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| STANDARD OPERATING PROCEDURE |
| |  |  | | --- | --- | | **Title: Multiple Reaction Monitoring (MRM) using a TSQ Vantage triple quadrupole mass spectrometer** | | | **Version #: 1.1** | **Author: Hui Zhang Laboratory – Johns Hopkins University** | | **Date: 06/10/2016** |  | |

**Purpose**

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

**Scope**

This procedure describes the setup of the mass spectrometer and the MRM method parameters for the TSQ Vantage triple quadrupole mass spectrometer. 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**

* Mass spectrometer: TSQ Vantage (Thermo Scientific)
* Ion source: HESI (Thermo Scientific)

**Procedure**

1. Setup MS method and tune file parameters
   1. ESI Source/Gas parameters
      1. Spray voltage: 4000 V
      2. Capillary temperature: 210 °C
      3. Sheath gas pressure: 15
      4. Collision gas pressure: 1.5 mTorr
   2. MS parameters
      1. Q1 & Q3 peak width (FWHM): 0.70
      2. Cycle time: 0.480 sec
      3. Declustering potential: 2 V
      4. Chrom filter peak width: 30.0 sec
      5. Scheduled window: 180 sec
      6. Signal trigger: 1.0 e5
2. Test system suitability with 100 fmol 6 protein digest standard, equimolar (Thermo Scientific; cat. # 88342) – QC sample
3. LC-MRM method preparation and execution
   1. Set up the autosampler and LC methods as indicated in the Liquid Chromatography SOP
   2. Inject the QC sample three times to assess LC-MRM-MS performance
   3. Create Skyline file containing .fasta files of assay targets
   4. Export unscheduled transition lists (≤ 60 transitions per list)
   5. Run heavy target peptides using unscheduled methods and obtain retention times
   6. Import .raw files into Skyline and refine transitions
   7. Add light peptides into Skyline document
   8. Export a scheduled MRM-MS transition list with a 3 min retention time window
   9. Run the scheduled method twice
   10. Import the raw files into Skyline and check the peak integration
   11. Run QC sample at least once every 16 hours