Experiment Method parameter Value

Experiment 1 Cycle time (s) 2

MS OT

Detector type Orbitrap

Orbitrap resolution 30,000

Mass range Normal

Use quadrupole isolation True

Scan range (m/z) 350–1,100

RF lens (%) 30

AGC target 1.0 × 106

Maximum injection time (ms) 100

Microscans 1

Data type Profile

Polarity Positive

Source fragmentation Disabled

Use EASY-IC False

Monoisotopic precursor selection (MIPS) filter

Monoisotopic peak determination Peptide

Relax restrictions when too few precursors are found True

Exclude undetermined charge states True

Charge state filter

Include charge state(s) 2–6

Include undetermined charge states False

Include charge states 25 and higher False

Dynamic exclusion filter

Exclude after n times 1

Exclusion duration (s) 5

Mass tolerance m/z

Low 0.55

High 1.55

Exclude isotopes False

Perform dependent scan on single charge state per precursor only False

Decisions

Data-dependent mode Top speed

Precursor priority Most intense

Number of scan event types 1

Scan event type 1 ddMS2 IT HCD

(continued)

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Table 2 | Orbitrap Fusion and Fusion Lumos method settings with 500,000 MS1 resolving power (continued).

Experiment Method parameter Value

MSn level 2

Isolation mode Quadrupole

Use isolation m/z offset False

Activation type HCD

HCD collision energy (%) 30

Stepped collision energy False

Detector type Ion trap

Scan range mode Define m/z range

Ion trap scan rate Turbo

Scan range (m/z) 200–1,200

AGC target 1.0 × 104

Injection ions for all available parallelizable time False

Maximum injection time (ms) 15

Microscans 1

Data type Centroid

Experiment 2 Cycle time (s) 3

MS OT

Detector type Orbitrap

Orbitrap resolution 500000

Mass range Normal

Use quadrupole isolation True

Scan range (m/z) 350–1,100

RT lens (%) 30

AGC target 1.0 × 106

Maximum injection time (ms) 100

Microscans 1

Data type Profile

Polarity Positive

Source fragmentation Disabled

Use EASY-IC False