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| STANDARD OPERATING PROCEDURE |
| |  |  | | --- | --- | | **Title: Parallel Reaction Monitoring (PRM) Mass Spectrometry, Orbitrap Fusion Lumos** | | |  |  | | **Version #: 1** | **Author: Akhilesh Pandey Lab** | | **Date: 01/15/2016** |  | |

# Purpose

The purpose of this document is to describe the mass spectrometry method for the quantitative analysis of peptides using parallel reaction monitoring (PRM).

# Scope

This procedure describes the setup of the mass spectrometer and the PRM method parameters for the Orbitrap Fusion Lumos. LC parameters are contained in a separate document.

# 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

* Source: EASY-Spray (Thermo Fisher Scientific)
* Emitter tip: Precision positioned glass emitter integrated into EASY-Spray column (Thermo Fisher Scientific)
* LC-to-source connection: nanoViper fitting; integrated zero dead volume union within EASY-Spray column (Thermo Fisher Scientific)
* Mass spectrometer: Orbitrap Fusion Lumos (Thermo Fisher Scientific)

# Materials

* Injection loop: 20 µL nanoViper sample loop (Thermo Fisher Scientific; cat. # 6826.2420)
* Easy nLC 1200 system (Thermo Fisher Scientific) Nano Flow system with integrated autosampler.
* Trap Column: 100 µm I.D. x 2 cm packed with Acclaim PepMap 100 5 µm, 100 Å C18 (Thermo Fisher Scientific; cat. # 164564)
* Analytical Column: 75 µm I.D. x 50 cm EASY-Spray column packed with Acclaim PepMap RSLC 100 Å C18, 2 µm (Thermo Fisher Scientific; cat. # ES803)

# Reagents

* Water: Optima LC/MS-grade (Fisher Scientific; cat. # W6-4)
* Acetonitrile: Optima LC/MS-grade (Fisher Scientific; cat. # A955-4)
* Formic Acid: LC-MS Ultra (Sigma-Aldrich; cat. # 14265)

# Solutions

* Loading pump, mobile phase A: 0.1% formic acid in water
* NanoFlow pump, mobile phase A: 0.1% formic acid in water
* NanoFlow pump, mobile phase B: 0.1% formic acid in 95% ACN

# Procedure

1. Setup MS method and tune file parameters
   1. NSI Source/Gas parameters
      1. Spray Voltage: 2200 V
      2. Capillary Temperature: 200 °C
      3. Sheath Gas: 0
      4. Auxiliary Gas: 0
      5. Sweep Gas: 2
   2. MS parameters
      1. Microscans: 1
      2. Resolution: 30,000
      3. AGC target: 1e5
      4. Isolation window: 2.0 m/z
      5. NCE: 32
      6. Lock masses: 445.12003 m/z
2. Test system suitability with 200 fmol bovine serum albumin tryptic digest once column is conditioned
3. Preparing isolation list for target peptides
   1. LC-PRM method preparation
      1. Load the Skyline file containing peptides that will be monitored during the QC analysis
      2. Export the unscheduled transition list as a single method
      3. Import the unscheduled transition list as an Inclusion list in a Targeted-MS2  (tMS2) acquisition method in Xcalibur with the parameters set as indicated in Step 1, above
   2. Peptide detection
      1. Set up the autosampler and LC methods as indicated in the Liquid Chromatography SOP
      2. Inject the QC sample 3x
      3. Import the data files into the Skyline file
      4. Check the automatic integration of all peaks
   3. QC sample analysis
      1. Import the isolation list into an Inclusion list in a Targeted-MS2  acquisition method in Xcalibur with the parameters set as indicated in Step 1, above
      2. Set up the autosampler and LC methods as indicated in the Liquid Chromatography SOP
      3. Inject the QC sample 3x

# Referenced Documents

N/A