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| STANDARD OPERATING PROCEDURE |
| |  |  | | --- | --- | | **Title: Cell lysate preparation** | | |  |  | | **Version #: 1** | **Author: Paulovich Lab** | | **Date: 5/1/2012** |  | |

# 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 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

* No specialized equipment used in this protocol.

# 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 Cocktail 1 Cat#P2850
* Sigma Phosphatase Cocktail 2 Cat#P5726

# 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.

# Procedure

Cell Lysates

1. Preparation:
   1. Turn on bench-top centrifuge and cool to 4oC (check that appropriate adaptors are in the buckets).
   2. Turn on refrigerated micro-centrifuge and cool to 4oC.
   3. Turn on Coulter Counter and prime aperture.
   4. Thaw Phosphatase inhibitors.
   5. Label and pre-cool 50 mL tubes.
   6. Label and pre-cool micro-centrifuge tubes.
   7. Label and pre-cool Cryo-vials.
   8. 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. / 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 above)
    1. Lyse at 0.5x10^8 cells / mL
    2. Lysate buffer needs to be made fresh just before use.
    3. 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)
    1. Wipe down probe with water and ethanol between samples.
    2. 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).
    1. 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.