Protein Lysates

Mammalian cell lines

Cell Number: It is recommended to use at least $1x10^6$ cells. The protein yield will vary, but this number of cells will yield approx. 100ug protein.

General notes:
1) Wear gloves
2) Keep things cold
3) Make the cell lysis buffer before harvesting cells. have cold PBS ready

A. Cell Harvest:

1. Harvest and count cells using trypsin (if adherent) or by centrifugation (suspension cells). fast cool centrifuge
2. Wash cells with cold PBS three times—usually this is done in 15 ml conical tubes using 10cc PBS. Centrifuge cells for 7-10 min. at 800xg (1300rpm), remove sup, and resuspend the pellet in residual supernatant before adding the next PBS wash.
3. After the last wash, place cells in Eppendorf tube and spin down ; remove PBS by inverting tube onto Kim-wipe. 5m @ 800xg = 2800xg.

B. Cell Lysis

1.Add cell lysis buffer to Eppendorf tube. For each million cells, add 100 ul. It is best not to use less than 200 ul, even if total cell count is less than 2 million. Resuspend pellet by gently mixing with pipette or tapping tube.

2. Incubate on ice for 15 minutes.
3. Precool Eppendorf centrifuge to 4 C
4. Centrifuge cells at 4000 rpm (xg) for 20 minutes
5. Harvest supernatant into fresh, labeled Eppendorf tube (you can save 20 ul in a separate tube for OD determination
6. Determine protein concentration by A-595.
7. Freeze at –80 C.

Protein lysate buffer

	Amount	**Final conc.**
DdH2O	7.4 ml	
10xPBS	1 ml	
5% deoxycholate	1 ml	0.5%
Triton X	100 ul	1%
10% SDS	100 ul	0.1%
complete mini tab		
rotate until mini-tab goes into solution		
100 mM NaOrthovanadate	100 ul	1 mM
1M NaF	300 ul	30 mM
0.5M EDTA pH 8.0	20 ul	1 mM