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Untargeted Top-down Proteomics by LC-MS/MS on Lumos

Untargeted Top-down Proteomics by LC-MS/MS on Eclipse

Bryon Drown[1], Jeannie Camarillo[1], Neil Kelleher[1]

[1]Northwestern University

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Human BioMolecular Atlas Program (HuBMAP) Method Development Community

Kelleher Research Group

Kelleher KRG Research Group
Northwestern University, National Resource for Translational...

Describes the LC-MS/MS data acquisition procedure for top-down proteomics samples using the Thermo Scientific Orbitrap Fusion Lumos Tribrid mass spectrometer

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In steps of
Overall protocol for top-down LC-MS/MS of human heart tissue
Overall protocol for top-down LC-MS/MS of human small intestine tissue
Overall protocol for top-down LC-MS/MS of human spleen tissue

PLRP-S 5-µm particles 1,000-Å pore size (Agilent Technologies)
Water Optima LC/MS Grade (Fisher Scientific #W64)
Acetonitrile Optima LC/MS Grade (Fisher Scientific #A955-4)
Formic Acid LC/MS Grade (Thermo Scientific #28905)
15 µm SilicaTip PicoTip Emitter (New Object #FS360-50-15-N-20-C12)

Buffer A: 94.8 % water, 5 % acetonitrile, 0.2 % formic acid
Buffer B: 4.8 % water, 95 % acetonitrile, 0.2 % formic acid

1 Samples were analyzed on a Thermo Scientific Orbitrap Fusion Lumos Tribrid mass spectrometer in line with a Dionex Ultimate 3000 RSLCnano system

2 Samples (**6 µL**) were injected via the autosampler and loaded onto a self-packed trap column (150 µm i.d. x 2 cm length packed with PLRP-S 5-µm particles 1,000-Å pore size) for **00:10:00** with 100% loading buffer (94.8% water:5% acetonitrile:0.2% formic acid) 10m

3 Following a valve switch and initiation of the nanopump at 300 nL/min (buffer A: 94.8 % water, 5 % acetonitrile, 0.2 % formic acid; buffer B: 4.8 % water, 95 % acetonitrile, 0.2 % formic acid), proteins were separated on a self-packed analytical column (75 µm i.d. x 25 cm length packed with PLRP-S 5-µm particles 1,000-Å pore size) according to the following gradient for fractions 1-4:

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A	B	C
Time (min)	%B	Valve Position
0	5	10_1
10	5	1_2
13	15	
70	45	
72	95	
76	95	
80	5	
90	5	

For fraction 5 and later, nanopump used the following gradient:

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S
Time (min)	%B	Valve Position																
0	5	10_1																
10	5	1_2																
13	15																	
70	50																	
72	95																	
76	95																	
80	5																	
90	5																	

4 Eluted proteins were ionized in positive ion mode nanoelectrospray ionization (nESI) using a pulled tip nanospray emitter (15-µm i.d. ×125 mm) packed with 1mm of PLRP-S 5-µm particles 1,000-Å pore size with a custom nano-source (https://proteomicsresource.washington.edu/docs/protocols05/UWPR_NSI_Source.pdf).

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A	B
	High-High
Spray voltage	1800
Sweep gas	0
Ion transfer tube temp	320
Application mode	Intact Protein
Pressure mode	Low Pressure
Advanced Peak Determination	True
Default charge state	15
S-lens RF	30
Source fragmentation	15 eV

Global MS parameters

5 Precursor (intact protein) spectra were acquired at 120k FTRP

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A	B
	High-High
Detector type	Orbitrap
Resolving power	120000
m/z RP measured	200 m/z
Scan range	600-2000
Mass range	Normal
AGC target	1000000
Normalized AGC target	250%
Max Injection Time	100 ms
Microscans	4
Data type	Profile
Polarity	Positive
Use wide quad isolation	True

Parameters for MS1 acquisition

6 The mass spectrometer was operated using a Top2 data-dependent acquisition mode. Precursor ions were filtered by intensity, charge state, and dynamic exclusion.

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A	B
Intensity minimum	20000
Intensity maximum	1E20
Included charge states	6-60
Include undetermined charge states	False
Dynamic exclusion after n times	1
Dynamic exclusion duration	60 s
Mass tolerance	1.5 m/z
Exclude isotopes	True

Precursor selection filters for DDA

7 Ions for fragmentations were isolated and fragmented via higher energy dissociation (HCD)

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A	B
	High-High
Detector type	Orbitrap
Isolation mode	Quadrupole
Resolving power	60000
m/z RP measured	200 m/z
Scan range	350-2000
AGC target	1000000
Normalized AGC target	2000%
Max injection time	400 ms
Microscans	4
Isolation window	3 m/z
Activation type	HCD
Collision energy	27
Collision energy mode	Fixed
Polarity	Positive

Parameters for MS2 acquisition

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