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| STANDARD OPERATING PROCEDURE |
| |  |  | | --- | --- | | **Title: MRM Mass Spectrometry, TSQ Vantage** | | |  |  | | **Version #: SRM3** | **Author: PNNL Lab** | | **Date: 06/30/2016** |  | |

# 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: at least 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. Identify scheduling times for target peptides/transitions
16. Set up the autosampler and LC methods as in the accompanying LC SOP file
17. Check the overall status by injecting QC sample
18. Export the transition list from Skyline with collision energy defined by the software for Thermo TSQ
19. Inject target peptide mixture into TSQ Vantage
20. Import the data files into Skyline and manual check the retention time of each peptide
21. Export the scheduled transition list with collision energy defined by the software for Thermo TSQ
22. Method performance evaluation
23. Spike the target peptide mixture into real complex matrix (cell lysate, tissue digest, plasma etc)
24. Inject the mixed sample
25. Import the data file into Skyline
26. Check the LC and transition condition:
27. Make sure scheduling is ok, no missing peaks
28. Make sure peak shape is acceptable, no tailing or fronting
29. Make sure each transition is ok, no obvious interference

# Referenced Documents

List any publications or documents referenced in the SOP.