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

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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.

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Protocol status: Working
We use this protocol and it's working

MATERIALS

- Dionex UltiMate 3000 liquid chromatographic system (Thermo Fisher Scientific)
- Orbitrap Eclipse Tribrid mass spectrometer (Thermo Fisher Scientific)
- Acclaim PepMap 100 C_{18} trap column (75 µm x 2 cm, 3 µm particle size) (Thermo Fisher Scientific, PN 164535)
- Acclaim PepMap 100 C_{18} 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

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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 C_{18} trap column over 10 min at 5 µL/min with 100% solvent A.

3 Elute the peptides with a C_{18} 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.e.*, 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.e.* 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.