Title: **Protein lysates from cultured cells for Mass Spectrometry**

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Purpose: Generate mass spec compatible protein lysates from cultured cells or isolated PBMC.

Revision History

Revision date	Revision Author	Revision notes
28 Feb 2020	Richard Ivey	

Background: This protocol assumes:

- Cells have been harvested from TC flasks or plates and transferred to 15 or 50 mL conical centrifuge tube(s).

A. Cell Lysates

1. Preparation:
   - Turn on refrigerated micro-centrifuge and cool to 4°C.
   - Turn on Coulter Counter and prime aperture (or use hemocytometer).
   - Thaw Phosphatase and protease inhibitor cocktails.
   - Label and pre-cool 15 or 50 mL conical centrifuge tubes.
   - Label and pre-cool 1.7 mL micro-centrifuge tubes.
   - Label and pre-cool Cryo-vials.
   - **Make fresh 1x urea lysis buffer**- see solution section below.

2. Wash cells 2x with ice-cold PBS to remove culture medium.
3. Place tube with cell pellet on ice and add lysis buffer to a final concentration of $5x10^7$ cells / mL.
4. Resuspend cells in lysis buffer by dragging tube along a microfuge tube rack- do not pipette.
5. Sonicate cells 2 x 12 sec. (550 Sonic Dimembrator, Fisher Scientific; knob set to 5)
   - Wipe down probe with water and ethanol between samples.
   - Place lysate on ice for ~20 sec. between sonications.
6. Transfer lysate by pipette tip to micro-centrifuge tube, vortex 15 sec..
7. Micro-centrifuge: 20k x g (14K RPM or full speed) / 10 min. / 4°C.
8. 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.
9. Store lysates in liq. N2.
10. 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 2
  - Add 1% Sigma phosphatase cocktail 3
  - Add 1% Sigma Protease Inhibitor
  - Mix well, keep on ice.

- 5x Lysis Buffer Stock Solution. May be made in advance and stored at room temp.
  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, BioXtra grade Cat# U0631
- 1 M Tris (pH8.0): Sigma, BioPerformance grade Cat# T2694
- EDTA: Sigma, molecular biology grade Cat# E7889
- EGTA: Bioworld Cat# 40520008-1
- LC/MS water: Fisher, Optima™ LC/MS Grade 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