STANDARD OPERATING PROCEDURE

Title: Cell lysate preparation for samples compatible with mass spectrometry

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Purpose

The purpose of this document is to describe preparation of a cell lysate for protein analysis compatible with mass spectrometry.

Scope

This procedure may be used to make a cell lysate from cell lines.

Responsibilities

It is the responsibility of person(s) performing this procedure to be familiar with laboratory safety procedures. The interpretation of results must be done by a person trained in the procedure and familiar with such interpretation.

Equipment

- Refrigerated micro-centrifuge
- Centrifuge for use with tubes and 96 well plates
- Coulter Counter or hemocytometer
- Sonic dismembrator, (model 550, ThermoFisher Scientific)
- Lyophilizer

Materials

- UREA: SIGMA ULTRA CAT# U0631
- 1 M TRIS (PH8.0): SIGMA CAT# T2194
- EDTA: SIGMA CAT# E7889
- EGTA: SIGMA CAT# E0396
- HPLC WATER: FISHER CAT# W6-4

- SIGMA PHOSPHATASE COCKTAILS (P2850, P5726)

Reagents and Solutions

1. 1×DPBS (Gibco, ThermoFisher Scientific) (138 mM NaCl, 2.7 mM KCl, 8 mM Na_2HPO_4-$2H_2O$, 1.5 mM KH_2PO_4).
2. 7.5 M Urea: Add 6 mL HPLC-grade water to a 15 mL Falcon conical tube. Add 4.50 g urea and mix until the urea is in solution. Bring final volume to 10 mL with HPLC-grade water. Must be made fresh daily. Store at room temperature.
3. 5×Lysis buffer stock solution: To Make 100 mL: Add 12.5 mL 1 M Tris (pH 8.0), 1.0 mL 0.5 M EDTA, and 1.0 mL 0.5 M EGTA. Bring to 100 mL with HPLC-grade water. Filter with 0.22 µm filter. Store at room temperature for 6 months.
4. Lysis buffer: Add 4 Parts 7.5 M Urea, 1 Part 5×Lysis buffer stock solution, 1% Sigma phosphatase cocktail 2, 1% Sigma phosphatase cocktail 3, and 1% Sigma Protease Inhibitor to a final volume of 100 mL. Mix well. Must be made fresh daily, store on ice.
5. 0.2 M Tris, pH 8.0. Add 4 parts HPLC-grade water and 1 part 1 M Tris, pH 8.0.

Procedure

1. Preparation:
    a. Turn on bench-top centrifuge and cool to 4°C (check that appropriate adaptors are in the buckets).
    b. Turn on refrigerated micro-centrifuge and cool to 4°C.
    c. Turn on Coulter Counter and prime aperture.
    d. Thaw Phosphatase inhibitors.
    e. Label and pre-cool 50 mL tubes.
    f. Label and pre-cool micro-centrifuge tubes.
    g. Label and pre-cool Cryo-vials.
    h. Make fresh urea lysis buffer- see solution section above.
2. Remove media and rinse cells with 10 mL DPBS.
3. Harvest ~ two T-175 flasks by Trypsinization (6 mL / T175 flask or 2 mL / plate)
4. Incubate cells at room temperature with occasional mixing until the cells lift from culture surface as seen under the microscope.
5. Add 20 mL / flask (or 8 mL / plate) Growth Media (10% FBS) to swamp out the Trypsin
6. If needed, use a cell lifter to remove all cells from culture surface.
7. Transfer cells to pre-cooled 50 mL tubes.
8. Spin cells 180 x g / 8 min. / 4°C, dump supernatant.
9. Resuspend and pool cells from the same cell line in 10 mL ice-cold DPBS, remove 50 uL for cell counting by Hemocytometer.
10. Add ice-cold DPBS to 50 mL
11. Spin cells 180 x g / 8 min. / 4°C.
12. Count cell aliquot while cells are spinning. Calculate total cell count.

13. Dump supernatant and resuspend cells in 50 mL of ice-cold DPBS.
14. Spin cells 180 x g / 8 min. / 4°C.
15. Dump supernatant and invert tubes on paper towel ~ 20 sec., tap tubes on paper towels and remove remaining supernatant w/ Kim-Wipe.
16. Prepare Lysate buffer (see Solution section above)
    a. Lyse at 0.5x10^8 cells / mL
    b. Lysate buffer needs to be made fresh just before use.
    c. Add phosphatase inhibitors to lysis buffer at 1% just before use.
17. Place tube with cell pellet on ice and add lysis buffer to a final concentration of 0.5x10^8 cells / mL.
18. Gently resuspend cells in lysis buffer by dragging tube along a microfuge tube rack-do not pipette.
19. Sonicate cells 2 x 10 sec. (550 Sonic Dimembrator, Fisher Scientific; knob set to (5)
    a. Wipe down probe with water and ethanol between samples.
    b. Place lysate on ice for ~10 sec. between sonications.
20. Transfer lysate by pipette tip to micro-centrifuge tube, vortex 15 sec., ice 10 min., vortex 15 sec.
21. Micro-centrifuge / 20k x g (14K RPM or full speed) / 10 min. / 4°C.
22. Transfer supernatant to 1.0 mL cryo-vial (Nunc Cat# 377267).
    a. 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.
23. Determine initial protein concentration by Bradford or BCA assay (dilute lysate 1:25 in H20).
24. Store lysates in liq. N2.
25. When all lysates are available for pooling (if necessary), thaw lysates on ice.
26. Determine protein concentration by BCA.
27. Pool lysates as desired.

Referenced Documents

n/a