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| STANDARD OPERATING PROCEDURE |
| |  |  | | --- | --- | | **Title: Response Curve** | | |  |  | | **Version #: SRM3** | **Author: PNNL Lab** | | **Date: 06/30/2016** |  | |

# 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. All light peptides were spiked in at the same concentration level 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 17 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 (0.1 ug/ul) in the following ratio: 1 (no dilution), 2, 4, 10, 20, 40, 100, 200,,400, 1000, 4000, 20000, 40000, 200000, 400000, 2000000, 4000000.
3. 2 ul of each concentration point of heavy mix is added to 36 ul of the digested tissue matrix. 5 ul of buffer A is added to the 90 ul of the digested tissue matrix, since more volume is needed for blank (15 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). 5 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