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| STANDARD OPERATING PROCEDURE |
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| **Title: Reproducible detection of endogenous analytes** |
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# Purpose

The purpose of this document is to describe the characterization of a set of assays to assess the overall reproducibility and the ability of the assays to detect the endogenous analytes using heavy peptides as standards.

# Scope

This procedure covers overall preparation and running of samples for generating the samples with regards to CPTAC Assay Characterization Guidance experiment #5.

# 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:**

Portions of the same ovarian tumor tissue sample were digested five times on each of the five days. Digested samples were 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. 2 ul of of heavy peptides mix is added to 38 ul of five digested tissue matrices without spiking light SIS mix.
2. Analyze five digested samples each day. (See SOP LC-1 for Liquid Chromatography and SOP PM-1 for Peptide MRM on TSQ Vantage).

# 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