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| **STANDARD OPERATING PROCEDURE** |
| |  |  | | --- | --- | | **Title: Cell Culture and Lysate Preparation** | | | **SOP#: P-01** |  | | **Version #: 1** | **Author: Paulovich Lab** | | **Date Approved:** | **Date Modified:** | |

1. PURPOSE

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

1. SCOPE

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

1. 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.

1. Equipment
2. Materials

Urea: Sigma Ultra Cat# U0631

1 M Tris (pH8.0): Sigma Cat# T2194

EDTA: Sigma Cat# E7889

EGTA: Sigma Cat# E0396

TCEP: Sigma Cat# C4706

Trypsin Gold: Promega Cat# V5280

HPLC water: Fisher Cat# W6-4

Formic Acid: EDM Cat# 11670-1

Acetonitrile: Fisher Cat# A955-4

Oasis desalting cartridge.

Sigma Phosphatase Cocktail 1 Cat#P2850

Sigma Phosphatase Cocktail 2 Cat#P5726

1. Reagents

**Solutions**:

Lysis Buffer. Must be made fresh daily (final: 6M Urea, 25 mM Tris (pH8), 1 mM EDTA, 1 mM EGTA):

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

Mix well.

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 4.50 g Urea to a 15 mL Falcon tube.

Add 6 mL HPLC water and mix until Urea is in solution

Add HPLC water to a final volume of 10 mL.

1M TCEP Stock Solution:

0.287 g TCEP (Sigma Cat# C4706)

1.0 mL HPLC water

Aliquot and store at -80 oC.

Trypsin Gold (Promega): 100 ug resuspended in 100 uL HPLC water.

SPE cartridge Buffer A: 0.1% Formic Acid in HPLC water.

SPE cartridge Buffer B: 80% Acetonitrile (ACN), 0.1% Formic Acid in HPLC water.

2% acetonitrile, 0.1% formic acid made in HPLC water.

1. Procedure

**Cell Lysates**

1. Preparation:
   * Turn on bench-top centrifuge and cool to 4oC (check that appropriate adaptors are in the buckets).
   * 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. 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. / 4oC, 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. / 4oC.
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. / 4oC.
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 below)
    * Lyse at 0.5x10^8 cells / mL
    * Lysate buffer needs to be made fresh just before use (see below).
    * Add phosphatase inhibitors (see below) 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)
    * Wipe down probe with water and ethanol between samples.
    * 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. / 4oC.
22. Transfer supernatant to 1.0 mL cryo-vial (Nunc Cat# 377267).
    * 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, thaw lysates on ice.
26. Determine protein concentration by BCA.
27. Pool lysates as desired.
28. Referenced Documents