method name	used for which samples	pages
HCD_105min_T20_grad3_TB	QuaNPA MAA bead and protein input optimizations	19-22
HCD_2H5M_T20_HI	DDA method for Hela SILAC benchmark	15-18
HCD_105min_DIA_26iw_TB	DIA method 1 for Hela SILAC benchmark	2-5
HCD_105min_DIA_3xhr_MS1_27iw_MS2_TB	DIA method 2 for Hela SILAC benchmark	6-10
HCD_90min_grad3_DIA_28iw_TB	DIA method for IFNg time course NSP samples	11-14

Thermo EASY-LC method print for QExactive_HF

Sample pickup:

Volume [µl]	: 1.00
Flow [µl / min]	: 20.00

Sample loading:

Volume [µl]	: 22.00
Flow [µl / min]	: (unspecified)
Max. pressure [Bar]	: 800.00

Gradient:

Time [mm:ss]	Duration [mm:ss]	Flow [nl/min]	Mixture [%B]
00:00	00:00	300.00	3.00
04:00	04:00	300.00	8.00
06:00	02:00	300.00	10.00
74:00	68:00	300.00	32.00
86:00	12:00	300.00	50.00
87:00	01:00	300.00	100.00
94:00	07:00	300.00	100.00
95:00	01:00	300.00	3.00
105:00	10:00	300.00	3.00

Pre-column equilibration:

Volume [µl]	: 12.00
Flow [µl / min]	: (unspecified)
Max. pressure [Bar]	: 800.00

Analytical column equilibration:

Volume [µl]	: 3.50
Flow [µl / min]	: (unspecified)
Max. pressure [Bar]	: 600.00

Autosampler wash:

Flush volume [µl]	: 100.00

Method of Q Exactive HF

Overall method settings

Global Settings		
Use lock masses	best	
Lock mass injection	–	
Chrom. peak width (FWHM)	20	s
Time		
Method duration	105.00	min
Customized Tolerances (+/-)		
Lock Masses	–	
Inclusion	–	
Exclusion	–	
Neutral Loss	–	
Mass Tags	–	
Dynamic Exclusion	–	

Experiments

Full MS – SIM

General		
Runtime	0 to 105	min
Polarity	positive	
In-source CID	0.0	eV
Full MS – SIM		
Microscans	1	
Resolution	60,000	
AGC target	3e6	
Maximum IT	20	ms
Number of scan ranges	1	
Scan range	400 to 1000	m/z
Spectrum data type	Profile	

DIA

General		
Runtime	0 to 105	min
Polarity	positive	
In-source CID	0.0	eV
Default charge state	2	
DIA		
Microscans	1	
Resolution	30,000	
AGC target	1e6	
Maximum IT	50	ms
Loop count	26	
MSX count	1	
MSX isochronous ITs	on	
Isolation window	23.3	m/z

Isolation offset 0.0 m/z
Fixed first mass 200.0 m/z
(N)CE / stepped (N)CE nce: 27
Spectrum data type Profile

Setup

Tunefiles
General
Switch Count 0
Base Tunefile C:\Xcalibur\methods\nanosource.mstune

Contact Closure
General
Used False
Start in Closed True
Switch Count 0

Syringe
General
Used False
Start in OFF True
Stop at end of run False
Switch Count 0
Pump setup
Syringe type Hamilton
Flow rate 3.000 µL/min
Inner diameter 2.303 mm
Volume 250 µL

Divert Valve A
General
Used False
Start in 1-2 True
Switch Count 0

Divert Valve B
General
Used False
Start in 1-2 True
Switch Count 0

Lock Masses
1 entry

Mass [m/z]	Polarity	Start [min]	End [min]	Comment
445.12003	Positive			

Inclusion List
26 entries

Mass [m/z]	Formula [M]	Species	CS [z]	Polarity	Start [min]	End [min]	(N)CE	MSX ID	Comment
413.65000				Positive					
435.95000				Positive					
458.25000				Positive					
480.55000				Positive					
502.85000				Positive					
525.15000				Positive					
547.45000				Positive					
569.75000				Positive					
592.05000				Positive					
614.35000				Positive					
636.65000				Positive					
658.95000				Positive					
681.25000				Positive					
703.55000				Positive					
725.85000				Positive					
748.15000				Positive					
770.45000				Positive					
792.75000				Positive					
815.05000				Positive					
837.35000				Positive					
859.65000				Positive					
881.95000				Positive					
904.25000				Positive					
926.55000				Positive					
948.85000				Positive					
971.15000				Positive					

Thermo EASY-LC method print for QExactive_HF

Sample pickup:

Volume [µl] : 1.00
Flow [µl / min] : 20.00

Sample loading:

Volume [µl] : 22.00
Flow [µl / min] : (unspecified)
Max. pressure [Bar] : 800.00

Gradient:

Time [mm:ss]	Duration [mm:ss]	Flow [nl/min]	Mixture [%B]
00:00	00:00	300.00	3.00
04:00	04:00	300.00	8.00
06:00	02:00	300.00	10.00
74:00	68:00	300.00	32.00
86:00	12:00	300.00	50.00
87:00	01:00	300.00	100.00
94:00	07:00	300.00	100.00
95:00	01:00	300.00	3.00
105:00	10:00	300.00	3.00

Pre-column equilibration:

Volume [µl] : 12.00
Flow [µl / min] : (unspecified)
Max. pressure [Bar] : 800.00

Analytical column equilibration:

Volume [µl] : 3.50
Flow [µl / min] : (unspecified)
Max. pressure [Bar] : 600.00

Autosampler wash:

Flush volume [µl] : 100.00

Method of Q Exactive HF

Overall method settings

Global Settings		
Use lock masses	best	
Lock mass injection	–	
Chrom. peak width (FWHM)	20	s
Time		
Method duration	105.00	min
Customized Tolerances (+/-)		
Lock Masses	–	
Inclusion	–	
Exclusion	–	
Neutral Loss	–	
Mass Tags	–	
Dynamic Exclusion	–	

Experiments

Full MS – SIM

General		
Runtime	0 to 105	min
Polarity	positive	
In-source CID	0.0	eV
Full MS – SIM		
Microscans	1	
Resolution	120,000	
AGC target	3e6	
Maximum IT	20	ms
Number of scan ranges	1	
Scan range	400 to 1000	m/z
Spectrum data type	Profile	

DIA

General		
Runtime	0 to 105	min
Polarity	positive	
In-source CID	0.0	eV
Default charge state	2	
DIA		
Microscans	1	
Resolution	30,000	
AGC target	1e6	
Maximum IT	50	ms
Loop count	9	
MSX count	1	
MSX isochronous ITs	on	
Isolation window	23.2	m/z

Isolation offset	0.0 m/z
Fixed first mass	200.0 m/z
(N)CE / stepped (N)CE	nce: 27
Spectrum data type	Profile

Full MS — SIM

General

Runtime	0 to 105 min
Polarity	positive
In-source CID	0.0 eV

Full MS — SIM

Microscans	1
Resolution	120,000
AGC target	3e6
Maximum IT	20 ms
Number of scan ranges	1
Scan range	400 to 1000 m/z
Spectrum data type	Profile

DIA

General

Runtime	0 to 105 min
Polarity	positive
In-source CID	0.0 eV
Default charge state	2

DIA

Microscans	1
Resolution	30,000
AGC target	1e6
Maximum IT	50 ms
Loop count	9
MSX count	1
MSX isochronous ITs	on
Isolation window	23.2 m/z
Isolation offset	0.0 m/z
Fixed first mass	200.0 m/z
(N)CE / stepped (N)CE	nce: 27
Spectrum data type	Profile

Full MS — SIM

General

Runtime	0 to 105 min
Polarity	positive
In-source CID	0.0 eV

Full MS — SIM

Microscans	1
Resolution	120,000
AGC target	3e6
Maximum IT	20 ms
Number of scan ranges	1

Scan range	400 to 1000 m/z
Spectrum data type	Profile

DIA
General

Runtime	0 to 105 min
Polarity	positive
In-source CID	0.0 eV
Default charge state	2

DIA

Microscans	1
Resolution	30,000
AGC target	1e6
Maximum IT	50 ms
Loop count	9
MSX count	1
MSX isochronous ITs	on
Isolation window	23.2 m/z
Isolation offset	0.0 m/z
Fixed first mass	200.0 m/z
(N)CE / stepped (N)CE	nce: 27
Spectrum data type	Profile

Setup

Tunefiles
General

Switch Count	0
Base Tunefile	C:\Xcalibur\methods\nanosource.mstune

Contact Closure
General

Used	False
Start in Closed	True
Switch Count	0

Syringe
General

Used	False
Start in OFF	True
Stop at end of run	False
Switch Count	0

Pump setup

Syringe type	Hamilton
Flow rate	3.000 µL/min
Inner diameter	2.303 mm
Volume	250 µL

Divert Valve A
General

Used False
Start in 1-2 True
Switch Count 0

Divert Valve B
General
Used False
Start in 1-2 True
Switch Count 0

Lock Masses
1 entry

Mass [m/z]	Polarity	Start [min]	End [min]	Comment
445.12003	Positive			

Inclusion List
27 entries

Mass [m/z]	Formula [M]	Species	CS [z]	Polarity	Start [min]	End [min]	(N)CE	MSX	ID	Comment
411.00000				Positive						
432.60000				Positive						
454.80000				Positive						
477.00000				Positive						
499.20000				Positive						
521.40000				Positive						
543.60000				Positive						
565.80000				Positive						
588.00000				Positive						
610.20000				Positive						
632.40000				Positive						
654.60000				Positive						
676.80000				Positive						
699.00000				Positive						
721.20000				Positive						
743.40000				Positive						
765.60000				Positive						
787.80000				Positive						
810.00000				Positive						
832.20000				Positive						
854.40000				Positive						
876.60000				Positive						
898.80000				Positive						
921.00000				Positive						
943.20000				Positive						
965.40000				Positive						
987.60000				Positive						

Thermo EASY-LC method print for QExactive_HF

Sample pickup:

Volume [µl] : 1.00
Flow [µl / min] : 20.00

Sample loading:

Volume [µl] : 22.00
Flow [µl / min] : (unspecified)
Max. pressure [Bar] : 800.00

Gradient:

Time [mm:ss]	Duration [mm:ss]	Flow [nl/min]	Mixture [%B]
00:00	00:00	300.00	5.00
01:00	01:00	300.00	6.00
51:00	50:00	300.00	27.00
70:00	19:00	300.00	44.00
70:10	00:10	300.00	95.00
80:00	09:50	300.00	95.00
81:00	01:00	300.00	5.00
81:10	00:10	300.00	5.00
90:00	08:50	300.00	5.00

Pre-column equilibration:

Volume [µl] : 12.00
Flow [µl / min] : (unspecified)
Max. pressure [Bar] : 800.00

Analytical column equilibration:

Volume [µl] : 3.50
Flow [µl / min] : (unspecified)
Max. pressure [Bar] : 600.00

Autosampler wash:

Flush volume [µl] : 100.00

Method of Q Exactive HF

Overall method settings

Global Settings		
Use lock masses	best	
Lock mass injection	–	
Chrom. peak width (FWHM)	20	s
Time		
Method duration	90.00	min
Customized Tolerances (+/-)		
Lock Masses	–	
Inclusion	–	
Exclusion	–	
Neutral Loss	–	
Mass Tags	–	
Dynamic Exclusion	–	

Experiments

Full MS – SIM

General		
Runtime	0 to 90	min
Polarity	positive	
In-source CID	0.0	eV
Full MS – SIM		
Microscans	1	
Resolution	60,000	
AGC target	3e6	
Maximum IT	40	ms
Number of scan ranges	1	
Scan range	400 to 1000	m/z
Spectrum data type	Profile	

DIA

General		
Runtime	0 to 90	min
Polarity	positive	
In-source CID	0.0	eV
Default charge state	2	
DIA		
Microscans	1	
Resolution	30,000	
AGC target	1e6	
Maximum IT	40	ms
Loop count	26	
MSX count	1	
MSX isochronous ITs	on	
Isolation window	22.0	m/z

Isolation offset 0.0 m/z
Fixed first mass 200.0 m/z
(N)CE / stepped (N)CE nce: 27
Spectrum data type Profile

Setup

Tunefiles
General
Switch Count 0
Base Tunefile C:\Xcalibur\methods\nanosource.mstune

Contact Closure
General
Used False
Start in Closed True
Switch Count 0

Syringe
General
Used False
Start in OFF True
Stop at end of run False
Switch Count 0
Pump setup
Syringe type Hamilton
Flow rate 3.000 µL/min
Inner diameter 2.303 mm
Volume 250 µL

Divert Valve A
General
Used False
Start in 1-2 True
Switch Count 0

Divert Valve B
General
Used False
Start in 1-2 True
Switch Count 0

Lock Masses
1 entry

Mass [m/z]	Polarity	Start [min]	End [min]	Comment
445.12003	Positive			

Inclusion List
28 entries

Mass [m/z]	Formula [M]	Species	CS [z]	Polarity	Start [min]	End [min]	(N)CE	MSX ID	Comment
411.00000				Positive					
432.00000				Positive					
453.00000				Positive					
474.00000				Positive					
495.00000				Positive					
516.00000				Positive					
537.00000				Positive					
558.00000				Positive					
579.00000				Positive					
600.00000				Positive					
621.00000				Positive					
642.00000				Positive					
663.00000				Positive					
684.00000				Positive					
705.00000				Positive					
726.00000				Positive					
747.00000				Positive					
768.00000				Positive					
789.00000				Positive					
810.00000				Positive					
831.00000				Positive					
852.00000				Positive					
873.00000				Positive					
894.00000				Positive					
915.00000				Positive					
936.00000				Positive					
957.00000				Positive					
978.00000				Positive					

Thermo EASY-LC method print for QExactive_HF

Sample pickup:

Volume [µl] : 1.00
Flow [µl / min] : 20.00

Sample loading:

Volume [µl] : 22.00
Flow [µl / min] : (unspecified)
Max. pressure [Bar] : 800.00

Gradient:

Time [mm:ss]	Duration [mm:ss]	Flow [nl/min]	Mixture [%B]
00:00	00:00	300.00	3.00
04:00	04:00	300.00	8.00
06:00	02:00	300.00	10.00
74:00	68:00	300.00	32.00
86:00	12:00	300.00	50.00
87:00	01:00	300.00	100.00
94:00	07:00	300.00	100.00
95:00	01:00	300.00	3.00
105:00	10:00	300.00	3.00

Pre-column equilibration:

Volume [µl] : 12.00
Flow [µl / min] : (unspecified)
Max. pressure [Bar] : 800.00

Analytical column equilibration:

Volume [µl] : 2.00
Flow [µl / min] : (unspecified)
Max. pressure [Bar] : 600.00

Autosampler wash:

Flush volume [µl] : 100.00

Method of Q Exactive HF

Overall method settings

Global Settings		
Use lock masses	best	
Lock mass injection	–	
Chrom. peak width (FWHM)	20	s
Time		
Method duration	105.00	min
Customized Tolerances (+/-)		
Lock Masses	–	
Inclusion	–	
Exclusion	–	
Neutral Loss	–	
Mass Tags	–	
Dynamic Exclusion	–	

Experiment

Full MS / dd-MS² (TopN)

General		
Runtime	0 to 105	min
Polarity	positive	
In-source CID	0.0	eV
Default charge state	2	
Inclusion	–	
Exclusion	–	
Tags	–	
Full MS		
Microscans	1	
Resolution	60,000	
AGC target	3e6	
Maximum IT	32	ms
Number of scan ranges	1	
Scan range	350 to 1500	m/z
Spectrum data type	Profile	
dd-MS² / dd-SIM		
Microscans	1	
Resolution	15,000	
AGC target	1e5	
Maximum IT	50	ms
Loop count	20	
MSX count	1	
TopN	20	
Isolation window	2.0	m/z
Isolation offset	0.0	m/z
Scan range	200 to 2000	m/z
Fixed first mass	110.0	m/z

(N)CE / stepped (N)CE	nce: 26
Spectrum data type	Profile
dd Settings	
Minimum AGC target	1.00e3
Intensity threshold	2.0e4
Apex trigger	–
Charge exclusion	unassigned, 1, 5 - 8, >8
Multiple charge states	all
Peptide match	preferred
Exclude isotopes	on
Dynamic exclusion	25.0 s
If idle ..	do not pick others

Setup

Tunefiles

General

Switch Count	0
Base Tunefile	C:\Xcalibur\methods\nanosource.mstune

Contact Closure

General

Used	False
Start in Closed	True
Switch Count	0

Syringe

General

Used	False
Start in OFF	True
Stop at end of run	False
Switch Count	0

Pump setup

Syringe type	Hamilton	
Flow rate	3.000	µL/min
Inner diameter	2.303	mm
Volume	250	µL

Divert Valve A

General

Used	False
Start in 1-2	True
Switch Count	0

Divert Valve B

General

Used	False
Start in 1-2	True
Switch Count	0

1 entry

Mass [m/z]	Polarity	Start [min]	End [min]	Comment
445.12003	Positive			

Thermo EASY-LC method print for QExactive_HF

Sample pickup:

Volume [µl] : 1.00
Flow [µl / min] : 20.00

Sample loading:

Volume [µl] : 22.00
Flow [µl / min] : (unspecified)
Max. pressure [Bar] : 800.00

Gradient:

Time [mm:ss]	Duration [mm:ss]	Flow [nl/min]	Mixture [%B]
00:00	00:00	300.00	4.00
01:00	01:00	300.00	6.00
70:00	69:00	300.00	27.00
85:00	15:00	300.00	44.00
85:10	00:10	300.00	95.00
95:00	09:50	300.00	95.00
95:10	00:10	300.00	5.00
104:50	09:40	300.00	5.00
105:00	00:10	300.00	5.00

Pre-column equilibration:

Volume [µl] : 12.00
Flow [µl / min] : (unspecified)
Max. pressure [Bar] : 800.00

Analytical column equilibration:

Volume [µl] : 2.00
Flow [µl / min] : (unspecified)
Max. pressure [Bar] : 600.00

Autosampler wash:

Flush volume [µl] : 100.00

Method of Q Exactive HF

Overall method settings

Global Settings		
Use lock masses	best	
Lock mass injection	–	
Chrom. peak width (FWHM)	20	s
Time		
Method duration	105.00	min
Customized Tolerances (+/-)		
Lock Masses	–	
Inclusion	–	
Exclusion	–	
Neutral Loss	–	
Mass Tags	–	
Dynamic Exclusion	–	

Experiment

Full MS / dd-MS² (TopN)

General		
Runtime	0 to 105	min
Polarity	positive	
In-source CID	0.0	eV
Default charge state	2	
Inclusion	–	
Exclusion	–	
Tags	–	
Full MS		
Microscans	1	
Resolution	60,000	
AGC target	3e6	
Maximum IT	32	ms
Number of scan ranges	1	
Scan range	350 to 1500	m/z
Spectrum data type	Profile	
dd-MS² / dd-SIM		
Microscans	1	
Resolution	15,000	
AGC target	1e5	
Maximum IT	50	ms
Loop count	20	
MSX count	1	
TopN	20	
Isolation window	2.0	m/z
Isolation offset	0.0	m/z
Scan range	200 to 2000	m/z
Fixed first mass	110.0	m/z

(N)CE / stepped (N)CE	nce: 26
Spectrum data type	Profile
dd Settings	
Minimum AGC target	1.00e3
Intensity threshold	2.0e4
Apex trigger	–
Charge exclusion	unassigned, 1, 5 - 8, >8
Multiple charge states	all
Peptide match	preferred
Exclude isotopes	on
Dynamic exclusion	25.0 s
If idle ..	do not pick others

Setup

Tunefiles

General

Switch Count	0
Base Tunefile	C:\Xcalibur\methods\nanosource.mstune

Contact Closure

General

Used	False
Start in Closed	True
Switch Count	0

Syringe

General

Used	False
Start in OFF	True
Stop at end of run	False
Switch Count	0

Pump setup

Syringe type	Hamilton
Flow rate	3.000 µL/min
Inner diameter	2.303 mm
Volume	250 µL

Divert Valve A

General

Used	False
Start in 1-2	True
Switch Count	0

Divert Valve B

General

Used	False
Start in 1-2	True
Switch Count	0

1 entry

Mass [m/z]	Polarity	Start [min]	End [min]	Comment
445.12003	Positive			