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| STANDARD OPERATING PROCEDURE |
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| **Title: Response Curve** |
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# Purpose

The purpose of this document is to describe the characterization of a set of assays by response curve.

# Scope

This procedure covers overall preparation and running of samples for generating the response curve.

# 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 sample was 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 response experiment.

# Procedure:

**Preparation of Samples for LC-MRM**

1. The following is designed to create 9 points of varying concentrations of analyte (crude heavy labelled peptides) and 1 blank.
2. The stock crude heavy peptide mix is serially diluted with tissue digest matrix (0.25 ug/ul) in the following ratio: 1 (no dilution), 10, 100, 1000, 10000, 20000, 50000, 100000, 200000.
3. 2 ul of each concentration point of heavy mix is added to 36 ul of the digested tissue matrix. 4 ul of buffer A is added to the 72 ul of the digested tissue matrix, since more volume is needed for blank (9 runs).
4. 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). 4 ul of light SIS mix is spiked into blank sample.
5. All samples are prepared in Waters glass vial. Shake the vial on thermomixer with 800 rpm, 4 ºC, 10 min.
6. 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).
7. 4 ul of sample is used for each run with the run order of blank, low concentration to high concentration as a batch, and acquire the data in three batches.

# 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