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| STANDARD OPERATING PROCEDURE |
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| **Title: Stability** |
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| **Version #: 2** | **Author: PNNL Lab** |
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# Purpose

The purpose of this document is to describe the characterization of a set of assays to evaluate the variation of the measurements after different samples storage times and conditions.

# Scope

This procedure covers overall preparation and running of samples for generating the samples with regards to CPTAC Assay Characterization Guidance experiment #4.

# 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:**

A background matrix consisting of ovarian cancer tumor tissue digest was 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:

**Preparation of Samples for LC-MRM**

1. The matrix is spiked with the medium concentration according to the Experiment 2(See SOP for Validation Samples).
2. 20 ul of heavy peptides mix is added to 360 ul of a digested tissue matrix.
3. 20 ul of light peptide IS mix (50 fmol/µL) is added to the sample, which makes a total volume of 400 ul. By doing this, both heavy and light peptide standard only account for 5% of final total volume.
4. The sample is prepared in Waters glass vial. Shake the vial on thermomixer with 800 rpm, 4 ºC, 10 min.
5. Aliquot into 12 vials and put three in auto-sampler, which are immediately injected in duplicate.
6. Analyze these samples again once after at least 6 hours and once after at least 24 hours. (See SOP LC-1 for Liquid Chromatography and SOP PM-1 for Peptide MRM on TSQ Vantage).
7. Store the other 9 vials in a freezer at -70 ºC.
8. Thaw three (fourth to sixth samples) of them at room temperature for 1 hour and analyze for LC-MRM-MS.
9. Thaw another three (seventh to ninth samples) at room temperature, put back to the freezer at -70 ºC, and thaw at room temperature again for LC-MRM-MS.
10. Analyze the last three samples after four weeks of storage at -70 ºC.

# 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