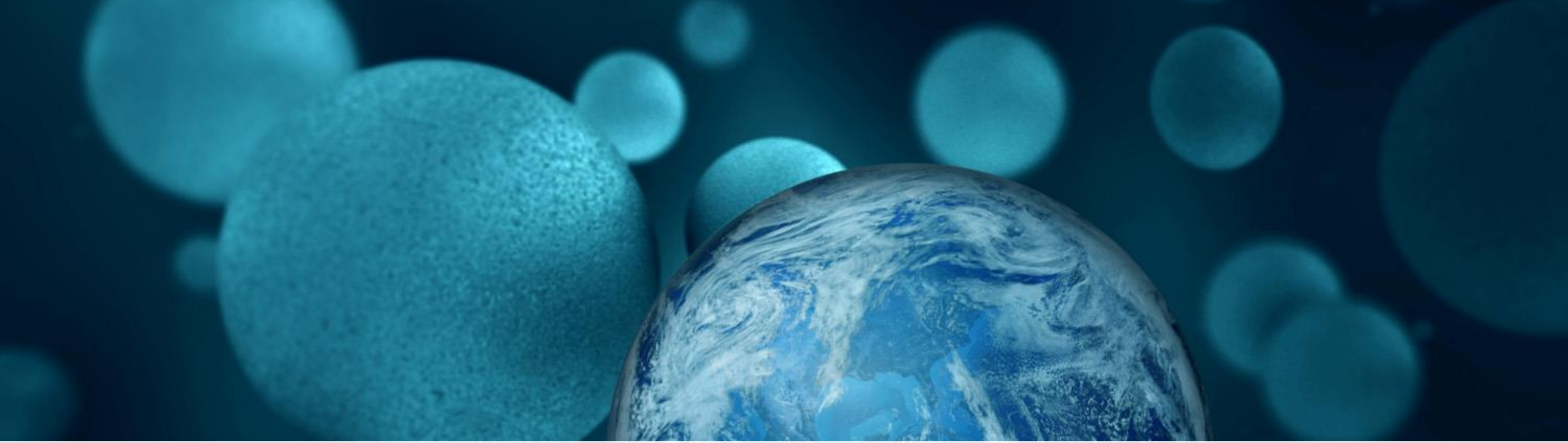


ThermoFisher SCIENTIFIC

TMT/TMTpro Instrument Acquisition

LC Method: EASY-Spray™ C18 50cm column & UltiMate™ 3000 RSLCnano UHPLC

120 min gradient

No	Time	Flow [µl/min]	%B	Curve
1	0.000	Equilibration		
2	0.000	0.300	2.0	5
3	*New Row*			
4	0.000	Run		
5	14.000	0.300	2.0	5
6	17.000	0.300	4.0	5
7	100.000	0.300	16.0	5
8	145.000	0.300	25.0	5
9	150.000	0.300	85.0	5
10	158.000	0.300	85.0	5
11	160.000	0.300	4.0	5
12	*New Row*			
13	185.000	Stop Run		

Solvent A: 0.1% formic acid

Solvent B: 100 % Acetonitrile, 0,1% formic acid

Flow rate: 300 nL/min

Injection volume: 1-2 µL

Direct injection

ThermoFisher SCIENTIFIC

LC Method: EASY-Spray™ C18 50cm column & UltiMate™ 3000 RSLCnano UHPLC

Loading pump conditions

Time(min)	Flow, ul/min
0	20
100, 185	20

120 min gradient

No	Time	Flow [µl/min]	%B	Curve
1	0.000	Equilibration		
2	0.000	0.300	2.0	5
3	*New Row*			
4	0.000	Run		
5	14.000	0.300	2.0	5
6	17.000	0.300	4.0	5
7	100.000	0.300	16.0	5
8	145.000	0.300	25.0	5
9	150.000	0.300	85.0	5
10	158.000	0.300	85.0	5
11	160.000	0.300	4.0	5
12	*New Row*			
13	185.000	Stop Run		

Solvent A: 0.1% formic acid

Solvent B: 100 % Acetonitrile, 0,1% formic acid

Flow rate: 300 nL/min

Injection volume: 1-2 µL

Trap loading

ThermoFisher SCIENTIFIC

LC Method using EASY-Spray™ C18 50cm column & EASY-nLC™ 1200 HPLC

120 min

Time (min)	Flow(nL/min)	%B
0	300	5
110	300	25
120	300	40
130	300	95
140	300	95

Solvent A:	0.1% formic acid
Solvent B:	80 % Acetonitrile, 0,1% formic acid
Flow rate:	300 nL/min
Injection volume:	1-2 µL

ThermoFisher SCIENTIFIC

LC Method using EASY-Spray™ C18 50cm column & EASY-nLC™ 1000 HPLC

120 min

Time (min)	Flow(nL/min)	%B
0	300	5
5	300	7
120	300	32
130	300	90
140	300	90

Solvent A:	0.1% formic acid
Solvent B:	100 % Acetonitrile, 0,1% formic acid
Flow rate:	300 nL/min
Injection volume:	1 µL

ThermoFisher SCIENTIFIC

Instrument Method Settings: QExactive Classic and QExactive Plus

Properties	QE classic 120 min TMT 11 plex	QE classic 120 min TMTpro 16 plex	QE + 120 min TMT 11 plex	QE + 120 min TMTpro 16 plex
Resolution Full MS	70000	70000	70000	70000
AGC target Full MS	3e6	3e6	3e6	3e6
MS max IT, ms	50	50	50	50
Scan range, *m/z*	350-1500	350-1500	350-1500	350-1500
Loop count	15	10	15	10
MS2 resolution	35000	35000	35000	35000
MS2 AGC target	1e5	**2e5**	1e5	**2e5**
MS2 max IT, ms	250 ms	250 ms	120 ms	120 ms
Isolation Window , Th	1.2	1.2	0.7	0.7
NCE, %	32-34	**28-32**	32-34	**28-32**
Intensity threshold	1e4	**2e4**	1e4	**2e4**
Peptide match	preferred	preferred	preferred	preferred
Dynamic exclusion, s	45 s	45 s	30 s	30 s
First mass, *m/z*	110	110	110	110

ThermoFisher SCIENTIFIC

Instrument Method Settings: QExactive HF and QExactive HF X

Properties	QE HF 120 min TMT 11 plex	QE HF 120 min TMTpro 16 plex	QE HF X 120 min TMT 11 plex	QE HF X 120 min TMTpro 16 plex
Resolution Full MS	120000	120000	120000	120000
AGC target Full MS	3e6	3e6	3e6	3e6
MS max IT, ms	50	50	50	50
Scan range, m/z	350-1500	350-1500	350-1500	350-1500
Loop count	15	15	15	15
MS2 resolution	60000(2.9- 45000)	60000(2.9- 45000)	45000	45000
MS2 target	1e5	**1.2e5**	1e5	**1.2e5**
MS2 max IT, ms	120	120	96	**120**
Isolation Window, Th	0.7 m/z	0.7 m/z	0.7 m/z	0.7 m/z
NCE, %	32-34	**28-32**	32-34	**28-32**
Intensity threshold	1e4	**1.5e4**	1e4	1e4
Peptide match	preferred	preferred	preferred , single charge	preferred , single charge
Dynamic exclusion, s	30	30	30	30
First mass, m/z	110	110	110	110

Tune 2.10 and higher

ThermoFisher SCIENTIFIC

Instrument Method Settings: Fusion (Tune 3.3)

Properties	Fusion SPS 120 min TMT 11 plex	Fusion SPS 120 min TMTpro 16 plex	Fusion MS2 120 min TMT 11 plex	Fusion MS2 120 min TMT pro 16 plex
Resolution Full MS	120000	120000	120000	120000
AGC target Full MS	4e5	4e5	4e5	4e5
MS max IT, ms	50	50	50	50
Scan range, m/z	400-1400	400-1400	400-1400	400-1400
Top Speed, s	3	3	3	3
MS2 max IT, ms	50	50	120	120
MS2 Isolation window, Th	1.2(2)-0.7(3)-0.5 (4+)	1.2(2)-0.7(3)-0.5 (4+)	0.7(2-3)-0.5 (4+)	**0.7**
MS2 NCE, %	35	35	38-40	**35**
MS2 Intensity threshold	5e3	5e3	5e4	5e4
Dynamic exclusion, s	60, single charge	60, single charge	60, single charge	60, single charge
MS2 Resolution	turbo	**rapid**	50000	50000
MS2 AGC target	1e4	1e4	1e5	**1.2e5**
MS3 AGC target	1e5	**1.2e5**		
SPS Isolation window, Th	1.3(2)-0.7(3)-0.5 (4+)	1.3(2)-0.7(3)-0.5 (4+)		
SPS NCE, %	65	**55**		
SPS max IT, ms	120	120		
SPS settings: # notches, mass range, Tag Exclusion	5-10-10, *m/z* 110-500, TMT	5-10-10, *m/z* 110-500, **TMTpro**	*m/z* 110	*m/z* 110

Fit Filter: 70+

APD on

ThermoFisher SCIENTIFIC

Instrument Method Settings: Lumos (Tune 3.3)

Properties	SPS 120 min TMT 11plex	SPS 120 min TMT pro 16 plex	MS2 120 min TMT 11plex	MS2 120 min TMTpro 16 plex
Resolution Full MS	120000	120000	120000	120000
AGC target Full MS	100%(4e5)	100%(4e5)	100%(4e5)	100%(4e5)
MS max IT, ms	50	50	50	50
Scan range, m/z	400-1400	400-1400	400-1400	400-1400
Top Speed, s	3	3	3	3
MS2 max IT, ms	50	50 (custom)	105	120
MS2 Isolation window, Th	1.2(2)-0.7(3)-0.5 (4+)	0.7	0.7(2-3)-0.5 (4+)	0.7
MS2 NCE, %	35	30	38-40	35
MS2 Intensity threshold	5e3	5e3	5e4	5e4
Dynamic exclusion, s	60, single charge	60, single charge	60, single charge	60, single charge
MS2 Resolution	turbo	rapid	50000	50000
MS2 AGC target	100%(1e4)	100%(1e4)	200%(1e5)	200%(1e5)
MS3 AGC target	200%(1e5)	200%(1e5)		
SPS Isolation window, Th	1.3(2)-0.7(3)-0.5 (4+)	0.7		
SPS NCE, %	65	55		
SPS max IT, ms	105 (custom)	120 (custom)		
SPS settings: # notches, mass range, Tag Exclusion	5-10-10 *m/z* 110-500, TMT	10 *m/z* 110-500, TMTpro	*m/z* 110	*m/z* 110

Fit Filter: 70+

APD on

FAIMS: CV-50/-70 1.5 sec/CV

ThermoFisher SCIENTIFIC

Instrument Method Settings: Eclipse (Tune 3.3)

Properties	SPS 120 min TMT 11plex	SPS 120 min TMT pro 16 plex	MS2 120 min TMT 11plex	MS2 120 min TMTpro 16 plex
Resolution Full MS	120000	120000	120000	120000
AGC target Full MS	100%(4e5)	100%(4e5)	100%(4e5)	100%(4e5)
MS max IT, ms	50	50	50	50
Scan range, m/z	400-1400	400-1400	400-1400	400-1400
Top Speed, s	3	3	3	3
MS2 max IT, ms	50	50 (custom)	105	120
MS2 Isolation window, Th	1.2(2)-0.7(3)-0.5 (4+)	0.7	0.7(2-3)-0.5 (4+)	0.7
MS2 NCE, %	35	30	38-40	35
MS2 Intensity threshold	5e3	5e3	5e4	5e4
Dynamic exclusion, s	60, single charge	60, single charge	60, single charge	60, single charge
MS2 Resolution	turbo	rapid	50000	50000
MS2 AGC target	100%(1e4)	100%(1e4)	200%(1e5)	200%(1e5)
MS3 AGC target	200%(1e5)	200%(1e5)		
SPS Isolation window, Th	1.3(2)-0.7(3)-0.5 (4+)	0.7		
SPS NCE, %	65	55		
SPS max IT, ms	105 (custom)	120 (custom)		
SPS settings: # notches, mass range, Tag Exclusion	5-10-10 *m/z* 110-500, TMT	10 *m/z* 110-500, TMTpro	*m/z* 110	*m/z* 110

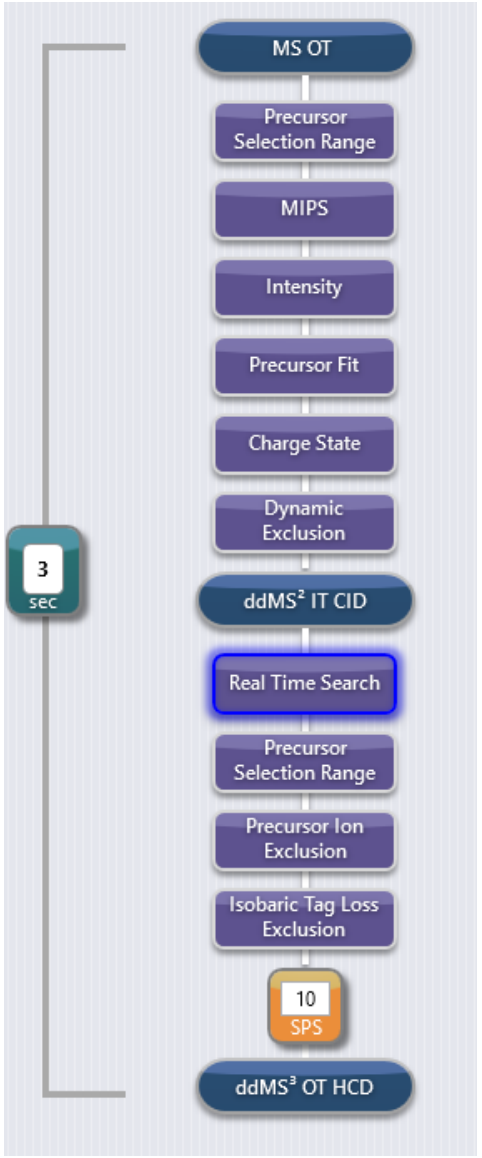
Fit Filter: 70+

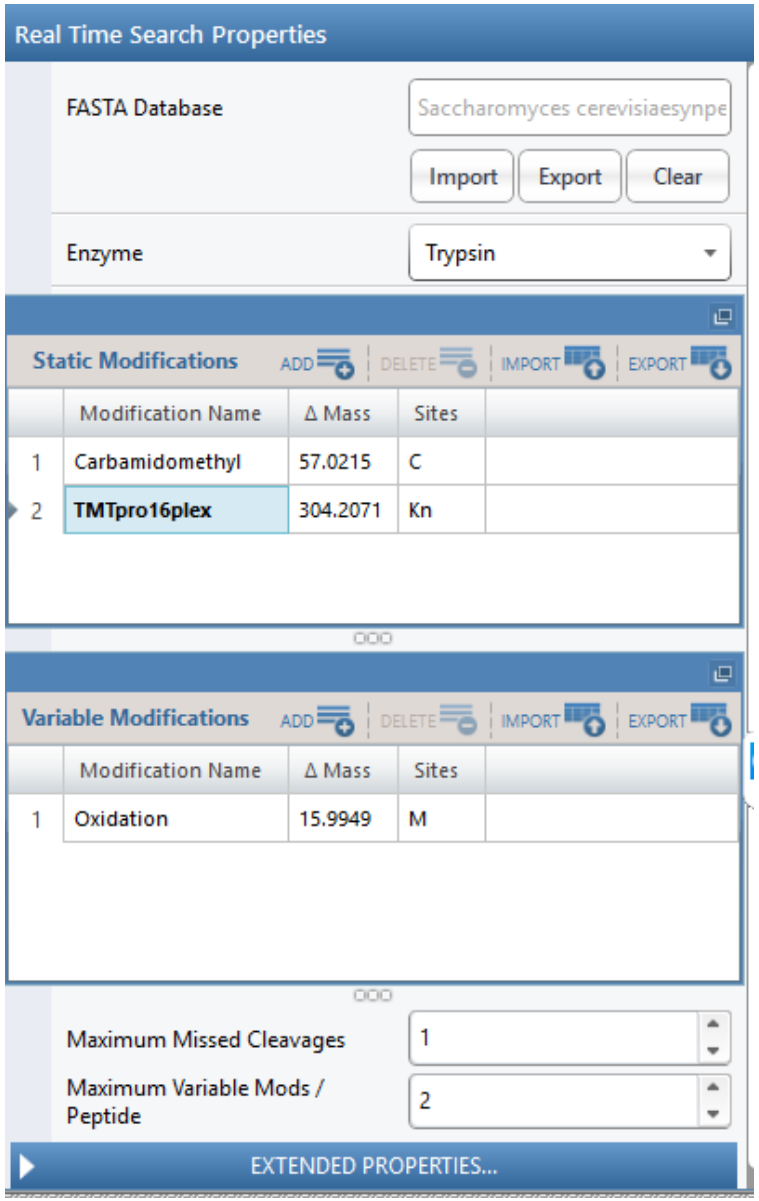
APD on

FAIMS: CV-50/-70 1.5 sec/CV

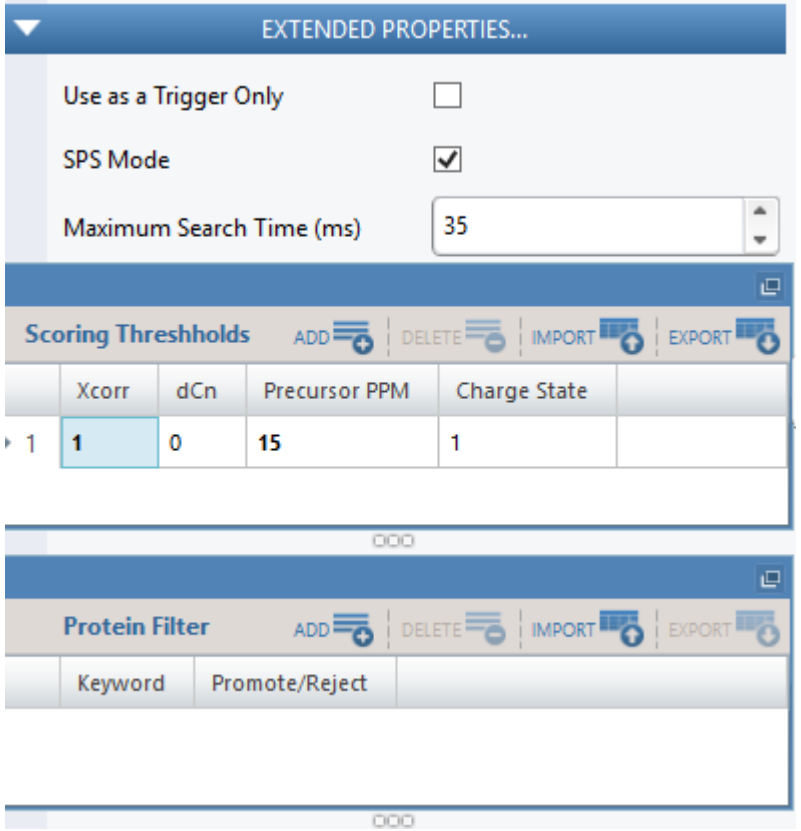
ThermoFisher SCIENTIFIC

Instrument Method Settings: Eclipse (Tune 3.3)





with RTS



ThermoFisher SCIENTIFIC

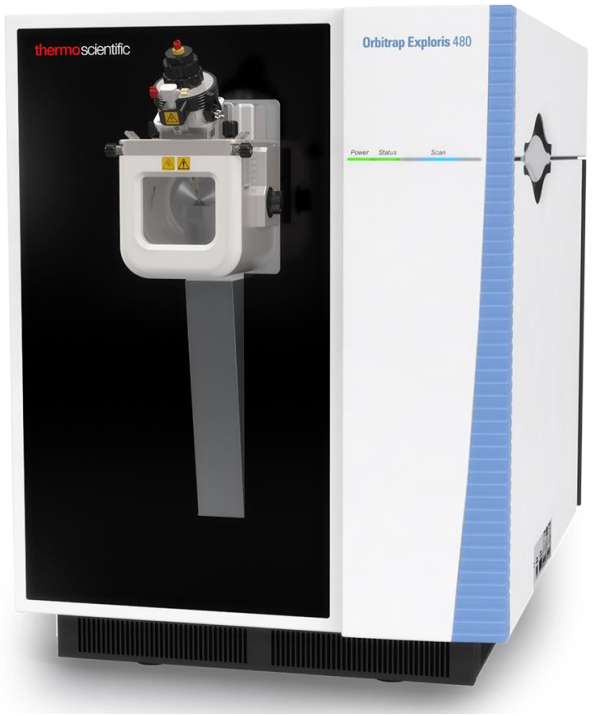
Instrument Method Settings: Exploris 480 (Tune 1.1)

Properties	MS2 120 min TMT 11plex	MS2 120 min TMTpro 16 plex
Resolution Full MS	120000	120000
AGC target Full MS	100%(4e5)	100%(4e5)
MS max IT, ms	50	50
Scan range, m/z	400-1400	400-1400
Top Speed, s	3	3
MS2 max IT, ms	105	120
MS2 Isolation window, Th	0.7(2-3)-0.5 (4+)	0.7
MS2 NCE, %	38-40	32
MS2 Intensity threshold	5e4	5e4
Dynamic exclusion, s	60, single charge	60, single charge
MS2 Resolution	50000	50000
MS2 AGC target	200%(1e5)	200%(1e5)
First mass	*m/z* 110	*m/z* 110
SPS Isolation window, Th		
SPS NCE, %		
SPS max IT, ms		

Fit Filter:
70+

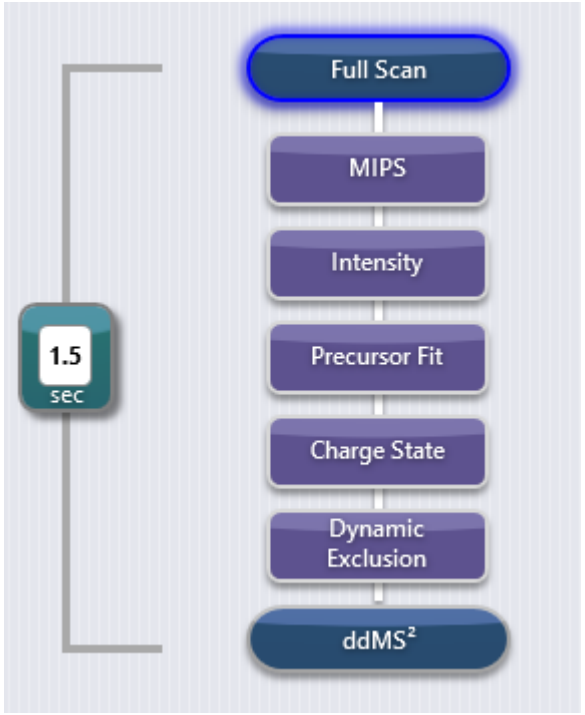
APD on

FAIMS:
CV-50/-70
1.5 sec/CV



ThermoFisher SCIENTIFIC

Instrument Method Settings: Exploris 480 (Tune 1.1)



Full Scan Properties

Orbitrap Resolution	60000
Scan Range (m/z)	375-1575
FAIMS Voltages	On
FAIMS CV (V)	-50
RF Lens (%)	40
AGC Target	Custom
Normalized AGC Target (%)	300
Maximum Injection Time Mode	Custom
Maximum Injection Time (ms)	50
Microscans	1
Data Type	Profile
Polarity	Positive
Source Fragmentation	☐
Scan Description	-50 CV

ThermoFisher SCIENTIFIC