|  |
| --- |
| STANDARD OPERATING PROCEDURE |
| |  |  | | --- | --- | | **Title: Cell lysate preparation** | | |  |  | | **Version #: 1** | **Author: Akhilesh Pandey Lab** | | **Date: 01/15/2016** |  | |

# Purpose

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

# Scope

This procedure is used to create 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

* Sonicator
* Benchtop Centrifuge

# Reagents

* Guanadine HCL (Sigma-Aldrich; cat. # G3272-500G)
* Dulbecco’s Phosphate-Buffered Saline 1X (DPBS) (Corning; cat. # 21-030-CVR)
* Water: Optima LC/MS-grade (Fisher Scientific; cat. # W6-4)

# Solutions

* 8 M Guanidine HCL Lysis Buffer
  + Add 7.2 g Guanidine HCL to a 15 mL Falcon tube.
  + Add HPLC water to a final volume of 10 mL and mix until Guanidine HCL is fully in solution.

# Procedure

1. Prepare for Lysis Procedure.
   1. Prepare 8 M Guanidine stock solution as per recipe provided in Solutions Section.
   2. Incubate benchtop centrifuge to achieve final temperature of 4**°**C.
2. Remove media from cell culture dish and rinse cells with 10 mL ice-cold DPBS. Repeat for a total of three washes.
3. Remove excess DPBS and add 1 mL Lysis Buffer to cell culture dish.
4. Use a cell scraper to remove all cells from culture dish surface.
5. Harvest cells
   1. Transfer cells to falcon tube (falcon tube size dependent upon lysate volume).
   2. To lyse cells, using a microtip Sonicator, sonicate cells at 30 W with 3 bursts of 15 sec each. (Sonicator, Fisher Scientific, output control set to “3”).
      1. Wipe down probe with HPLC water and ethanol between samples.
      2. Cool lysate on ice for ~20 sec between each burst.
   3. Clear lysate by centrifugation in refrigerated benchtop centrifuge at 4**°**C at 12,500 x g at for 15 minutes.
   4. Preserve supernatant and discard pellet.
6. Store Cells
   1. If intended for storage, transfer supernatant to new tube (tube size dependent upon lysate volume).
      1. Note: If storing aliquots of a lysate, first transfer the lysate to a fresh tube to ensure homogenous mixing of the lysate before generating aliquots.
   2. Store lysates in -80**°**C.
7. Determine Protein Concentration
   1. Prior to Step 7, determine initial protein concentration by BCA assay (dilute lysate 1:5 in HPLC water).
   2. If lysates are to be used for subsequent processing/experimentation, thaw frozen lysates on ice.

# Referenced Documents

N/A