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| STANDARD OPERATING PROCEDURE |
| |  |  | | --- | --- | | **Title: Parallel Reaction Monitoring (PRM) using a Q-Exactive mass spectrometer** | | | **Version #: 1.1** | **Author: Hui Zhang Laboratory – Johns Hopkins University** | | **Date: 02/18/2016** |  | |

# 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 Q-Exactive. 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: Q-Exactive (Thermo Fisher Scientific)

# Materials

* Injection loop: 20 µL Dionex nanoViper sample loop (Thermo Fisher Scientific; cat. # 6826.2420)
* Column compartment: UltiMate 3000 Binary Rapid Separation Nano Flow Pump with Ternary Loading Pump and Column Compartment (Thermo Fisher Scientific; cat. # NCS-3500RS)
* Autosampler: UltiMate 3000 Thermostatted Rapid Separation Pulled Loop Wellplate Sampler (Thermo Fisher Scientific; cat. # WPS-3000)
* Solvent Degasser: UltiMate 3000 Integrated Solvent and Degasser Rack, 4 Channels (Thermo Fisher Scientific; cat. # SRD-3400)
* Trap Column: 300 µm I.D. x 5 mm packed with Acclaim PepMap 100 5 µm, 100 Å C18 (Thermo Fisher Scientific; cat. # 160454)
* Analytical Column: 75 µm I.D. x 50 cm EASY-Spray column packed with Acclaim PepMap RSLC 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: 2% ACN/0.1% formic acid in water
* NanoFlow pump, mobile phase A: 2% ACN/0.1% formic acid in water
* NanoFlow pump, mobile phase B: 0.1% formic acid in 90% ACN

# Procedure

1. Setup MS method and tune file parameters
   1. NSI Source/Gas parameters
      1. Spray Voltage: 1800 V
      2. Capillary Temperature: 250 °C
      3. Sheath Gas: 0
      4. Auxiliary Gas: 0
   2. Scheduled PRM parameters
      1. PRM detection window (sec): 300
      2. Maximum IT: 100 ms
      3. Inclusion list: On
   3. MS parameters
      1. Microscans: 1
      2. Resolution: 70,000
      3. AGC target: 5e5
      4. Isolation window: 2.0 m/z
      5. NCE: 29
      6. Lock masses: 445.12003 m/z; 371.10123 m/z
2. Test system suitability with 900 ng bovine fetuin tryptic digest once column is conditioned
3. Identify scheduling time for target peptides/transitions
   1. LC-PRM method preparation
      1. Load the Skyline file containing peptides and transitions that will be monitored during the QC analysis
      2. Note: For the Q-Exactive, Thermo does not use a true collision offset in their instrument method editor; thus, there is no Q-Exactive-specific collision energy equation in Skyline
      3. Export the unscheduled transition list as a single method
      4. Import the unscheduled transition list as an Inclusion list in a Targeted-MS2  acquisition method in Xcalibur with the parameters set as indicated in Step 1, above
   2. Timing the 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
      5. Export the scheduled transition list using 300 sec scheduled windows
   3. QC sample analysis
      1. Import the scheduled transition 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

1. Thomas SN, Harlan R, Chen J, Aiyetan P, Liu Y, Sokoll LJ, Aebersold R, Chan DW, Zhang H. Multiplexed Targeted Mass Spectrometry-Based Assays for the Quantification of N-Linked Glycosite-Containing Peptides in Serum. Anal Chem. 2015 Nov 3;87(21):10830-8. doi: 10.1021/acs.analchem.5b02063. Epub 2015 Oct 21. PubMed PMID: 26451657; PubMed Central PMCID: PMC4708883.