

Thermo Scientific Orbitrap Exploris 4.2 Instrument Control Software (OES 4.2 ICSW) –

Overview – Updated With Defect Fixes in SP

November 2023

The world leader in serving science



Thermo Scientific Orbitrap Exploris MS Portfolio – <u>One</u> ICSW





Thermo Scientific[™] Orbitrap Exploris[™] MX Mass Detector



Thermo Scientific[™] Orbitrap Exploris[™] 120 Mass Spectrometer



Thermo Scientific[™] Orbitrap Exploris[™] GC Mass Spectrometer

Thermo Scientific™ Orbitrap Exploris™ 240 Mass Spectrometer



For Research Use Only. Not for use in diagnostic procedures.



Thermo Scientific[™] Orbitrap Exploris[™] 480 Mass Spectrometer



Thermo Scientific[™] Orbitrap Exploris[™] GC 240 Mass Spectrometer

Comparison of Orbitrap Exploris Portfolio

	*								
	Orbitrap Exploris MX	Orbitrap Exploris 120	Orbitrap Exploris 240	Orbitrap Exploris 480					
Max Resolution (FWHM) @ m/z 200	180,000	120,000	240,000	480,000					
Mass range	40 – 3,000 (8,000 *)	40 – 3,000	40 - 6,00	00 (8,000 *)					
Precursor ion selection	n/a		≤ 2,500						
Sensitivity		S/N 250 @ 20	0 fg reserpine (tSIM)	S/N 150 @ 50 fg reserpine (tSIM)					
MSMS scan rate (Hz)	22 Hz (Full Scan)		22 Hz	40 Hz					
Mass accuracy - external		< 3 ppm RMS drift over 24 hours							
Mass accuracy w/ EASY-IC - internal			< 1 ppm over 5 days						
Spectral multiplexing	n/a	20							
Polarity switching * : one cycle equals (pos./switch/neg./switch)	60 k Full Scan* < 700 ms (equals > 1.4 Hz)	60 k Full Scan* < 700 ms (equals > 1.4 Hz) 60 k tSIM Scan* < 600 ms (equals > 1.6 Hz)							
Calibration		One-click calibration with FlexMix and dedicated calibration probe - with harmonization and improved user experience across all TNG platforms (TSQs, Hybrids, Tribrids)							
One-Point Mass Calibration		One-Point (Self) Mass Ca	libration achieves < 3 ppm RMS drift over at least 4 we	eks					
Scan modes Full MS AIF t-SIM DIA MS2 combinable within in one single experiment, such as:	• Full Scan In addition, multiple experiments can be created combining various Full Scan experiments	 Full Scan ddMS2 (Top1-4) tSIM (targeted mass list) ddMS2 (Top1-4) Full Scan ddMS2 (targeted list) (Top1-4) In addition, up to 5 experiments can be created combining the above listed scan types	 Full Scan ddMS2 (topN) Full Scan ddSIM tSIM (targeted mass list) ddMS2 Full Scan ddMS2 (targeted mass list) With options for 'Number of Scans' (= TopN) 'Cycle Time' In addition, multiple experiments can be created combining the above listed scan types 	 Full Scan ddMS2 (topN) Full Scan ddSIM tSIM (targeted mass list) ddMS2 Full Scan ddMS2 (targeted mass list) With options for 'Number of Scans' (= TopN) 'Cycle Time' 'Scans per Outcome' (branching) In addition, multiple experiments can be created combining the above listed scan types 					
			AcquireX, APD, AcquireX AB						
Advanced acquisitions	APD	AcquireX (chargeable option)	TMT @ 45k resolution setting	 16 msec transient (7,500 min resolution) System Templates supporting BoxCar and SureQuant approaches TurboTMT with TMT reagents up to 18-plex 					

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Software Release

Flexera Orbitrap Exploris Series ICSW 4.2 SP4 is available to customers using Flexera software distribution site.

Customers new to the Flexera site should use the following link: https://thermo.flexnetoperations.com/control/thmo/RegisterMemberToAccount

After setting up an account, customers can access the site using the following link: <u>https://thermo.flexnetoperations.com/control/thmo/login</u>

In the 'Product List' page, find 'Instrument – Orbitrap Exploris Series' and identify Orbitrap Exploris Series 4.2 SP4 in the subfolder.

Software & Services Product List Product Search Order History Search Line Items Files Not Downloadd Product Search Series 4.2 Product Series 4.2	<u>vnload Help</u>
Recent Files Recent Files Recent Emails Licensing Search Licenses	s website
Offline Activation Download Activation	2 Files
Codes + File Description + File Size + File Name	*
Account Members 1.7 GB Exploris Series 4.2 iso	
Join Account Change Password Email Preferences Download Schedol Elloc	
Product Preferences Download Preferences Vour Profile	
Information	
FAQs	
ESDM User Manual Support	
Sessions	
Switch Account	
Logout	

Software Release

Thermofisher.com & AnalyteGuru



Updates: <u>AnalyteGuru.com</u>

To receive focused updates, subscribe to the pertinent labels (e.g., *Orbitrap Exploris MS Instrument Control Software*)

 AnalyteGuru > Knowledgebase > Scientific Library > Download Orbitrap Exploris Series Instrument Contr...

 Download Orbitrap Exploris Series Instrument Control software 4.0 SP1 here

 Image: Comparison of the comparison of

- Information: <u>Thermofisher.com</u>
 - Software information
 Known Issues
 - Links for download
 - New Features
- Discovered issues
- Fixed Defects

Release Notes

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Thermo Scientific Orbitrap Exploris Series 4.2 SP4 Instrument Control Software Release Notes

This document lists installation notes, new features and improvements regarding the Thermo Scientific™ Orbitrap Exploris™ Series 4.2 SP4 Instrument Control Software release. For information regarding the installation, features, functionality, and use of this product, refer to the following sources of information:

- Orbitrap Exploris Series Operating Manual
- Orbitrap Exploris GC and Orbitrap Exploris GC 240 Operating Manual

Version	Version No.	Orbitrap Exploris 480	Orbitrap Exploris 240	Orbitrap Exploris 120	Orbitrap Exploris GC	Orbitrap Exploris GC 240	Orbitrap Exploris MX
1.0	1.0.77.7	~	—	—	—	—	—
1.1	1.1.117.22	✓	_	—	—	—	—
1.1 SP1	1.1.117.26	✓	_	_		_	_
2.0	2.0.182.18	✓	✓	✓	_	—	_
2.0 SP1	2.0.182.25	✓	✓	✓	_	—	_
2.0 SP2	2.0.182.35	✓	✓	✓	_	_	_
3.0	3.0.261.13	✓	✓	✓	✓	✓	_
3.1	3.1.279.9	✓	✓	✓	~	✓	_
4.0	4.0.309.27	✓	✓	✓	✓	✓	✓
4.0 SP1	4.0.309.28	✓	✓	✓	~	✓	✓
4.1	4.1.335.19	✓	✓	✓	~	✓	✓
4.2	4.2.362.16	✓	✓	✓	~	✓	✓
4.2 SP1	4.2.362.21	✓	✓	✓	✓	✓	✓
4.2 SP2	4.2.362.26	✓	✓	✓	✓	✓	✓
4.2 SP3	4.2.362.36	✓	✓	✓	✓	✓	✓
4.2 SP4	4.2.362.42	✓	✓	✓	✓	✓	✓

Source: Release Notes for OES 4.2 SP4 ICSW

System Requirements

Thermo Scientific Orbitrap Exploris Series 4.2 SP4 Instrument Control Software Release Notes

Installation Notes

Supported Target Systems

Thermo Scientific Orbitrap Exploris 120 mass spectrometer

Thermo Scientific Orbitrap Exploris 240 mass spectrometer

Thermo Scientific Orbitrap Exploris 480 mass spectrometer

Thermo Scientific Orbitrap Exploris MX mass spectrometer

Thermo Scientific Orbitrap Exploris GC mass spectrometer

Thermo Scientific Orbitrap Exploris GC 240 mass spectrometer

System
RequirementsThe minimum hardware and software configurations required for the Orbitrap Exploris Series 4.2 SP4
Instrument Control Software operation are as follows:

	System	Requirements								
	PC	3.0 GHz Quad Core Intel [™] Processor								
		512 GB SSD Hard Drive								
		Display Monitor Resolution of 1920 × 1080								
_		Two Network Interface Cards (NIC), 1000 MBit/s								
ſ	Software	Microsoft [™] Windows [™] 10 Enterprise 2016 LTSB, 2019 LTSC or 2021 LTSC Thermo Scientific Xcalibur 4.7								
l	Tip The Orbitrap Explo	ris Series 4.2 SP4 Instrument Control Software was only tested within the delivered								

Note: Xcalibur 4.7 software applies Foundation 3.1 SP9.

List of New Features and Improvements in OES 4.2 ICSW

New Features

General

- Operating Manual, Pre-Installation Requirements Guide, and Software manuals are updated
- AcquireX support is provided for peptide mapping (AcquireX Ab) with OE 240 and OE 480
- Scheduled one-point mass calibration (via Tune Preferences)
- Additional resolution settings are accessible for OE 240 and OE 480 (11.25k, 22.5k, and 90k) in Tune and Method Editor

Tune

- OE GC: Tune Calibration pane provides a Manual Calibration panel
- OE GC: Method Editor provides a "Run Start Mass Calibration" template in system template

Method Editor

- New management of tables in DIA scan
- New table format is available for SIM scan. SIM with multiple broad scan ranges is provided for OE 120, 240 and GC
- Options are provided to select the order with which precursors are selected for data-dependent scans
- TMT 18-plex is supported by TurboTMT on OE 480
- New option "Auto-Extended" is provided for MS2 Scan Range Mode (Small Molecule Application Mode)

8 Note: It is recommended upgrading the system to benefit from these improvements

Improvements (selection)

Tune and Method Editor : Optimized default ESI/HESI gas flow settings for OE 120, 240, and MX

Tune Diagnostics : FlexMix Spray Optimization (neg+pos) applies Source Gases independent from polarity

Tune

- Calibration pane enables One-Point Mass and Customized Mass procedures which are compatible with FAIMS attached
- Source gas and temperature are set independently from polarity
- OE GC: Tune and ion source optimization reports contain a leak check history plot, filament current and emission current plots, and an emission current set value plot
- Calibration Status panel: reworked the update of Recommended Calibration dates depending on the procedure outcome
- Calibration Status panel: clarified display of Last Successful Calibration to 'Outdated' after Venting and Bake-out

Method Editor : User experience is enhanced when changing "Max. number of multiplexed ions"

Method Execution : Spray Voltage stays "On" during the execution of Run Start EASY IC

- **General** : The Orbitrap Exploris Series 4.2 Installer updates the MongoDB from version 4.0.6 to version 4.0.28
- Note: MongoDB needs to be uninstalled manually when downgrading from version 4.2 to an older version. The ISO image contains a MongoDB uninstaller.

Source: Release Notes for OES 4.2 SP4 ICSW

Resolved Issues in OES 4.2 SP1, SP2, SP3, and SP4

• Resolved Issues between OES 4.2 and OES 4.2 SP1

- Method execution Full Scan DDA with Targeted Mass Filter with CE per compound and Fixed/Stepped Collision Energies (CEs) in the
 properties pane of the MS2: fixed CEs in the Targeted Mass Filter are erroneously executed as stepped CEs
- Method transfer: Cannot open certain older methods with Orbitrap Exploris Series 4.2 ICSW index out of range error message
- Orbitrap Exploris GC: License Error with 60k GCHCD license prevents running methods
- Orbitrap Exploris GC: Method Editor: Corrected acquisition delay tooltip in Global Settings
- Orbitrap Exploris GC: Method Editor: Factor 10x UI recommendation rule is erroneously applied to all GC System Templates
- Orbitrap Exploris GC: Method Editor: Restore Default context menu does not work for parameters under Ion Source Properties
- Full Scan acquisition with Mild Trapping option (Small Molecule Application Mode) leads to signal drop in positive mode

Resolved Issues between OES 4.2 SP1 and OES 4.2 SP2

- System Calibration: C-Trap RF frequency calibration selects a frequency outside the optimum
- System Calibration: Exploris GC: C-trap RF Amplitude Min. Calibration failed with SW failure during run time
- Tune Status pane: Improved Turbo Pump readback robustness

Resolved Issues in OES 4.2 SP1, SP2, SP3, and SP4

Resolved Issues between OES 4.2 SP2 and OES 4.2 SP3

- FAIMS Cooling Gas flow jumps to 20 L/min
- System Calibration: During Quad DC offset calibration, one point is sometimes too far off
- System Calibration: Quad isolation shape m/z 1034 iso 500 is not always detected
- System Calibration: Quad RF DDS frequency does not get set always on instrument boot

Resolved Issues between OES 4.2 SP3 and OES 4.2 SP4

- Software upgrade to version > 4.2 SP2 may result in a severe issue with loss of instrument configuration
- After software upgrade from version 4.2 to a newer version, Tune displays obsolete warning alerts ([SSPI Link] Reconnect during run-time) after firmware has been updated

Setting up Mass Self-Calibration



- 'One-Point Mass Self-Calibration' Procedure calibrates positive and negative ion mode - <u>Unattended</u>
- Fluoranthene from the EASY-IC source is used for the 'One-Point Mass' calibration procedure
- Infusion of FlexMix solution is not needed
- Running the 'One-Point Mass Self-Calibration' procedure updates the master calibration file. Its updated content is applied to upcoming scans and raw data files without further user interaction. The *Recommended Calibration* date (and color indicator) is updated accordingly.

Mass Self-Calibration Options System Self-Calibration is enabled. To abort or change options, please uncheck the system self calibration check box below Image: The system self-Calibration Schedule Self-Calibration Day Fridays Time 2 PM

Mass Self – Calibration Options

 'Run One-Point Mass Self-Calibration' can be activated

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Day and time for Self-Calibration is defined

Self-Calibration Pre-Conditions

- Self-Calibration procedure is pursued only if
 - The instrument is in standby or scanning ("On")
 - 'Run One-Point Mass Self-Calibration' can be activated in Tune Preferences
- Self-Calibration procedure is deferred and subsequently run if
 - There is an acquisition in progress (acquisition sequence or tune recording) at the time of scheduled Self-Calibration
 - Acquisition queue gets empty within the next 24 hours of scheduled Self-Calibration (check every 5 min)
- Self-Calibration is executed when MS is controlled under Xcalibur or Chromeleon

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Self-Calibration Procedure

- 5-min delay Self-Check preparation
 - Notification is displayed in Tune bottom panel that Self-Check is about to start; text is updated every minute

🖉 Self-Calibration will start automatically in 5 minutes at 03:00 PM. Please go to the calibration panel to initiate now or abort.

- 'Start Self-Calibration' / 'Abort Self-Calibration' buttons displayed and 'Start' button in calibration panel disabled
- Self-Calibration execution



- Started after 5-min preparation has elapsed
- Instrument is switched to 'On' if currently in standby
- During preparation, calibration UI is disabled
- During Self-Calibration execution
 - Tune operations are disabled, procedure can be aborted by pressing relevant button (aborted Self-Calibration not run until next scheduled calibration)
 - Self-Calibration running status is displayed (progress bar, notification panel)

After Completion of Self-Calibration

• PDF report is generated

Self-Calibration Report thermoscientific											
Date & Time Wednesday, October 5, 2022 09:15:01 AM											
Instrument Model Orbitrap Exploris 240											
Instrument Serial MM10003C											
Software Version 4.2.357.0											
Name Result Comment											
OnePointMass Calibration (positive and neg	ative Mode)		Passed								
OnePointMass Calibration (positive and negative Mode)											
Type – – – Calibration											
ICS Mass Calibration passed. System Configuration											
Name		Value									
Ion Source Type			ESI								
Internal Calibration (EASY-IC) Source			enabled								
Options			EASY-IC, BioPharm	EASY-IC, BioPharma							
Embedded System Information											
Label			Version								
Instrument Embedded Software			4.2.316.0								
Instrument Hardware Hash			c9463e6c4c2a18724	14bd80b2d74da4e4f5c724a							

• Notification in Tune bottom panel and notification area of calibration tab

•	Calibration	
	Mode	Calibrate •
	Polarity	Positive •
	Туре	One-Point Mass 🔹
	Starting Self-Calibration	
	One-Point Mass is finished.	
	000	
	Passed	

For a good and reliable mass accuracy/stability:

- Run experiments with RunStart EASY-IC
- Schedule unattended self-calibration: daily
- Run a system calibration once per month

Internal Mass Calibr	ation	EASY-IC™	•									
Mode		Run Start	•									
Mass Self-Calibration O	Mass Self-Calibration Options											
System Self-Calibration is	System Self-Calibration is enabled. To abort or change options, please											
Run One-Point Mass	Uncheck the system self calibration check box below											
Schedule Self-Calibratio	Schedule Self-Calibration											
Day Daily	Day Daily											
System												
 Recommende 	d Calibration:	11/19/2022										
Last Successfu	Last Successful Calibration: 10/20/2022											
-	▼ Calibration											
Mode		Calibrate	•									
Polarity		Positive	•									
Туре		Mass & System	•									

AcquireX Data Acquisition Workflow Enhancements

New Features

- New intelligent data acquisition workflow for Biopharma applications: AcquireX Ab
 - Available with Xcalibur 4.6 in Peptide Application Mode of Orbitrap Exploris 240 and 480 MS systems
 - One AcquireX Ab workflow: Custom Workflow

- New AcquireX workflow for Small Molecule applications: Custom Workflow
 - Available with Xcalibur 4.6 in Small Molecule Application Mode of Orbitrap Exploris 240 and 480 MS systems (chargeable option for Orbitrap Exploris 120)
 - Custom Workflow replaces Advanced Deep Scan Workflow





New AcquireX Ab Workflow for Peptide Mapping

New Features in Method Editor

	Global Parameters	Scan Parameters	Summa	ary		
	Method Timeline	Experiment ACTIO	NS 🗸	Settings		
Application Mode	1	30 40 50 MS		Infusion Mode	Liquid Chron	natography
Peptide 🔻				Expected LC Peak Width (s)	10	
Method Duration (min)				Advanced Peak Determination	n 🔽	
60				Default Charge State	2	
				Enable Xcalibur AcquireX Ab		
			Q	method modifications		
			Ð	Internal Mass Calibration	Off	
	Experiment #1	0-60 CLEAR	Ì			4
			Targeter	Mass Properties		
Precursor Fit		_	largetee	i mass i roperties		
	Full S	can		MASS I	IST	
Charge State	Targeter	Mass	Mas	is List Type	m/z	*
Dynamic Exclusion		20 scans	Tim	e Mode	Start/End Time	•
	Targetec Exclus	sion	Inclu	ude Intensity Threshold	~	
Targeted Inclusion >	ddM	s ²	Add by X	Mass List Targets Determined (calibur AcquireX Ab	\checkmark	
Targeted Exclusion 🕨						6
				ADD C		C EXPORT
Apex Detection			Co	mpound m/z t start (min)	t stop (min) Int	ensity Threshold
Isotope Exclusion		BOB	▶ 1 MR	FA 524.265 0	60 0.0	90
				000		

AcquireX Ab workflows introduced with Xcalibur 4.6

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- AcquireX Ab available in Peptide Application Mode
- Controls implemented in
 - Global Settings
 - Targeted Mass and Targeted Mass Exclusion filters
- Constraints
 - Time Mode = Start/End Time
 - Include Intensity Threshold = TruePrecursor Mass Range
 - Mass List Type = m/z OR m/z & z

<u> </u>		1	MASS LIST						
	Mass List Typ	e	ľ	m/z & z			•		
	Time Mode		-	Start/End 1	Time		•		
	Include Inten	sity Threshold	\checkmark	\checkmark					
I	Add Mass List by Xcalibur A	: Targets Determi cquireX Ab	^{ned} 🗹	9					
		Λ					C		
					ІМРО		c		
_		Α	DD - O D						
	Compound	Formula	Adduct	m/z	z	t start (min)	ts		

Small molecules

- Advanced Deep Scan has been replaced by Custom Workflows
 - Allows for the use of multiple groups in a single workflow
 - Reuse and combine inclusion and exclusion lists from previous groups
 - Reminder: option to use new component detection from Thermo Scientific™ Compound Discoverer™ software



 Create workflows specific to your requirement What Xcalibur Does: Generates an inclusion or exclusion list by combining up to 5 injections per group Provides options to reuse inclusion and exclusion lists from previous groups Incorporates various experiment types in a single experiment 		MS OT Targeted Mass Desired Targeted Mass Custom WORKFLOWS
 What Xcalibur Does: Generates an inclusion or exclusion list by combining up to 5 injections per group Provides options to reuse inclusion and exclusion lists from previous groups Incorporates various experiment types in a single experiment 	Create workflows spe	ecific to your requirement
 Generates an inclusion or exclusion list by combining up to 5 injections per group Provides options to reuse inclusion and exclusion lists from previous groups Incorporates various experiment types in a single experiment 	What Xcalibur Does:	
	 Generates an incl injections per gro Provides options previous groups Incorporates varie 	lusion or exclusion list by combining up to 5 hup to reuse inclusion and exclusion lists from ous experiment types in a single experiment
		SELECT



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		Acc	quireX Work		Mode High O											
		AcquireX Template Injections		AcquireX Template Injections Group 2 🔻		Group 2 🔻 💉	🛍 🛛 Blan	ks 2 Excl. Ref 1	Incl. Ref 0	ID Ir	D Injections 4 # Groups to Add 1 Add					
		#	Name	Туре	Group	Instrument Method	Apply Excl.	List	Apply Incl. List	Vial	Inj Vol (µl)					
		1	Blank_01	Blank	1	Instrument Method		ſ	Dight slight slight	D.A.4	10.00 <i>µl</i>					
Group 1 "Deep Scan"		2	ExclusionRef_01	Exclusion	1	Instrument Method			down, insert injec	for copy tion, and	↑ Insert Inj Abo	ve				
		3	Sample_01	Inclusion	1	Instrument Method			undo			0W				
		4	ID_01	ld	1	Instrument Method	[1]	T	[1] •	R:A1	Dundo					
		5	ID_02	ld	1	Instrument Method				R:A1	Display Com	ment Column				
		6	ID_03	ld	1	Instrument Method				R:A1	Apply Name	Extension				
		7	Blank_01	Blank	2	Instrument Method	In H	High m	node- Option to	R:A1	10.00 <i>µl</i>					
		8	Blank_02	Blank	2	Instrument Method		grou	ip 1 and 2	R:A1	10.00 <i>µl</i>					
up 2		9	ExclusionRef_01	Exclusion	2	Instrument Method	7			R:A1	10.00 <i>µl</i>					
Iterative	\langle	10	ID_01	ld	2	Instrument Method	1, [2]	T	1 🔹	R:A1	10.00 <i>µl</i>					
usion"		11	ID_02	ld	2	Instrument Method	✓ 1			R:A1	10.00 <i>µl</i>					
		12	ID_03	ld	2	Instrument Method	✓ 2			R:A1	10.00 <i>µl</i>					
		13	ID_04	ld	2	Instrument Method				R:A1	10.00 <i>µl</i>					

Peptide and protein workflows

- New workflow for peptides/proteins, and other biopharma applications
- 1 workflow called "Custom Ab Workflow"
- Component detection is based on Thermo Scientific[™] Biopharma Finder[™] software Mass Analyzer algorithm
- Use MSn methods for exclusion/inclusion generation
- Reuse and combine exclusion and inclusion lists from previous groups



Create peptide mapping workflows specific to your requirement

What Xcalibur Does:

- Creates one exclusion list per group to reduce background fragmentation in your ID runs
- Creates one inclusion list per group to fragment more relevant precursor ions in multiple ID injections
- Injects ID samples iteratively for groups with an inclusion list until all ions in the inclusion list are fragmented or a user-defined number of ID injections is reached
- Injects ID samples iteratively for groups without an inclusion list until all ions in the sample are fragmented or a user-defined number of ID injections is reached
- Provides options to reuse inclusion and exclusion lists from previous groups
- Submits an experiment with several groups

	MS OT		/	AcquireX Ab Wo	rkflow Edito	or			Ма	ode 💶 🗤 🔞) × (
	Targeted Mass Internation	CUSTOM Ab WORKFLOWS		AcquireX Template Injections	Turne	Blanks	0 Excl. Ref 0	Incl. Ref 0	ID Injections 0	# Groups t/ dd 0	Add =
	ddMS ² OT HCD			# Name	Туре	Adding or removing	g ter injectio	ns auto-populates th	is table		voi (µi) <u>−</u> -
	Experiment Details Experiment Folder	Browse				/					
	Experiment Name	browse			Full customization of number of each				h		
	Group Parameters Group # 1-			50						L	
Fully customizable		Apply To All Groups					Low-	reuse excl	usion and	inclusion	list
	Instrument Methods Method for Blank/Exclusion/Inclusion se	Browse New					High-	reuse use from any	any list (e previous	ven multip group	ole)
	Template method for ID samples	Browse New	• II.	Method for	Blank/Ex	clusion/Inc	lusion car		-		
	Experiment Parameters			b	e MSn no	ot ONLY MS					
	Component Detection Se	ttings	0								
detection	Exclusion List Parameters	5	0	Acquire X Ab sequence can be expo as a .csv and imported later					1 (
parameters	Inclusion List Parameters		0					be export d later	orted		
								V			
					Ba	ck Cancel	Export	Import	Save	Save As	Submit

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	Acc	quireX Ab Wo	orkflow Edit	or			Μ	ode 🕒	ligh 🔵 🪺	
	AcquireX	Template Injections	Group 2 🔻 💉 🚺	🗊 🛛 Blan	ks 2 Excl. Ref 1	Incl. Ref 1	D Injections 3	# Groups to	Add 1 Add	
	#	Name	Туре	Group	Instrument Method	Apply Excl. Lis	st Apply Incl. List	Vial	Inj Vol (µl)	Ē
	1	Blank_01	Blank	1	Instrument Method	Rig	ht click allow for copy	R:A 🔒	Insert Inj Above	
	2	ExclusionRef_01	Exclusion	1	Instrument Method		undo	R:A 🔸	Insert Inj Below	•
	3	Sample_01	Inclusion	1	Instrument Method			R:A	Copy Down	
Each group	4	ID_01	ld	1	Instrument Method	[1]	▼ [1] ▼	R:A	Display Comment Column	· •
can have a	5	ID_02	ld	1	Instrument Method			R:A 🖜	Apply Name Extension	•
number to	6	Blank_01	Blank	2	Instrument Method			R:A1	10.00 <i>µl</i>	
sample types	7	Blank_02	Blank	2	Instrument Method	In	High mode- Option to	R:A1	10.00 <i>µl</i>	
	8	ExclusionRef_01	Exclusion	2	Instrument Method	Cho	group 1 and 2	R:A1	10.00 <i>µl</i>	
	9	Sample_01	Inclusion	2	Instrument Method	7		R:A1	10.00 <i>µl</i>	
	10	ID_01	ld	2	Instrument Method	1, [2]	• [2] •	R:A1	10.00 <i>µl</i>	
	11	ID_02	ld	2	Instrument Method	✓ 1		R:A1	10.00 <i>µl</i>	
	12	ID_03	ld	2	Instrument Method	✓ 2		R:A1	10.00 <i>µl</i>	

Scan Range Mode - Auto-Extended (tMS2 or ddMS2)



- Scan Range Mode "Auto-Extended"
- Applies factor 15 rule for all precursor masses
- Low fragments for precursor masses between 500 and 1500 are integrated in the scan filter (better comparability with Q Exactive spectra for low fragment masses)

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Examples – Auto vs. Auto-Extended

Last mass/ Precursor Mass [m/z]	Rule in Scan range mode "Auto"	Scan Range Mode "Auto"	Scan Range Mode Auto "Auto-Extended" (Factor 15 rule)
200	Factor 5	40	40
240	Factor 5	48	40
250	Factor 5	50	40
260	Factor 10	50 \rightarrow fm increase to 50 (note not to 40!)	40
400	Factor 10	50 < 50 \rightarrow fm increase to 50	40
900	Factor 10	90	60
1000	Factor 10	100	67
1500	Factor 10	150	100
1600	Factor 15	150 < 150 → fm increase to 150	107
2000	Factor 15	150 < 150 → fm increase to 150	133

Management of Tables in DIA Scan

New DIA Window Type

- DIA Window Type = Auto
 - DIA windows calculated from
 - Precursor Mass Range
 - Isolation Window
 - Window Overlap
 - DIA windows in Table cannot be
 - Modified by direct typing
 - Modified by adding / deleting rows
 - Modified by importing files
- DIA Window Type = User Defined
 - DIA windows defined by
 - Direct typing
 - Adding / deleting rows
 - Importing files

DIA Window Type = Auto



DIA Window Type = User Defined

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			_
Da	ta-Independent Acquisition Properties	Show Favorit	es
	DIA Window Type	User Defined 🔹	k
	Multiplex lons		*
	DIA Window Mode	m/z Range 🔹 🦿	*
	Collision Energy Type	Normalized •	*

		DIA m/z window	Li I		DIA m/z window	
	m/z range			m/z rang	2	
1	100-200			1 100-200		
2	200-300			2 200-300		
3	300-400			3 300-400		
4	400-500			4 400-500		
5	500-600			5 500-600		
6	600-700			6 600-700		
7	700-800			7 700-800		
8	800-900			8 800-900		
9	900-1000			9 900-1000		
10	1000-1100			10 1000-110		
						1

Management of Tables in DIA Scan



New DIA Window Mode

- DIA Window Mode = m/z Range
 - One column in Table
 - m/z Range
- DIA Window Mode = Center Mass
 - Two columns in Table
 - Center Mass
 - Window Width
- DIA Windows are converted when switching between the two modes

8 9 10

 The two DIA Window Modes are compatible with the two DIA Window Types

DIA Window Mode = m/z Range

	Da	ta-Independent Acquisition Properties		Show Favorites	Dat	ta-Independent Acc
		DIA Window Type	User Defined	• *		DIA Window Type
		Multiplex lons		*		Multiplex lons
		DIA Window Mode	m/z Range	- *		DIA Window Mode
>		Collision Energy Type	Normalized	• *		Collision Energy Type

DIA Window Mode = Center Mass

a	ta-Independent Acquisition Properties		Show Favorites
	DIA Window Type	User Defined	• *
	Multiplex lons		*
	DIA Window Mode	Center Mass	- *
	Collision Energy Type	Normalized	•

	DIA m/z window				DIA m/z window	
m/z range				Center Mass (m/z)	Window Width (m/z)	
100-200			1	150	100	
200-300			2	250	100	
300-400			3	350	100	
400-500			4	450	100	
500-600			5	550	100	
600-700			6	650	100	
700-800			7	750	100	
800-900			8	850	100	
900-1000			9	950	100	
1000-1100			10	1050	100	
		1				

New Table Format For SIM Scan

New SIM Window Mode

					×	Targeted	SIM Scan Properties		Show Favor	ites							Targeted S	SIM Scan Properties		Show Favo	rite
	M	ss List Table	ADD =			Multi	plex lons			*		Mass List Ta	ible				Multip	plex lons			7
	Compound	Formula Addu	ct Center Mass (r	1/z) z		💷 Isolat	ion Window (m/z)	2		*	Comp	ound Formula	Adduct	Center Mass (m/z)	z	Isolation Window (m/z)	📰 Isolatio	ion Window (m/z)	Defined in Table		7
1			524.2649	1	_	lsolat	ion Offset	Off	•	*	1			524.2649	1	2	Isolatio	ion Offset	Off	-	7
3			922	1		SIM V	Vindow Mode	Center Mass	-	+	3			922	1	2	SIM W	Vindow Mode	Center Mass	-	4
	1						Targeted SIM Scan	I Properties		Show Favorites		•	SIN	1 Wind	do Im	w Mode	e = m	n/z Ranę	ge		
		Mass Li	st Table				Multiplex lons	Γ		*			• 0								
		m/z Range					SIM Window Mo	ode	m/z Range	- *			•	m/z Rar	nge						
	1	523.2649-525.2649 261.6-263.6					📰 Orbitrap Resolut	tion	60000	•		•	SIN	/I Wind		w Mode	2 – C	Center M	lass		
	3	521-525					III RF Lens (%)		50	*					JU		- 0		iuuu		

- Two columns in Table
 - Center Mass
 - Window Width (with Table icon selected)
- SIM Windows converted when switching between the two modes

Multiple Full Scan Ranges (tSIM)

ime Range (min)	0	-15			switch 😂 🛛 Clear 👔	Ū				٥,
						Tar	geted SIM Scan	Properties		
(C	tSIM					Multiplex lons	[
					×		SIM Window Mo	de	m/z Range	•
		Mass Lis	st Table				Orbitrap Resolut	ion	60000	-
		m/z Range	RT Time (min)	Window (min)			DE 1 (0/)		70	
	1	100-300	7.5	15			KF Lens (%)	ļ	70	
	2	300-600	7.5	15			AGC Target	l	Standard	-
	▶ 3	600-1000	7.5	15			Maximum Inject Mode	ion Time	Auto	•
							Microscans		1	
							Data Type		Profile	•
							Polarity	ſ	Positive	-
							Source Fragmen	tation [
							Scan Description	ı (
							Time Mode		Retention Time Wind	• •
							Select table icon	to add propert	y to mass list table.	
						Ma	ass List Table ADD			13
							m/z Range	RT Time (mi	in) Window (min)	_ ^
						1	100-300	7.5	15	
						2	300-600	7.5	15	
						3	600-1000	7.5	15	
								000		

- Multiple Scan ranges can be entered in the new table format for tSIM
- Extension of "Isolation Window" width from 0.4-50 to 0.4-2000 m/z units on OE120 and OE240
- Possibility to set parameters scan range dependent

Advantages:

- Increase dynamic range of the calibration curve
- Better overview parameters for all scan ranges are on one view (opposed to setup the experiment with different experiments in the timeline)

Note: For Full Scan ranges consider to set the AGC Target value to 1000 (1e6). Standard = $100 \cong 1e5$

Additional Resolution Settings

Show All

Tune

thermo scientific		0	Full	Scan Properties	
Orbitrap Exploris	s 480 🕑	0		Orbitrap Resolution	120000
ION SOURCE DEFINE S	CAN CALIBRATION			RF Lens (%)	7500
Scan Type	Full Scan	•			11250
Orbitrap Resolution	15000	•		Polarity	15000
Scan Range (m/z)	7500				22500
RF Lens (%)	11250				30000
AGC Target	15000				45000
Maximum Injection Time	30000				60000
Time (ms)	45000				90000
Time (ms)	60000				120000
Microscans	90000				120000
Source Fragmentation	120000				180000
Use EASY-IC™	180000				240000
	240000				480000
	480000				

Method Editor

• Available for OE 480 and OE 240

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• Available for all scan types

TMT 18-plex Support by TurboTMT

One More Window Covered by TurboTMT / TMTpro Reagent

Orbitrap Exploris 480 Method Edite	or 4.2.338		- 🗆 X
File Orbitrap Exploris 480			
Method Editor	Global Parameters Scan Paramet	ters Summary	
	Method Timeline Experiment AC	TIONS V Settings	e
Application Mode	# 10, 20, 30, 40, 50 1 MS	0 60 Infusion Mode	Liquid Chromatography 🔻
Peptide 🔻		Expected LC Peak Width (s)	10
Method Duration (min)		Advanced Peak Determination	
60		Default Charge State	2
		~	
	Experiment #1ın <u>c</u> 0-60	CLEAR 🔟	Q ,
Precursor Selection		Data-Dependent MS ² Scan P	Properties <u>Show Favorites</u>
Runge	Full Scan	Multiplex lons	□ ★
MIPS		Isolation Window (m/z)	2
Intensity	Targeted Mass	20 Isolation Offset	Off • ★
Precursor Fit	Targeted Mass Exclusion	Collision Energy Type	Normalized 🔹 🖈
Charge State	ddMS ²	HCD Collision Energy (%)	30 🖈
Durantia funduation		Orbitrap Resolution	15000 🔹 🖈
		TurboTMT	Off 🔹 🖈
Targeted Inclusion >		Scan Range Mode	Off 🗶
Targeted Exclusion 🕨		AGC Target	TMT Reagents
Apex Detection		Maximum Injection Time Mode	Auto
		Microscans	1
		Data Type	Profile 🔹 🛧
Triggers			

TurboTMT only available for OE 480

- Additional reporter ions
 - TMTpro-134C: 134. 154565 (covered by existing window 134.0414 134.2614)
 - TMTpro-135N: 135.151600 (covered by new window 135.0446-135.2646)

TurboTMT is specifically applied to increase the resolving power to six windows of 0.22 Da width, centered around the reporter masses for TMT and TMTpro in the mass range of 125-136 Da.

▲ Learn more...

TurboTMT uses an advanced spectral processing algorithm that increases resolving power within a specified reporter ion mass range without requiring a longer transient acquisition. Using TurboTMT specifically to the Tandem Mass Tags™ (TMT[™]) reporter ions increases the resolution sufficient to baseline resolve isotopologues even when using transients that produce a 30,000 resolution. For TMT Reagent, Turbo TMT is specifically applied to 6 windows of 0.22 Da centered around the masses 126.1309, 127.1279, 128.1313, 129.1346, 130.1380, 131.1413.

For TMTpro Reagent, Turbo TMT is specifically applied to 10 windows of 0.22 Da centered around the masses 126.1309, 127.1279, 128.1313, 129.1346, 130.1380, 131.1413, 132.1447, 133.1480, 134.1514, 135.1546)

Note: Using 15,000 resolving power may require additional data analysis tools.

Alternate Precursor Sorts

Additional Options to Define Precursor Selection Priority in DDA



OE 480, OE 240

OE 120



- Alignment with Orbitrap Tribrid Series ICSW
- No sorting for OE MX
- No Charge State Sorting for OE 120
- Allowed combinations
 - OE 480, OE 240: Sort by Charge (1st) AND Sort by Intensity OR Sort by m/z

hermo

• OE 120: None

New and Updated Templates

- New Chimerys templates (Peptide Application Mode)
- New AcquireX Ab templates (Peptide Application Mode)
- Updated/Corrected Ion Source settings for several small molecule templates





Updates to Manuals for Orbitrap Exploris Series

Pre-Installation Requirements Guide

Operator Manuals

Model specific Software Manuals and online help

The world leader in serving science



Pre-Installation Requirements Guide And Operating Manual

Pre-Installation Requirements Guide



Operating Manual



Orbitrap Exploris Series

Orbitrap Exploris 120, Orbitrap Exploris 240, Orbitrap Exploris 480, and Orbitrap Exploris MX Operating Manual

BRE0014471 Revision F October 2022

Software Manuals for these Orbitrap Exploris models

Thermo Fisher



Thermo Scientific™ Orbitrap Exploris™ MX Mass Detector



Updated Software Manuals and updated online help are part of the delivered ISO-Image and installed upon the installation of OES 4.2 SP4 ICSW

Orbitrap Exploris MX

Software Manual



Thermo Fisher s c i e n t i f i c



The world leader in serving science



OES 4.2 SP4 ICSW and Chromeleon CDS Software

LC-MS data acquisition under Chromeleon

- OES 4.2 SP4 driver validated for use with Chromeleon CDS 7.2.10 MUg software and Chromeleon CDS 7.3.2
- Improvements : Method Editor menu bar is now available and allow
 - Import Method from Raw Data File
 - Import Mass Lists from Q Exactive Method File

	Orbitrap Exploris 240	
Impo	t Method from Raw Data File	ran
Impo	t Mass Lists from Q Exactive Method File	

- Defect fixes: The previous issue observed with Orbitrap Exploris Series 4.1 ICSW, which prevented Workstation Method Editor to be launched without Foundation installed and therefore the use of the MS client driver on Chromeleon enterprise systems using Terminal Server / Citrix clients, has been fixed.
- The Chromeleon Driver Compatibility matrix is updated when new combinations of software versions are tested. For more information and to view the compatibility matrix, sign on to <u>https://support.thermoinformatics.com/downloads/default.aspx</u>, and then select Chromeleon > Chromeleon > Related Drivers > Driver Compatibility Matrix.