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| STANDARD OPERATING PROCEDURE |
| |  |  | | --- | --- | | **Title: Cell sample preparation** | | |  |  | | **Version #: 3** | **Author: PNNL Lab** | | **Date: 06/01/2016** |  | |

# Purpose

The purpose of this document is to describe the procedure for cell lysate sample preparation.

# Scope

This procedure may be used to make lysate from cell lysate samples.

# 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.

# Cell line

# PA-1

# SKOV3

# Equipment

# Pellet Pestle Homogenizer

# Sonication bath (Branson)

# Refrigerated centrifuge (Eppendorf)

# Speed-Vac (Thermo)

# Materials

# 0.6, 1.5, and 2.0-mL microcentrifuge tubes (Fisher)

# 4.0-mL cryovials (Corning)

# Reagents

# Urea (Sigma Aldrich)

# Complete Protease Inhibitor tables (Roche)

# BCA Assay Reagents A and B (Thermo Pierce)

# Solutions

# Lysis Buffer (8M urea (2.4g/5mL), 75mM NaCl in 100 mM NH4HCO3 pH 7.8 (to 5 mL), 10 mM NaF (100 ul of 2 g/dL stock per 5 mL), phosphatase inhibitor cocktail 2 (50ul per 5 mL Sigma P5726) and cocktail 3 (50 ul per 5 mL, Sigma P0044), cOmplete (Roche 05 892 791 001 -1/2 tablet per 5 mL))

# Procedure

# Chill cultured cell pellets and lysis buffer on ice.

1. Add 500 ul lysis buffer to cell pellets.
2. Vortex on high for 3 min and sonicate in a sonication bath for 3 min
3. 12000 g centrifuge 15min, transfer supernatant to another tube for digestion and BCA assay
4. Take aliquot for BCA assay

# Referenced Documents

List any publications or documents referenced in the SOP.