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| STANDARD OPERATING PROCEDURE |
| |  |  | | --- | --- | | **Title: Liquid Chromatography, Waters nanoACQUITY UPLC system** | | |  |  | | **Version #: PRISM** | **Author: PNNL lab** | | **Date: 07/20/2016** |  | |

# Purpose

The purpose of this document is to describe the Liquid Chromatography (LC) method of 1st dimension PRISM fractionation for development of CPTAC PRISM-SRM assays.

# Scope

This procedure is designed to help the setup of LC gradient and method parameters on Waters nanoACQUITY UPLC system of the PRISM fractionation method.

# 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

UPLC: nanoACQUITY (Waters, Part Number 176016000)

Collect Pal: LEAP Technologies

# Materials

Injection loop: 50 uL peeksil

Column: 3 μm Jupiter C18 bonded particles, 200 μm i.d. × 50 cm long packed in house

Collection plate: twin.tec PCR Plate 96, skirted, colorless (Eppendorf cat. No. 951020401)

Acetonitrile, HPLC grade (ACN) (Fisher Scientific, A955-4)

Formic Acid (0.1%)/Acetonitrile (EMD, FX0437P-1)

Formic Acid (FA) (Agilent Technologies, G2453-85060)

Ammonium formate, for HPLC, >= 99.0% (Sigma-Aldrich 540-69-2)

# Reagents

Mobile phase A: 10mM Ammonium formate in H2O, pH 9

Mobile phase B: 10mM Ammonium formate in 90% ACN, pH 9

Strong Needle Wash buffer: 100% ACN

Weak Needle Wash buffer: 0.1% FA in H2O

Seal Wash buffer: 10% ACN in H2O

# Procedure

**PRISM fractionation (see details in Reference 1)**

1. nanoACQUITY autosampler parameters:

Under General tab:

‘Partial loop’ under ‘sample loop option’

Wash Solvent: Weak 600 ul, Strong 200 ul

Autosampler temperature: 4 ºC

1. PRISM fractionation gradient methods:

* *Column Temperature: room temperature*
* 45 uL of each sample (0.5 μg/μL) were loaded onto the column and separated using a binary gradient of 1-10%B in 2 min, 10-15% B in 15 min, 15-25% B in 35 min, 25-35% B in 25 min, 35-45% B in 13 min and 45-90% B in 25 min. The flow rate is 2.2 ul/min

1. Collection:

* Following the LC separation, the eluate from the capillary column is collected every 1min into a 96 well plate using a Collect Pal system over the course of 96 min.
* Prior to peptide fraction collection, 18 μL of water is added to each well in the plate to avoid peptide loss and also to dilute the peptide fraction for LC-SRM analysis.
* The fraction containing a target peptide is intelligently selected based on the retention time of the peptide obtained by on-line monitoring.
* The fractions are transferred to individual Waters glass vials and stored in autosampler. Now they are ready for 2nd dimension LC-SRM analysis.
* The following is a list of peptides and their locations in terms of fraction number.

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| Protein | Peptide | ResponseCurveSamples | ValidationSamples |
| LAX1\_HUMAN | TDDPGTHVQCVK | 6 | 5 |
| CTNA2\_HUMAN | IASSEFADDPCSSVK | 7 | 6 |
| RAF1\_HUMAN | LVADCVK | 12 | 10 |
| AMPD1\_HUMAN | YNPVGASELR | 12 | 11 or 10 |
| FOSB\_HUMAN | IPYEEGPGPGPLAEVR | 16 | 14 |
| SLAF7\_HUMAN | VDFPDGGYSLK | 22 | 20 |
| AMPD1\_HUMAN | TDNLPENLGYHLK | 23 | 22 |
| CTNA2\_HUMAN | WDDSGNDIIVLAK | 24 | 23 |
| SRF\_HUMAN | ALIQTCLNSPDSPPR | 28 | 25 |
| DUS1\_HUMAN | DGTLALAAGALCR | 37 | 35 |
| ARI4B\_HUMAN | LGGFDNIESGAVWK | 40 | 39 |
| ATF3\_HUMAN | NLFIQQIK | 44 | 42 |
| ELMO1\_HUMAN | LLDLENIQIPDAPPPIPK | 52 | 51 |
| ELMO2\_HUMAN | LLDLENIQIPEAPPPIPK | 53 | 51 |

# Referenced Documents

1. Shi T, TL Fillmore, X Sun, R Zhao, AA Schepmoes, M Hossain, F Xie, S Wu, JS Kim, NJ Jones, RJ Moore, L Pasa-Tolic, J Kagan, KD Rodland, T Liu, K Tang, DG Camp, II, RD Smith, and W Qian. 2012. "Antibody-free, targeted mass-spectrometric approach for quantification of proteins at low picogram per milliliter levels in human plasma/serum." Proceedings of the National Academy of Sciences of the United States of America 109(38):15395-15400. doi:10.1073/pnas.1204366109