Making Protein Lysates from Cells

1. Gently wash cells in ice-cold 1X PBS

2. Collect cells in ice-cold 1X PBS by scrapping (0.5 mL – 5 mL depending on dish size)

3. Pellet cells by spinning at ~ 200 xg for 5 min at 4 ˚C

4. Remove supernatant

5. Resuspend cell pellet in ice-cold cell lysis buffer.
**Choice of buffer is critical and depends on downstream application. For example, RIPA lysis buffer is harsher and may not be appropriate for maintaining weak protein-protein interactions that may be preserved in a milder buffer (such as NP-40 lysis buffer). Altering the salt and/or detergent concentrations may be required for optimizing your specific application/protein(s). Additionally, if your protein of interest is chromatin bound, you may need to digest the DNA to efficiently solubilize it.*

**The volume of lysis buffer will primarily depend on size of dish (# of cells) and ultimate application of the lysate. For example, 100 µL per well from a 6-well is a good starting point for a concentrated lysate.*

6. Allow cells to lyse on ice for 30 min
**Depending on viscosity of solution (determined by # of cells, lysis buffer, and ultimate application for the lysate) you may want to pass cell lysate through 23-gauge needle, ~5-times. Keep in mind that cell lysates should always be kept cold.*

7. After lysing, spin down insoluble material for 10 min at max speed in a microcentrifuge at 4 ˚C

8. Collect supernatant containing the soluble protein lysates from cells. Discard pellet.

9. Use protein lysates immediately or snap freeze in liquid nitrogen and store at -80 ˚C

RIPA Lysis Buffer (harsh)
50 mM Tris-HCl pH 7.5
150 mM NaCl
1 % NP-40 (Igepal CA-630)
0.5 % Sodium Deoxycholate
0.1 % SDS
1 mM DTT (ADD FRESH AT TIME OF USE)
1 X Protease inhibitor cocktail (ADD FRESH AT TIMIE OF USE)

NP-40 Lysis Buffer (mild)
50 mM Tris-HCl pH 7.5
150 mM NaCl
0.5 % NP-40 (Igepal CA-630)
1 mM DTT (ADD FRESH AT TIME OF USE)
1 X Protease inhibitor cocktail (ADD FRESH AT TIMIE OF USE)