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| STANDARD OPERATING PROCEDURE |
| |  |  | | --- | --- | | **Title: Stability** | | |  |  | | **Version #: 2** | **Author: PNNL Lab** | | **Date: 09/01/2015** |  | |

# Purpose

The purpose of this document is to describe the characterization of a set of assays according to its selectivity of peptides in 6 different biological replicates of the matrix

# Scope

This procedure covers overall preparation and running of samples for generating the samples with regards to CPTAC Assay Characterization Guidance experiment #3.

# 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

Eppendoff Thermomixer

# Materials

Waters glass vial

# 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:**

Both pure heavy stable isotope-labeled peptides and sequence matched pure light versions were synthesized. 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. Those pure peptides were purified to >95% purity by HPLC from the vendor. They were quantified by amino acid analysis and aliquots were stored in 5% acetonitrile/0.1% formic acid at -80°C until use. Pure light peptides are spiked in as internal standards (IS). The stock of light internal standard was stored in -80 ºC freezer. Pure heavy peptides were mixed together at three different concentrations, including low, median, and high and further stored in -80 ºC until use.

**Matrix:**

Six biological replicates of a background matrix consisting of ovarian cancer tumor tissue digest were freshly prepared and diluted with buffer A (0.1% FA) to a concentration of 0.1 ug/ul. Tissue samples were 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 stored in -80 for the experiment.

# Procedure:

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

1. Peptides were multiplexed according to the Experiment 2(See SOP for Validation Samples) to create three different points of varying concentrations of analyte. Crude heavy peptides were spiked into six biological replicates of the digested tissue matrix: blank, medium concentration and half of the medium concentration.

Blank: no heavy peptides mix spiking in

Medium concentration: 70x LOQ

Half of the medium concentration: 35x LOQ

1. 2 ul of each concentration point of heavy peptides mix is added to 36 ul of six digested tissue matrix.
2. 2 ul of light peptide IS mix (50 fmol/µL) is added to each sample, and the final volume of each sample is 40 µl, while both heavy and light peptide mix account for 5% of final volume. The final light peptide concentration is 25 fmol/µg.
3. All samples are prepared in Waters glass vial. Shake the vial on thermomixer with 800 rpm, 4 ºC, 10 min.
4. Put all samples into autosampler and get ready for LC-MRM detection (See SOP LC-1 for Liquid Chromatography and SOP PM-1 for Peptide MRM on TSQ Vantage).
5. 4 ul of sample is used for each run. Analyze a total of 18 prepared samples in a randomized order on the same day in duplicate.

# Referenced Documents

SOP TD-1 for Trypsin Digestion of tissue sample.pdf

SOP TP-1 for Tissue Sample Preparation.pdf

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

SOP for Validation Samples.pdf