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

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

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1 Works for me dx.doi.org/10.17504/protocols.io.btxdnpi6

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ABSTRACT

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

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PROTOCOL CITATION

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FORK NOTE

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MATERIALS TEXT

CE vendor: Sciex
CE model: CESI 8000 Plus
CE capillary: Neutral OptiMS™ Capillary Cartridges (30 µm ID, L = 90 cm), neutral-coated
Background Electrolyte: 3% Acetic Acid
Conductive Liquid: 3% Acetic Acid
Optima Acetic Acid (Fisher Scientific, cat. no. A11310X1AMP)
Optima Water (Fisher Scientific, cat. no. W6-4)

1 Samples were analyzed on a Thermo Scientific Orbitrap Eclipse Tribrid mass spectrometer in line with a Sciex CESI 8000

Plus

2 Samples were prepared in (**10 µl**) 0.3% acetic acid and transferred to sample vials.

3 Setup buffers in tray:

A	B
Location	**Buffer composition**
BI:A1	Background electrolyte
BI:B1	Background electrolyte
BI:C1	0.1 M HCl
BI:D1	Water
BO:A1	Conductive liquid
BO:B1	Water

Background electrolyte: 3.0% acetic acid; Conductive liquid: 3.0% acetic acid

4 Prior to first use, the CESI cartridge were conditioned. This process can be divided into two methods: 1) washing method, 2) electrical conditioning method. Each morning, a shortened version of the electrical conditioning method was performed (+15 kV for 30 min). For long term storage, capillaries were rinsed with water and kept at 4 °C.

A	B	C	D	E	F	G
Time [min]	**Event**	**Value**	**Duration**	**Inlet**	**Outlet**	**Summary**
	Rinse - Pressure	100 psi	5.00 min	BI: C1	BO: B1	forward
	Rinse - Pressure	100 psi	10.00 min	BI: A1	BO: B1	forward
	Rinse - Pressure	100 psi	5.00 min	BI: D1	BO: B1	reverse
	Rinse - Pressure	100 psi	30.0 min	BI: D1	BO: B1	forward

Washing method

A	B	C	D	E	F	G
Time [min]	**Event**	**Value**	**Duration**	**Inlet**	**Outlet**	**Summary**
	Rinse - Pressure	100 psi	3.00 min	BI: A1	BO: A1	reverse
	Rinse - Pressure	100 psi	5.00 min	BI: A1	BO: A1	forward
0.00	Separate - Voltage	15.0 kV	60.00 min	BI: B1	BO: A1	1.00 min ramp, normal polarity, both
60.00	Separate - Voltage	1.0 kV	5.00 min	BI: B1	BO: A1	5.00 min ramp, normal polarity, both
65.00	End					

Electrical conditioning method

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5 Prior each sample injection, the capillary is flushed with 0.1 M HCl, filled with new background electrolyte, and conductive liquid was replaced. Sample is injected hydrodynamically at 2.5 psi for 60 sec (estimated 20 nL injection volume). Separation is performed at 15kV with 0.5 psi supplemental pressure for 60 minutes.

Method for CESI 8000 Plus is summarized below:

A	B	C	D	E	F	G
Time [min]	**Event**	**Value**	**Duration**	**Inlet**	**Outlet**	**Summary**
	Rinse - Pressure	100 psi	5.00 min	BI: C1	BO: A1	forward
	Rinse - Pressure	100 psi	3.00 min	BI: A1	BO: A1	reverse
	Rinse - Pressure	100 psi	5.00 min	BI: A1	BO: A1	forward
	Inject - Pressure	2.5 psi	60 sec	sample vial	BO: A1	forward
	Wait		0.00 min	BI: D1	BO: A1	dipping
	Inject - Pressure	2.5 psi	10 sec	BI: B1	BO: A1	forward
0.00	Separation - Voltage	15 kV 0.5 psi	60.00 min	BI: B1	BO: A1	1.0 min ramp, normal polarity, both
1.00	Relay On					
60.00	Separation - Voltage	1.0 kV 5 psi	5.00 min	BI: B1	BO: A1	5.0 min ramp, normal polarity, both
65.00	End					

CESI 8000 method

6 Eluted proteins were ionized in positive ion mode nanoelectrospray ionization (nESI) using a sheathless emitter.

A	B
	High-High
Spray voltage	1400 - 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

7 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	450-2000
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

8 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:

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	45 s
Mass tolerance	0.5 m/z
Exclude isotopes	True

Precursor selection filters for DDA

9 Ions for fragmentation 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	400000
Normalized AGC target	800%
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

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