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Protein Lysates (from cultured cells)

Solutions:

- 1x DPBS (Mediatech, Inc., #21-031-CV)
- Protease inhibitor cocktail (complete, Mini tablets, Roche Applied Science)
- Phosphatase inhibitor cocktail (Sigma-Aldrich, P5726).
- RIPA Buffer (25 mM Tris pH 7.4, 150 mM NaCl, 1 % NP-40, 1 % Na-deoxycholate, 0.1 % SDS) for final volume of 50 ml:

Reagent:	**[Final]**
25 ml ddH_2O	
1500 µl of 5M NaCl	150 mM
1250 µl of 1M Tris-Hcl pH 7.4	25 mM
500 µl NP-40	1%
5000 µl of 10 % sodium-deoxycholate	1%
500 µl of 10 % SDS	0.1%
500 µl of 0.5M EDTA	5 mM

- bring up volume to 50 ml with ddH_2O; mix well by inverting; chill on ice

Procedure:

1. Wash cells with once with 2 ml of cold 1X DPBS. Keep plates on ice as much as possible
2. For a 12-well plate, pipet **250 µl** of RIPA buffer + protease inhibitors + phosphatase inhibitors directly onto the cells. Make sure buffer completely covers cells.
3. Scrape the cells and collect the lysate in cold, labeled microcentrifuge tubes.
4. Nutate cells at 4 ℃ for 30 minutes (in cold room).
5. Centrifuge samples at 12000g for 15 minutes at 4 °C.
6. Transfer the supernatant to new labeled cold microcentrifuge tubes
7. Store the samples at -80°C