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| STANDARD OPERATING PROCEDURE |
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| **Title: Trypsin digestion of tissue sample** |
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| **Version #: PRISM** | **Author: PNNL Lab** |
| **Date: 07/20/2016** |  |

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# 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

Tecan GENios Plate Reader (Tecan Boston, Medford, MA)

Milli-Q Integral Water Purification System (EMD Millipore, Darmstadt, Germany, Catalog # ZRXQ010TO)

Ultrasonic Cleaner (Branson Ultrasonics Corp., Danbury, CT, Catalog # 5510R-DTH)

Vortex-genie 2 Lab Mixer (Scientific Industries Inc., Bohemia, NY, Catalog # G-560)

Thermomixer R (Eppendorf AG, Hamburg, Germany, Catalog # 5355)

HulaMixer Sample Mixer (Thermo Scientific, Rockford, IL, Catalog # 15920D)

Supelco Visiprep (Sigma Aldrich, St. Louis, MO, Catalog # 57030-U)

SpeedVac Concentrator (Thermo Scientific, Rockford, IL, Catalog # SC250EXP)

# Materials

High-clarity Polypropylene Conical Tube, 15 mL (Corning Inc., Corning, NY, Catalog # 352096)

Flat-Top Graduated Microcentrifuge Tubes (Fisher Scientific, Asheville, NC, Catalog # 02-681-268 & 02-681-331 & 02-681-332)

Internal Threaded Polypropylene Cryogenic Vial (Corning Inc., Corning, NY, Catalog # 430491)

Discovery DSC-18, 50mg/1mL (Supelco, Bellefonte, PA, Catalog # 52601-U)

# Reagents

DL-Dithiothreitol, >=98% (Sigma Aldrich, St. Louis, MO, Catalog # D0632)

Iodoacetaminde, BioUltra (Sigma Aldrich, St. Louis, MO, Catalog # I1149)

Trypsin (Affymetrix, Santa Clara, CA, Catalog # 22720)

Ammonium Bicarbonate, BioUltra >=99.5% (Sigma Aldrich, St. Louis, MO, Catalog # 09830)

Calcium Chloride Dihydrate, >=99% (Sigma Aldrich, St. Louis, MO, Catalog # C3306)

Methanol, Optima (Fisher Scientific, Asheville, NC, Catalog # A454-4)

Trifluoroacetic Acid, ReagentPlus 99% (Sigma Aldrich, St. Louis, MO, Catalog # T6508)

Acetonitrile, Optima LC/MS Grade (Fisher Scientific, Asheville, NC, Catalog # A955-4)

Pierce BCA Protein Assay Kit (Thermo Scientific, Rockford, IL, Catalog # PI23225)

# 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.