

Lambda phosphatase treatment of immunoprecipitated proteins

*Perform experiments at 4°C or on ice unless otherwise specified.

Immunoprecipitation

From $\sim 5 \times 10^6$ cells (2 x 100mm dish \sim 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 a 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 {100ul, 50% slurry, pre-washed in lysis buffer (500ul x 3)}

Rotate sample at 4°C for 1-2h

Spin down beads and wash beads once with lysis buffer

Spin down beads and wash beads twice with lysis buffer w/o phosphatase inhibitors

Add lysis buffer w/o phosphatase inhibitors (final total volume should be 100ul)

30ul aliquots into 3 tubes

Spin down beads and remove supernatant

Phosphatase treatment

	A (Mock)	B (+ Lambda PPase)	C (+ Lambda PPase+PPase inhibitors)
10 x λ PPase buffer	10ul	10ul	10ul
H ₂ O	65	64	40
20mM MnCl ₂	10	10	10
Prepare mixture above and add to beads			
Beads	15	15	15
0.5M NaF	-	-	10
0.1M NaVnO ₄	-	-	1
0.5M EDTA	-	-	1
0.5M NEM	-	-	10
1000x Okadaic Acid	-	-	1
400x Microcystine	-	-	1
Lambda PPase	-	1 (200 units)	1 (200 units)
Total	100ul	100ul	100ul

Incubate @30°C, for 20 min (mix occasionally)

Add 50ul of 3x SDS-PAGE loading buffer to stop reaction

Boil for 5min (vortex after 2min)

Cool down on ice

Store @ -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%			
1mM Na ₃ VO ₄ *	0.1M			
50mM NaF*	0.5M			
5mM EDTA	0.5M			
1uM microcystin*	400 X			
0.1uM Okadaic acid*	1000 X			
Complete-mini EDTA free	Tablet			
0.2mM pAPMSF	0.2M			
1mM DTT	1M			

* phosphatase inhibitor