Immunoprecipitation of proteins from S. pombe cells

Culture cells in 50 ml YES medium @ 30˚C for O/N

If cell density is more than 1.5 OD/ml, dilute cells in YES at 0.3 OD/ml, and culture cells another 3-5hr

Collect cells in a 50-ml conical tube by centrifugation. (2400 rpm, 4 min, 4˚C)

*Optimal cell density should be 0.5~1.5 OD/ml

Decant medium, and re-suspend cells in 10 ml Stop Buffer

Collect cells by centrifugation. (2400 rpm, 4 min, 4˚C)

Aspirate medium, and re-suspend cells in 1 ml Stop Buffer.

Transfer cell suspension to a 1.5 ml screw-top tube

Centrifuge to collect cells. (7000 rpm, 15 sec)

Remove sup by aspiration

*The cell pellet can be snap-frozen in liquid nitrogen (or EtOH/dryice) and kept at -80˚C at this point

Re-suspend cells in 400 µl of lysis buffer (ice-cold)

Add glass beads until over the surface of liquid.

Fast Prep. (Speed: 6.0, Time: 20 sec x 2, 2min interval)

Pierce the bottom of the tube with heated needle (25G).

Place the tube on top of a new 1.5 ml tube.

Centrifuge. (3000 rpm, 30 sec, RT)

The protein extract can be collected in the bottom tube

Remove the top tube and centrifuge the bottom tube at 13krpm, for 10min, at 4˚C

Transfer sup (WCE: whole cell extract) to a new micro tube

Save 20µl of the sup for WCE SDS-PAGE sample

(Mix: 20ul of WCE + 10µl of 3xSDS-PAGE loading buffer, boil 5min, on ice)

* Optional: Measure protein concentration using BCA protein assay kit (use 10ul), and adjust the protein concentration to the least concentrate sample. All samples must have the same volume.

Add additional 400 µl of Lysis buffer to the tube

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

Rotate sample at 4˚C for 1-2h

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

Rotate sample at 4˚C for 1-2h

Spin down Sepharose/Agarose beads (7000 rpm, 10 sec)

Carefully aspirate sup (don't aspirate beads), re-suspend (by inverting beads) the beads in 500ul of lysis buffer, and spin down the beads (7000 rpm 10 sec).

Repeat the above step two more times

Carefully aspirate sup (don't aspirate beads), and add 100ul of lysis buffer to beads

Add 60ul of 3 X SDS loading buffer, and mix sample

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 Na_3VO_4	0.1M			
5mM EDTA	0.5M			
5mM N-methylmaleimide	0.5M			
1uM microcystin	400x			
0.1uM Okadaic acid	1000x			
Complete-mini EDTA free	Tablet			
0.2mM pAPMSF	0.2M			

* If Na_3VO_4 is unnecessary, add 1mM DTT (DTT inhibits Na_3VO_4) instead.

Antibodies

Preferably, for immunoprecipitation, use an antibody raised in a different animal than the antibody for the following Western blotting. For example, use a rabbit antibody when a mouse antibody will be used for Western. If the size of immunoprecipitated protein is significantly different from the sizes of antibody heavy and light chains, you may be able to use same animal source.