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| STANDARD OPERATING PROCEDURE |
| |  |  | | --- | --- | | **Title: Trypsin digestion of tissue sample** | | |  |  | | **Version #: SRM3** | **Author: PNNL Lab** | | **Date: 06/30/2016** |  | |

# Purpose

The purpose of this document is to describe the procedure of trypsin digestion of tissue sample.

# Scope

This procedure may be used to perform trypsin digestion of tissue sample.

# 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

Plate reader (Tecan)

Sonicator Bath

Vortexer

Thermomixer (Eppendorf)

Hula Mixer (Invitrogen)

Vacuum SPE Station (Supelco)

Speed-Vac (Thermo)

# Materials

15-mL centrifuge tube (Corning)

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

4.0-mL cryovials (Corning)

Discovery C-18 50 mg/1 mL SPE cartridges (Supelco)

# Reagents

DTT (Sigma Aldrich)

Iodoacetamide (Sigma Aldrich)

Trypsin (USB)

Methanol (Fisher)

TFA (Sigma Aldrich)

ACN (Fisher)

BCA Assay Reagents A and B (Thermo Pierce)

# Solutions

DTT (500 mM, 77 mg DTT per mL of Nanopure water)

IAA (400 mM, 74 mg IAA per mL of Nanopure water)

# Procedure

1. Add sufficient amount of the stock solution DTT to the samples to have a final concentration of 5 mM. Incubate at 37 ºC for 1 hr, 1200 rpm shaker speed
2. Add sufficient amount of the stock solution IAA to have a final concentration of 10 mM. Incubate at RT for 1 hr in the dark, 1200 rpm shaker speed
3. Dilute samples 2x using Nanopure water
4. Add 1M CaCl2 to have a final concentration of 1 mM CaCl2 in the samples
5. Add 20 µI of 50 mM NH4HCO3 to a vial of 20 µg of trypsin. Incubate for 10 min at 37 ºC to activate.
6. Add trypsin to samples in a 1:50 enzyme:protein ratio and incubate at 37 ºC for 4 hr, 700 rpm shaker speed
7. Dilute samples 4x using Nanopure water
8. Repeat steps 8-10, except incubate overnight at RT
9. Acidify samples right after digest with TFA to pH 2-2.5 (-0.5% TFA final concentration)
10. Proceed to SPE clean-up
11. Centrifuge samples at 4000 x g for 10 min
12. C-18 SPE

a. Prewash with 3 ml of MeOH

b. Prewash with 2 ml of 0.1% TFA

c. Slowly put sample through the column

d. Wash with 4 ml of 95:5 H2O: ACN, 0.1% TFA

e. Elute with 1 ml of 80:20 ACN: H2O, 0.1% TFA

13. BCA on concentrated sample

# Referenced Documents

List any publications or documents referenced in the SOP.