Immunoprecipitation of proteins from tissue cultured cells

From ~5 x 10^6 cells (2 x 100mm dish~1x 150mm dish)

Wash cells twice with ice-cold 1xPBS

Transfer cells to microtube using cell scraper

Spin down cells by centrifugation, 2000rpm, 5min, 4˚C

Cell pellet may be frozen at this point at -80˚C

Add 1ml of lysis buffer (ice-cold) to cell pellet

Suspend cells by pipetting

Sonicate cells for 15s (Optional) (Branson; output 9%, on time 2sec, Off time 5sec)

Incubate 10min, on ice, occasional brief vortex (once or twice)

Centrifuge, 10min, 4˚C, 13krpm

Transfer sup to new microtube

Save 50ul for WCE SDS-PAGE sample

(50ul of WCE + 25ul of 3xSDS-PAGE loading buffer

Measure protein concentration using BCA protein assay kit (use 10ul)

Add antibody (1-3ug antibody to 1mg of protein)

Rotate sample at 4˚C for 1-2h

Add protein A-Sepharose {30ul, 50% slurry, pre-washed in lysis buffer (500ul x 3)}

Rotate sample at 4˚C for 1-2h

Spin down beads and wash beads 3 times in 500ul of lysis buffer

Add 100ul of lysis buffer to beads

Add 60ul of 3XSDS

Boil for 5min

Chill on ice

Store at -20˚C

Lysis buffer

	Stock soln.	For 10ml		
50mM Tris-HCl, pH8.0	1M			
150mM NaCl	5M			
10% Glycerol	60%			
0.1% NP-40	10%			
50mM NaF	0.5M			
1mM Na3VO4	0.1M			
1mM DTT	1M			
5mM EDTA	0.5M			
1uM microcystin	400 X			
0.1uM Okadaic acid	1000 X			
Complete-mini EDTA free	Tablet			
0.2mM pAPMSF	0.2M			