|  |
| --- |
| STANDARD OPERATING PROCEDURE |
| |  |  | | --- | --- | | **Title: Mini-validation of Samples (Repeatability)** | | |  |  | | **Version #: PRISM** | **Author: PNNL Lab** | | **Date: 07/20/2016** |  | |

# Purpose

The purpose of this document is to describe the characterization of a set of assays according to its repeatability of measurement over 5 days. This is to estimate the performance of the assay measured in a complex sample over multiple days.

# Scope

This procedure covers overall preparation and running of samples for generating the validation samples with regards to CPTAC Assay Characterization Guidance experiment #2.

# 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

Microcentrifuge

Vortex-Genie 2 Lab Mixer

# Materials

Waters glass vial

PCR-Clean microcentrifuge tube

# Solutions

Mobile phase A: 0.1% FA in H2O

Mobile phase B: 0.1% FA in ACN

# Reagents

Water, HPLC grade (H2O)

Acetonitrile, HPLC grade (ACN) (Fisher Scientific, A955-4)

Formic Acid (0.1%)/Acetonitrile (EMD, FX0437P-1)

Formic Acid (FA) (Agilent Technologies, G2453-85060)

**Peptide Standards:**

Crude heavy stable isotope-labeled peptides and sequence matched pure light versions were synthesized. Pure light peptides were purified to >95% purity by HPLC from the vendor and spiked in as internal standards (IS). Light peptides were quantified by amino acid analysis and aliquots were stored in 5% acetonitrile/0.1% formic acid at -80°C until use. All light peptides were spiked in at the same concentration level and served as light stable isotope standard (SIS). The stock of light internal standard was stored in -80 ºC freezer. Crude heavy peptides incorporated a fully atom labeled 13C and 15N isotope at the C-terminal lysine (K) or arginine (R) position of each (tryptic) peptide, resulting in a mass shift of +8 or +10 Da, respectively. Crude heavy peptides were mixed together at three different concentrations, including low, median, and high and further stored in -80 ºC until use. And the concentration of crude heavy peptides was estimated from the ratio of heavy/light ratio times the known concentration of pure light peptides.

**Matrix:**

A background matrix consisting of ovarian cancer tumor tissue digest was used for experiment 2. Tissue sample was processed as described in SOP TP-1 (Tissue sample Preparation). Digestion was performed according to SOP TD-1 (Trypsin Digestion of tissue sample). The tissue digest was aliquoted and independent digestions were performed for each replicate of repeatability validation to make complete process replicates for each sample. Following digestion, tissue digest was first diluted to a concentration of 0.5 ug/ul and prepared for future use.

# Procedure

**Determination of spike levels and preparation of samples**

1. Peptides were multiplexed according to the LLOQ and linear range determined from the response curves (experiment 1) in order to prepare validation samples at an appropriate concentration. Crude heavy peptides were spiked into tissue digest matrix with the following spike levels:

Low: 5-20 LOQ

Med: 80-320 LOQ

High: 1.875 times the concentration of Medium

1. The stock crude heavy peptide mix is serially diluted with tissue digest matrix (0.5 ug/ul) to create the required concentration above.
2. The stock pure light SIS peptide mix is prepared at 40 fmol/uL.
3. 2.75 ul of each concentration point of heavy mix is added to 50.875 ul of the digested tissue matrix. And 1.375 ul of light SIS mix is further added to each sample, which makes each sample a total volume of 55 ul. (By doing this, both heavy and light peptide standard account for less than 5% of final total volume).
4. All samples are prepared in Eppendorf 0.6 mL plastic tube and then transferred to Waters glass vial after brief vortexing and centrifuge.
5. Put all samples into autosampler and get ready for 1st dimension PRISM fractionation.
6. 45 ul of sample is used for 1st dimension PRISM fractionation and 3uL out of 20 ul is used for each 2nd dimension LC-SRM analysis with triplicate injection runs (See SOP LC-1 for Liquid Chromatography and SOP PM-1 for Peptide MRM on TSQ Vantage).
7. Each day 3 samples (Low, Med and High) are prepared and fractioned by PRISM. That is done in 5 consecutive days. The fractions are analyzed by triplicates.

# Referenced Documents

SOP TD-1 for Trypsin Digestion of tissue sample

SOP TP-1 for Tissue Sample Preparation

SOP LC-1 for Liquid Chromatography  
SOP PM-1 for Peptide MRM on TSQ Vantage

SOP Frac-1 for Sample Fractionation Peptide