STANDARD OPERATING PROCEDURE

Title: Response curve

Version #: 1

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Date: 8/17/2015

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.

Materials

- HPLC water: Fisher Cat# W6-4
- Formic Acid: EDM Cat# 11670-1
- Acetonitrile: Fisher Cat# A955-4

Reagents

Standards:

- Heavy stable isotope-labeled standards (SIS) were synthesized with a fully atom labeled 13C and 15N isotope incorporated 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. Pure standards were purified to >95% purity by HPLC and quantified by amino acid analysis. Aliquots were stored in 3% acetonitrile/0.1% formic acid at -80°C until use.

Matrix:

- An equal mix (by total protein mass) of lymphoblast cell lines (LCL, Coriell, GM07057, GM07026) treated and untreated by 10Gy ionizing radiation, was used as a background matrix. Lysates were prepared as described in SOP P-02 (Cell line lysis). The pooled lysate was aliquotted and independent digestions were performed in an automated fashion as described in SOP D-05 (Trypsin digestion of cell lysate) for each replicate of concentration points to make complete process replicates for each response curve. Following digestion, heavy synthetic peptide was added to the samples at one of eight concentrations, the samples were desalted and the phosphopeptides enriched by IMAC as described in SOP P-04 (Peptide immunoaffinity enrichment). Measurements of heavy:light (endogenous) ratio were used to characterize the assays at the concentrations described below. Digests for different days are performed >= 24 hours apart.

Procedure

1. Heavy peptides were spiked into 500µg of the digested lysate at 8 points of varying concentrations of analyte. Points were prepared by serial dilution. All prepared in triplicate (3 days). Light peptide was spiked into each sample at a constant amount of 100 fmol.
    a. Point 1 – 0.35 fmol
    b. Point 2 – 0.69 fmol
    c. Point 3 – 1.39 fmol
    d. Point 4 – 4.17 fmol
    e. Point 5 – 12.5 fmol
    f. Point 6 – 50 fmol
    g. Point 7 – 200 fmol
    h. Point 8 – 2000 fmol
2. Blanks were prepared in triplicate for each day (total 9 blanks)
3. Immunoaffinity enrichment was performed on separate days (x3).
4. Samples were run in increasing order of heavy peptide level with one injection per replicate, followed by a blank run to estimate total system carryover.

Referenced Documents

SOP D-05 Trypsin digestion of cell lysate.pdf
SOP P-02 Cell line lysis.pdf
SOP P-04 Peptide immunoaffinity enrichment.pdf