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| STANDARD OPERATING PROCEDURE |
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| **Title: Response Curve** |
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| **Version #: 2** | **Author: PNNL Lab** |
| **Date: 09/01/2015** |  |

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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:**

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. Different heavy peptides were spiked in at different concentration level depending on the response of 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 0.1 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 9-13 points of varying concentrations of analyte (pure heavy labelled peptide) depending on the peptide response and 1 blank.
2. The pure heavy peptide stock is serially diluted with tissue digest matrix (0.1 µg/µl).
3. 4 µl of each concentration point of heavy stock is added to 34 µL of the digested tissue matrix. (By doing this, heavy peptide standard only account for less than 10% of final total volume).
4. 2 µl of light IS peptide mixture stock (50 fmol/µL) is further added to each sample, which makes each sample a total volume of 40 µl (By doing this, light peptide only account for less than 5% of final total volume). The final concentration of the IS peptides is 25 fmol/µg. 4 µl of buffer A is added to the 72 µl of the digested tissue matrix, since more volume is needed for blank (>=9 runs).
5. All samples are prepared in Waters glass vial. Shake the vial on thermomixer with 800 rpm, 4 ºC for 10 min.
6. Put all samples into LC 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 µl 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 replicates at each concentration point.

# 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