

Oct 20, 2023

Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) Acquisition by Data-Independent Acquisition (DIA) on an Orbitrap Eclipse Tribrid Mass Spectrometer

DOI

dx.doi.org/10.17504/protocols.io.36wgq3m15lk5/v1

J P Rose¹, M A Watson¹, B Schilling¹, Joanna Bons¹

¹Buck Institute for Research on Aging

Metabolomics Protocols & Workflows
Tech. support email: bbmisraccb@gmail.com



M A Watson

Buck Institute

Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

Create free account

OPEN  ACCESS



DOI: <https://dx.doi.org/10.17504/protocols.io.36wgq3m15lk5/v1>

Protocol Citation: J P Rose, M A Watson, B Schilling, Joanna Bons 2023. Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) Acquisition by Data-Independent Acquisition (DIA) on an Orbitrap Eclipse Tribrid Mass Spectrometer. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.36wgq3m15lk5/v1>

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working

We use this protocol and it's working

Created: October 20, 2023

Last Modified: October 20, 2023

Protocol Integer ID: 89690

Keywords: LC-MS/MS, DIA, Orbitrap Eclipse, Proteomics, Mass Spectrometry, orbitrap eclipse tribrid mass spectrometer proteolytic peptide measurement, proteolytic peptide measurement, tandem mass spectrometry, orbitrap eclipse tribrid mass spectrometer, using liquid chromatography, liquid chromatography, protein identification, protein, acquisition by data

Abstract

Proteolytic peptide measurement using Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) Acquisition by Data-Independent Acquisition (DIA) on an Orbitrap Eclipse Tribrid Mass Spectrometer for peptide/protein identification and quantification.

Materials

- Dionex UltiMate 3000 liquid chromatographic system (Thermo Fisher Scientific)
- Orbitrap Eclipse Tribrid mass spectrometer (Thermo Fisher Scientific)
- Acclaim PepMap 100 C₁₈ trap column (75 µm x 2 cm, 3 µm particle size) (Thermo Fisher Scientific, PN 164535)
- Acclaim PepMap 100 C₁₈ analytical column (75 µm x 50 cm, 3 µm particle size; Thermo Fisher Scientific, PN 164570)
- Autosampler vials and caps
- LC-MS-grade water
- Indexed retention time peptides (iRT, Biognosys)
- Solvent A: 2% acetonitrile (ACN), 0.1% formic acid (FA) in water
- Solvent B: 98% acetonitrile (ACN), 0.1% formic acid (FA) in water

Troubleshooting

- 1 Dilute the peptide sample with solvent A (2% ACN, 0.1% FA) to the desired concentration. Transfer the samples to an autosampler vial and add iRT peptides to the sample according to the manufacturer's instructions.
- 2 Inject the sample onto a Dionex UltiMate 3000 liquid chromatographic system coupled to an Orbitrap Eclipse Tribrid mass spectrometer and load the peptides on a C₁₈ trap column over 10 min at 5 µL/min with 100% solvent A.
- 3 Elute the peptides with a C₁₈ analytical column heated at 35°C and at a flow rate of 300 nL/min over 215 min using the following gradient of solvent B:
 - 3.1 2% for 10 min
 - 3.2 From 2% to 20% in 125 min
 - 3.3 From 20% to 32% in 40 min
 - 3.4 From 32% to 80% in 1 min
 - 3.5 80% for 9 min
 - 3.6 From 80% to 2% in 1 min
 - 3.7 2% for 29 min.
- 4 Acquire the data in data-independent acquisition (DIA) mode using the settings described in **Table 1**. One cycle consists of one full MS scan collected in the Orbitrap analyzer followed by DIA-MS/MS scans collected in the Orbitrap analyzer based on an isolation scheme of 26 variable windows covering the 350 - 1,650 m/z range with an overlap of 1 m/z (**Table 2**).

Ion Source Properties	
Spray Voltage (Positive Ion)	2100 V
Sweep Gas	0
Ion Transfer Tube Temperature	300°C
MS Scan Properties	
Detector Type	Orbitrap
Orbitrap Resolution	120,000
Scan Range	350-1,650 m/z
AGC Target	Custom
Normalized AGC Target	750% (<i>i.e.</i> , 3e6 ions)
Maximum Injection Time Mode	Custom
Maximum Injection Time	60 ms
Microscans	1
Data Type	Profile
Polarity	Positive
Targeted MS ⁿ Properties	
MS ⁿ Level	2
Isolation Mode	Quadrupole
Isolation Window	1.6 m/z
Activation Type	HCD
Collision Energy Mode	Fixed
HCD Collision Energy	27%
Detector Type	Orbitrap
Orbitrap Resolution	30,000
Scan Range Mode	Define First Mass
First Mass	200 m/z
AGC Target	Custom
Normalized AGC Target	6000% (<i>i.e.</i> 3e6 ions)
Maximum Injection Time Mode	Auto
Maximum Injection Time	60 ms
Microscans	1
Data Type	Profile
Polarity	Positive
Loop Control	N
N (Number of Spectra)	26
Dynamic Retention Time	Off
Time Mode	Start/End Time

Table 1. Settings for data-independent acquisition on an Orbitrap Eclipse Tribrid mass spectrometer.

Window	Start m/z	Stop m/z	Center m/z	z	Isolation Width
1	350	383	366.5	3	33
2	382	408	395	3	26
3	407	429	418	3	22
4	428	448	438	3	20
5	447	467	457	3	20
6	466	484	475	3	18
7	483	503	493	3	20
8	502	521	511.5	3	19
9	520	539	529.5	3	19
10	538	557	547.5	3	19
11	556	575	565.5	3	19
12	574	594	584	3	20
13	593	614	603.5	3	21
14	613	634	623.5	3	21
15	633	656	644.5	3	23
16	655	678	666.5	3	23
17	677	701	689	3	24
18	700	726	713	3	26
19	725	756	740.5	3	31
20	755	787	771	3	32
21	786	823	804.5	3	37
22	822	862	842	3	40
23	861	914	887.5	3	53
24	913	979	946	3	66
25	978	1077	1027.5	3	99
26	1076	1650	1363	3	574

Table 2. Isolation scheme of the data-independent acquisition method.