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| STANDARD OPERATING PROCEDURE |
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| **Title: Stability** |
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| **Date: 04/23/2015** |  |

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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 sample 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

# 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:**

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 0.25 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:

**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 SIS mix is further 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