

RIPA Lysates of Cells

Solutions Needed:

RIPA Buffer

150 mM NaCl

10 mM Tris, pH 7.2

0.1% SDS

1.0% Triton X-100

1% Deoxycholate

5 mM EDTA

Add protease/phosphatase inhibitors in final concentrations of:

Protease inhibitors:

1 mM phenylmethylsulfonyl fluoride, 10 mM benzamidine, 2 μ g/ml leupeptin

Phosphatase inhibitors:

100 μ M sodium orthovanadate, 10 mM p-nitrophenylphosphate

Procedure:

1. Place cells on ice
2. Wash cells with ice cold PBS to remove media
3. Add 1 ml RIPA buffer to 100 mm plate. Scale up or down as necessary
4. Scrape cells into RIPA buffer and transfer to small centrifuge tube
5. Let stand on ice for 10 min, vortexing every few minute to dissolve material. Lysates can also be passed through a 22 g needle to aid in solubilization.
6. Centrifuge in Sorvall using the SS34 rotor with adaptors at 17,000 rpm for 10 min
7. Remove supernatant for protein assays and discard the pellet.

For experiments in which it is not desirable to denature proteins and possibly break protein:protein interactions, the RIPA buffer can be replaced with a non-denaturing NP40 Solubilization Buffer:

150 mM NaCl

20 mM Tris, pH 7.5

1% NP40

5 mM EDTA

If this non-denaturing buffer is used, lysates should be homogenized or passed through a needle several times to ensure adequate solubilization.