock for 1 hr in 4oC.
7. add 30 ul of protein A or protein A/G beads to each sample (note: gentle tap and resuspend all the beads), Rock for overnight at 4oC.

Day 2

1. Prepare resolving Gel (typical 10%), takes 30 min to solidify.
2. Prepare Stacking Gel (mostly 5%), add in comb to generate wells. Take 5-10 min to solidify.
3. centrifuge the beads for 5 min at 3000 rpm at 4oC. aspirate the supernatant and add in 1ml lysis buffer for each sample.
4. repeat step 9 for 2 times, centrifuge the beads for 5 min at 3000 rpm at 4oC. aspirate the supernatant.
5. add in 6X SDS protein loading buffer to each sample

SDS Gel-loading buffer:	4X	6X
Tris.Cl (PH 6.8) (mM)	200	300
Dithiothreltol (DTT, mM)	400	600
SDS (Electrophoresis)	8%	12%
Bromophenol Blue	0.4%	0.6%
Glycerol	40%	60%