

Supplementary Figure 1

A

Properties of Full MS / dd-MS² (TopN)

General	
Runtime	0 to 76 min
Polarity	positive
In-source CID	0.0 eV
Default charge state	2
Inclusion	—
Exclusion	—
Tags	—
Full MS	
Microscans	1
Resolution	70,000
AGC target	1e6
Maximum IT	120 ms
Number of scan ranges	1
Scan range	300 to 1750 m/z
dd-MS² / dd-SIM	
Microscans	1
Resolution	17,500
AGC target	5e5
Maximum IT	60 ms
Loop count	12
MSX count	1
TopN	12
Isolation window	1.2 m/z
Fixed first mass	100.0 m/z
NCE	25.0
Stepped NCE	—
dd Settings	
Underfill ratio	1.3 %
Intensity threshold	1.0e5
Apex trigger	—
Charge exclusion	unassigned, 1, >8
Peptide match	—
Exclude isotopes	on
Dynamic exclusion	30.0 s

B

Properties of Full MS / dd-MS² (TopN)

General	
Runtime	0 to 76 min
Polarity	positive
In-source CID	0.0 eV
Default charge state	2
Inclusion	—
Exclusion	—
Tags	—
Full MS	
Microscans	1
Resolution	70,000
AGC target	1e6
Maximum IT	120 ms
Number of scan ranges	1
Scan range	300 to 1750 m/z
dd-MS² / dd-SIM	
Microscans	1
Resolution	35,000
AGC target	5e5
Maximum IT	120 ms
Loop count	12
MSX count	1
TopN	12
Isolation window	1.2 m/z
Fixed first mass	100.0 m/z
NCE	25.0
Stepped NCE	—
dd Settings	
Underfill ratio	2.5 %
Intensity threshold	1.0e5
Apex trigger	—
Charge exclusion	unassigned, 1, >8
Peptide match	—
Exclude isotopes	on
Dynamic exclusion	30.0 s

Supplementary Figure 1: Overview of the settings used in the (A) 'fast' and (B) 'sensitive' method. Note the difference in underfill ratio is set to keep the intensity threshold for peak picking to be constant.