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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 5×10^7 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