|  |
| --- |
| STANDARD OPERATING PROCEDURE |
| |  |  | | --- | --- | | **Title: MRM Mass Spectrometry, TSQ Vantage** | | |  |  | | **Version #: PRISM** | **Author: PNNL Lab** | | **Date: 07/20/2016** |  | |

# Purpose

The purpose of this document is to describe the Mass Spectrometry (MS) method for developing peptide multiple reaction monitoring (MRM) assays.

# Scope

This procedure encompasses the setup of the MS and method parameters on Thermo TSQ Vantage.

# 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: in-house built nano-sprary source

Emitter tip: In-house made emitter (20um ID, 360um OD)

Mass spectrometer: Thermo TSQ Vantage

# Materials

# Reagents

# Procedure

1. Setup MS method parameters:
2. Source Parameters:
3. Ion Spray Voltage: 2400 V
4. Capillary Temperature: 335 ºC
5. Scheduled MRM Parameters:
6. MRM detection window (sec): 240
7. Dwell time: 10 ms
8. MS parameters:
9. Use tuned S-lens value
10. Collision gas pressure: 1.5 mTorr
11. Q1 and Q3 unit resolution
12. Collision energy: generated by Skyline software
13. Intensity threshold reference: not set
14. Test system suitability with appropriate QC standard once column is conditioned.
15. Method performance evaluation
16. Spike the target peptide mixture into real complex matrix (cell lysate, tissue digest, plasma etc)
17. Inject the mixed sample
18. Import the data file into Skyline
19. Check the LC and transition condition:
20. Make sure peak shape is acceptable, no tailing or fronting
21. Make sure each transition is ok, no obvious interference

# Referenced Documents

List any publications or documents referenced in the SOP.