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| STANDARD OPERATING PROCEDURE |
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| **Title: Selectivity** |
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| **Version #: 1** | **Author: PNNL Lab** |
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# 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

# Reagents

Water, HPLC grade (H2O)

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. Different light peptides were spiked in at different concentration level depending on the response of peptides 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 high concentration and 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:**

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 0.25 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: 50-60x LOQ

Half of the medium concentration: 25-30x 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 SIS mix is further added to each sample, which makes each sample a total volume of 40 ul. By doing this, both heavy and light peptide standard only account for 5% of final total volume.
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