WHOLE CELL EXTRACT PREPARATION

Materials:

plate of cells (80-100% confluent)
cold 1xPBS
cell scraper (Midwest Scientific #TP9902)
dry ice/methanol
Buffer C
Bio-Rad Protein Assay Dye Reagent Concentrate (cat#500-0006)

Procedure:

1. Aspirate off media from 10 cm TC plate.
2. Rinse with cold 1xPBS (pipet about 2ml onto plate and swirl around gently). Aspirate off 1xPBS.
3. Add 1 ml cold 1xPBS to plate.
4. Scrape cells to one area (at the edge of the plate).
5. Pipet cells into microfuge tube.
6. Spin cells for 10 seconds in microfuge tube.
7. Aspirate off 1xPBS (be careful not to lose the pellet).
8. Freeze cell pellet in dry ice/methanol.
9. Resuspend pellet in 20µl Buffer C.

Buffer C (100ml)	Stock	Volume
25% glycerol	100%	25 ml
0.42 M NaCl	4 M	10.5 ml
1.5 mM $MgCl_2$	1 M	150 µl
0.2 mM EDTA	0.5 M	40 µl
20 mM HEPES (pH 7.9)	1 M	2 mls
• add distilled water up to 100 ml • add DTT and PMSF to 0.5 mM before use		

10. Spin tube for 15 seconds.
11. The proteins are suspended in the supernatant. Collect the supernatant to determine the protein concentration by BioRad assay.

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