Title: **Protein lysates from cultured cells for Mass Spec.**
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Purpose: Generate mass spec compatible protein lysates from cultured cells or isolated PBMC.
Background: This protocol assumes:

- Cells have been harvested and washed in DPBS.

A. Cell Lysates

1. Preparation:
    - Turn on refrigerated micro-centrifuge and cool to 4ºC.
    - Turn on Coulter Counter and prime aperture.
    - Thaw Phosphatase inhibitors.
    - Label and pre-cool 50 mL tubes.
    - Label and pre-cool micro-centrifuge tubes.
    - Label and pre-cool Cryo-vials.
    - **Make fresh urea lysis buffer**- see solution section below.

2. Place tube with cell pellet on ice and add lysis buffer to a final concentration of $4.5x10^7$ cells / mL.
3. Resuspend cells in lysis buffer.
4. Sonicate cells in cold water cup horn sonicator. Set to 50% for 5 min
5. Vortex 15 sec
6. Micro-centrifuge: 20k x g (14K RPM or full speed) / 10 min. / 4ºC.
7. Transfer supernatant to 1.0 mL cryo-vial
    - Note: if storing aliquots of a lysate, first transfer the lysate to a fresh micro-centrifuge tube to ensure homogeneous mixing of the lysate before aliquoting.
8. Store lysates in liq. N2.
9. Determine protein concentration by BCA .

Solutions and Reagents:

Solutions:

- Lysis Buffer. **Must be made fresh daily**:
    - 4 Parts 7.5 M Urea (see below)
    - 1 Part 5x Lysis Buffer Stock Solution (see below).
    - Add 1% Sigma phosphatase cocktail 1
    - Add 1% Sigma phosphatase cocktail 2
    - Add 1% Sigma Protease Inhibitor
    - Mix well.

- 5x Lysis Buffer Stock Solution. May be made in advance and stored at room temp. or 4ºC. To Make 100 mL:
    - 12.5 mL 1M Tris (pH8.0)
    - 1.0 mL 0.5 M EDTA
    - 1.0 mL 0.5 M EGTA
    - HPLC water to 100 mL
    - Sterilize with 0.22 um filter.
- **7.5 M Urea. Make fresh daily**.
    - Add 6.5 mL HPLC water to a 15 mL Falcon tube.
    - Add 4.50 g Urea to the 15 mL Falcon tube.

- Mix until Urea is in solution, final volume should be 10 mL.

Reagents:

- Urea: Sigma Ultra Cat# U0631
- 1 M Tris (pH8.0): Sigma Cat# T2694
- EDTA: Sigma Cat# E7889
- EGTA: Sigma Cat# E0396
- HPLC water: Fisher Cat# W6-4
- Sigma Protease Inhibitor Cat# P8340
- Sigma Phosphatase Cocktail 2 Cat# P5726
- Sigma Phosphatase Cocktail 3 Cat# P0044

Final Urea buffer: 6M Urea, 25 mM Tris (pH8.0), 1 mM EDTA, 1 mM EGTA