Feb 28, 2022

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

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

[1]Northwestern University

dx.doi.org/10.17504/protocols.io.bttknnkw

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 Eclipse Tribrid mass spectrometer

DOI

dx.doi.org/10.17504/protocols.io.bttknnkw

Bryon Drown, Jeannie Camarillo, Rafael Melani, Neil Kelleher 2022. Untargeted Top-down Proteomics by LC-MS/MS on Eclipse. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bttknnkw

Office of the Director of the National Institutes of Health
Grant ID: UH3 CA24663

National Institute of General Medical Sciences of the National Institutes of Health
Grant ID: P41 GM108569

National Institute of Cancer of the National Institute of Cancer of the National Institutes of Health
Grant ID: F32 CA246894

protocol ,

Mar 30, 2021

Feb 28, 2022

48716

In steps of

Overall protocol for top-down LC-MS/MS of human lung 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 Eclipse 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) at 3 uL/min. 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:

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	

Citation: Bryon Drown, Jeannie Camarillo, Rafael Melani, Neil Kelleher Untargeted Top-down Proteomics byÃÂ LC-MS/MS on Eclipse https://dx.doi.org/10.17504/protocols.io.bttknnkw

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

A	B	C
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).

A	B
	High-High
Spray voltage	1600
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

Citation: Bryon Drown, Jeannie Camarillo, Rafael Melani, Neil Kelleher Untargeted Top-down Proteomics byÃÂ LC-MS/MS on Eclipse https://dx.doi.org/10.17504/protocols.io.bttknnkw

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

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	2000000
Normalized AGC target	500%
Max Injection Time	50 ms
Microscans	1
Data type	Profile
Polarity	Positive
Use wide quad isolation	True

Parameters for MS1 acquisition

6 The mass spectrometer was operated using a TopN 3 sec data-dependent acquisition mode Precursor ions were filtered by intensity, charge state, and dynamic exclusion:

Citation: Bryon Drown, Jeannie Camarillo, Rafael Melani, Neil Kelleher Untargeted Top-down Proteomics byÃÂ LC-MS/MS on Eclipse https://dx.doi.org/10.17504/protocols.io.bttknnkw

A	B
Intensity minimum	5000
Intensity maximum	1E20
Included charge states	4-60
Include undetermined charge states	False
Dynamic exclusion after n times	1
Dynamic exclusion duration	60 s
Mass tolerance	0.5 m/z
Exclude isotopes	True

Precursor selection filters for DDA

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

Citation: Bryon Drown, Jeannie Camarillo, Rafael Melani, Neil Kelleher Untargeted Top-down Proteomics byÃÂ LC-MS/MS on Eclipse https://dx.doi.org/10.17504/protocols.io.bttknnkw

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	600 ms
Microscans	1
Isolation window	3 m/z
Activation type	HCD
Collision energy	32
Collision energy mode	Fixed
Polarity	Positive

Parameters for MS2 acquisition

Citation: Bryon Drown, Jeannie Camarillo, Rafael Melani, Neil Kelleher Untargeted Top-down Proteomics byÃÂ LC-MS/MS on Eclipse https://dx.doi.org/10.17504/protocols.io.bttknnkw