

# Scale up your science Orbitrap Ascend Tribrid mass spectrometer

### **Benefits**

- Improved capture of labile compounds
  while maintaining optimal ion transmission
- Exceptional performance with shorter gradients due to higher MS/MS sensitivity and scan rate
- Unmatched depth, accuracy, and precision for Tandem Mass Tag<sup>™</sup> (TMT<sup>™</sup>) analysis
- Higher Mass Range MS<sup>n</sup> (HMR<sup>n</sup>+) option for comprehensive analysis of native protein complexes
- The fully integrated Auto-Ready ion source allows for complete, unattended system calibration, without the need for manual hardware reconfiguration
- Full experimental flexibility with revolutionary instrument control software
- A wide range of optional functionalities for unprecedented versatility
- Common user interface with Thermo Scientific<sup>™</sup> Orbitrap Exploris<sup>™</sup> and Thermo Scientific<sup>™</sup> TSQ<sup>™</sup> Triple Quadrupole mass spectrometers

The Thermo Scientific<sup>™</sup> Orbitrap<sup>™</sup> Ascend Tribrid<sup>™</sup> mass spectrometer combines industry-leading versatility and usability with the exceptional performance afforded by its built-in intelligence and revolutionary Thermo Scientific<sup>™</sup> Tribrid<sup>™</sup> architecture to deliver an instrument designed to address the most difficult analytical challenges. The newest Tribrid platform includes a softer ion source, a new Front Ion Routing Multipole, Auto-Ready ion source for calibration, and a new optional High Mass Range MS<sup>n</sup>+ (HMR<sup>n</sup>+) mode. Collectively, these features make this instrument uniquely suited for accurate and high-throughput full-proteome quantitation, characterization of complex peptide and protein mixtures, small molecules and deciphering higher-order protein structures.



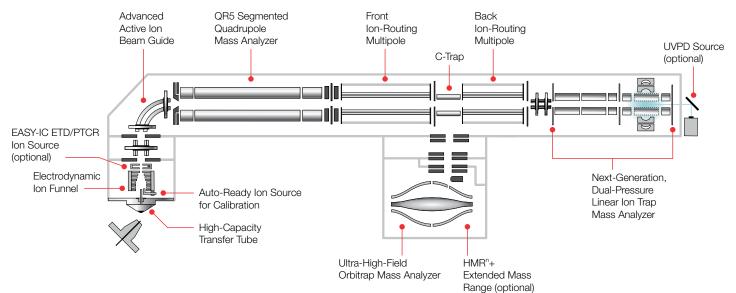
Be ready for tomorrow's most challenging demands with the Orbitrap Ascend Tribrid mass spectrometer

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## Key capabilities

- Improved high-sensitivity atmospheric pressure interface combines a high capacity transfer tube and a modified electrodynamic ion funnel, providing an increased ion flux and lower limits of detection for a wider range of molecules
- Active Ion Management (AIM+) Technology that includes the QR5 segmented quadrupole mass filter with hyperbolic surfaces, maximizes ion transmission to achieve unprecedented levels of qualitative and quantitative performance
- Dual Ion Routing Multipoles (IRMs) improves ion transfer, capture and dissociation
- Automated, remote, and hands-free calibration with the Auto-Ready ion source, allowing the user to spend more time obtaining results and less time on instrument setup
- Optional Higher Mass Range MS<sup>n</sup> (HMR<sup>n</sup>+) mode provides expanded mass range capabilities for comprehensive MS<sup>n</sup> characterization of native proteins and protein complexes
- Real-Time Search of a database or a spectral library enables faster data acquisitions and improved specificity, significantly increasing analytical performance, including TMT-based quantification experiments
- Thermo Scientific<sup>™</sup> TurboTMT mode improves HRAM MS<sup>n</sup> acquisition rates in TMT experiments
- Thermo Scientific<sup>™</sup> Precursor Fit filter enables precursor ion selection for a defined specificity
- Enhanced Vacuum Technology improves Thermo Scientific<sup>™</sup> Orbitrap<sup>™</sup> mass analyzer performance
- Improved accumulation and transmission of high molecular weight ions to the ion trap and Orbitrap mass analyzers
- Unique Tribrid architecture includes a quadrupole mass filter, dual-pressure linear ion trap and Orbitrap mass analyzers, allowing for high acquisition rates to provide maximum experimental flexibility for dissociation and detection of fragment ions
- DDA MS/MS acquisition rates of up to 45 Hz with 7500 resolution @ *m/z* 200 for Orbitrap mass analyzer and 50 Hz in the linear ion trap experiments

- Orbitrap resolution up to 480,000 FWHM at m/z 200
- Large surface area ion trap detector for significantly improved robustness and lifetime
- Full parallelization of MS and MS<sup>n</sup> analyses with Dynamic Maximum Injection Time
- Synchronous Precursor Selection (SPS) for MS<sup>n</sup> experiments
- Optional Compact Electron Transfer Dissociation and Proton Transfer Charge Reduction (ETD/PTCR) ion source based on Townsend discharge with extremely stable anion flux for improved usability and reagent longevity
- Multiple fragmentation techniques: collision-induced dissociation (CID), higher-energy collisional dissociation (HCD), optional electron-transfer dissociation (ETD, including electron-transfer higher-energy collision dissociation, or EThcD, and electron-transfer collision-induced dissociation, or ETciD) and optional ultraviolet photodissociation (UVPD), available at any stage of MS<sup>n</sup> with detection in the Orbitrap mass or linear ion trap analyzers
- Optional Proton Transfer Charge Reduction (PTCR) simplifies MS<sup>n</sup> spectra of multiply charged analytes
- Advanced Peak Determination (APD) for improved precursor annotation in data-dependent experiments
- Universal method maximizes peptide identifications without method optimization when sample concentration is unknown
- Intuitive, flexible drag-and-drop instrument method editor simplifies method development and creation of unique analytical workflows
- Extensive pre-built and optimized method templates that are fully customizable for a variety of application workflows
- Thermo Scientific<sup>™</sup> AcquireX data acquisition workflow provides all-inclusive, automated small molecule sample profiling on an LC timescale
- Streamlined calibration routines with improved Orbitrap calibration for ions with m/z < 200



Orbitrap Ascend Tribrid mass spectrometer ion path

### Hardware features

### Active Ion Management (AIM+) Technology

AIM<sup>+</sup> maximizes ion transmission, from injection to detection, using a novel hardware design for precise management of electrical fields and removal of noise to achieve unprecedented levels of quantitative performance

### Ion source

### Thermo Scientific<sup>™</sup> Ion Max NG<sup>™</sup> ion source

- Adjustable heated electrospray ionization (HESI) probe provides ultimate sensitivity
- Flow rates 1-2000 µL/min
- Designed for maximum performance for a broad range of molecular ions
- Enhanced ruggedness
- Sweep gas reduces chemical noise
- Optional APCI probe compatible with liquid flow rates of 50–2000 µL/min without splitting; the APCI probe can be upgraded to optional APPI capability

### Thermo Scientific<sup>™</sup> Auto-Ready ion source

- Integrated source with dedicated emitter and ion transfer tube simplifies maintenance and calibration
- Trigger on-demand or scheduled system checks and calibrations without disruption to your LC-MS configuration
- Calibration and maintenance results are controlled and reported directly in the instrument control software interface
- Internal Thermo Scientific<sup>™</sup> Pierce<sup>™</sup> FlexMix<sup>™</sup> Calibration solution consumable lasts ≥3 months with weekly mass calibration and monthly system calibration, plus intermittent checks

### Generation V ion optics and mass analyzers

The new API interface consists of a High-Capacity Transfer Tube (HCTT) and an Electrodynamic Ion Funnel (EDIF)

### High-capacity ion transfer tube (HCTT)

- The HCTT increases ion flux into the vacuum system for improved sensitivity
- Vent-free maintenance

### **Electrodynamic Ion Funnel**

- A modified ion funnel captures the ions under more gentle conditions, improving the transfer of labile compounds while maintaining optimal transmission
- Automatic tuning results in broad ion transmission curves with reduced ion losses, increasing sensitivity

### Advanced active ion beam guide (AABG)

AABG with an axial field and low-pass filter prevents neutrals and high-velocity clusters from entering the quadrupole mass filter

# QR5 segmented quadrupole mass filter with hyperbolic surfaces

Next generation quadrupole mass filter with 5.25 mm field radius delivers industry leading ion transmission across m/z 50–2000 range for efficient precursor selection with m/z 0.4–1200 isolation width

### A new front ion-routing multipole

- Ion storage capabilities
- Higher-energy collisional dissociation (HCD)
- Variable pressure control from 0.5 to 20 mTorr with HMR<sup>n</sup>+ option, for small and large molecule workflows
- Efficient and reliable ion transfer between the ion-routing multipole, Orbitrap mass analyzer and linear ion trap mass analyzer

### Orbitrap mass analyzer

- Next-generation ultra-high-field Orbitrap mass analyzer
- Low noise detection pre-amplifier
- Automatic calibration of all ion transfer parameters

#### Dual-pressure linear ion trap mass analyzer

- Extended front section of the high-pressure cell for improved ETD and PTCR reaction control
- For precursors between *m/z* 50 and 1800, isolation widths range from *m/z* 0.2 to 3000
- Minimum precursor isolation width for ions with *m/z* >1800 between *m/z* 2 at *m/z* 1800 and up to *m/z* 100 at *m/z* 8000, with a maximum precursor isolation width of *m/z* 4000
- Collision-induced dissociation (CID)
- ETD/EThcD/ETciD and UVPD fragmentation techniques
- PTCR ion-ion reaction
- Low pressure cell for improved scan rates, resolving power, and mass accuracy
- Dual-dynode detector with high linear dynamic range for improved quantification, and a large surface area for increased lifetime and robustness

### Vacuum system

- Split-flow turbomolecular pump provides vacuum in three regions
- Stainless steel and aluminum high-vacuum analyzer chambers
- Enhanced vacuum technology reduces the pressure in the ultra-high vacuum region to <10<sup>-10</sup> Torr
- Improved Orbitrap mass analyzer performance at a wider range of collision gas settings

### **Analog inputs**

Channel 1 analog input (0–10 V); Channel 2 analog (0–2 V)

### **Optional hardware**

#### Thermo Scientific<sup>™</sup> NanoSpray Flex NG<sup>™</sup> ion source

- Supports static and dynamic electrospray experiments
- Compatible with liquid flow rates of 50 nL/min-2 µL/min

### Thermo Scientific<sup>™</sup> EASY-Spray<sup>™</sup> ion source

Maximum nanoelectrospray performance with no need for adjustments

### Thermo Scientific<sup>™</sup> EASY-ETD<sup>™</sup> ion source

- Generates fluoranthene anions for ETD
- Townsend discharge provides extremely stable and robust ionization
- Compact size, located entirely within the footprint of the instrument
- Active reagent ion filtering using the quadrupole mass filter
- Charge-state dependent calibration of reaction time to maximize spectral quality
- Improved dynamic range and signal-to-noise ratio (S/N) for fragments using high dynamic range EASY-ETD HD

#### Thermo Scientific<sup>™</sup> PTCR ion source

- Extension of the EASY-ETD ion source
- Generates perfluoroperhydrophenanthrene (PFPP) ions for subsequent gas-phase ion-ion reactions
- Simplifies interpretation of complex spectra by decreasing the charge state of precursors and/or product ions

### Thermo Scientific<sup>™</sup> EASY-IC<sup>™</sup> ion source

- Generates internal calibrant ions for real-time mass calibration of spectra in positive and negative modes
- Provides <1 ppm RMS drift over 24 hours, measured with FlexMix Calibration Solution

### Thermo Scientific<sup>™</sup> UVPD ion source

- Includes a Class 1, 213 nm CryLaS laser system with 2.5 kHz repetition rate, delivering >1.2 µJ per pulse
- Performs dissociation of precursors at any stage of MS<sup>n</sup>, with detection in either the ion trap or Orbitrap mass analyzer
- Compact size, located entirely within the footprint of the instrument

### Thermo Scientific<sup>™</sup> FAIMS Pro Duo interface

- Performs online gas-phase fractionation based on differential ion mobility
- Optimized performance for 100 nL/min to 1 mL/min flow rates

#### Higher mass range with MS<sup>n</sup> (HMR<sup>n</sup>+)

- Extends the mass range to *m/z* 16,000 for detection of precursor and product ions in the Orbitrap mass analyzer
- Precursor ion isolation up to *m/z* 8000 using the linear ion trap
- MS<sup>n</sup>, n = 1 through 10 using any available fragmentation technique

## Software features

### Data system

- High-performance PC with Intel® microprocessor
- High-resolution LED color monitor
- Microsoft<sup>®</sup> Windows<sup>®</sup> 10 operating system

### Thermo Scientific<sup>™</sup> Xcalibur<sup>™</sup> software

- Xcalibur software is the control software for the nextgeneration Thermo Scientific mass spectrometer portfolio
- Accelerates familiarization and reduces training needs

### Orbitrap Ascend Tribrid MS instrument control software

- Tune application for instrument calibrations and checks, diagnostics, and manual data acquisition
- Method editor with a comprehensive application-specific template library, method setup supported by tooltips, and a drag-and-drop user interface to facilitate method development

### **Optional software**

### Thermo Scientific<sup>™</sup> Proteome Discoverer<sup>™</sup> software

Flexible, expandable platform for the qualitative and quantitative analysis of proteomics data

### ProSightPD<sup>™</sup> software

Processing node within Proteome Discoverer software that allows execution of ProSight searches of top-down and middle-down data

### Thermo Scientific<sup>™</sup> ProSightPC<sup>™</sup> software

Stand-alone software for analyzing top-down, middle-down, and bottom-up data

### Thermo Scientific<sup>™</sup> BioPharma Finder<sup>™</sup> software

Integrated solution for protein-based biotherapeutic analysis through molecular and sub-structural data processing utilizing deconvolution and predictive fragmentation pattern algorithms

### Thermo Scientific<sup>™</sup> Compound Discoverer<sup>™</sup> software

Platform for small-molecule structural identification, qualitative, and quantitative data analysis

## **Operation modes**

### Top speed

Maximizes the number of high-quality MS<sup>n</sup> spectra in each cycle by intelligently scheduling MS and data-dependent MS<sup>n</sup> scans based on a user-defined time between adjacent survey spectra

### TopN

Enables a user-defined maximum number of high-quality MS<sup>n</sup> spectra from each cycle

### Dynamic maximum injection time

Maximizes the amount of high-quality data acquired by synchronizing operation of the quadrupole mass filter, ion-routing multipole, and linear ion trap and Orbitrap mass analyzers

### Advanced Peak Determination (APD)

Precursor annotation algorithm for improved charge state assignment to increase the number of precursors available for data-dependent analysis

### **Exclusive technologies**

### Dynamic scan management

Enables intelligent, real-time scheduling, parallelization, and prioritization of acquisition events, and selection, sorting, and routing of precursors to different fragmentation modes and analyzers based on user-selected parameters, including precursor m/z, intensity, and/or charge

### Multiple dissociation options

Precursor dissociation can be performed in the ion-routing multipoles (HCD), the linear ion trap (CID, optional ETD/ETciD, optional UVPD) or both (optional EThcD), with product ion detection in the linear ion trap or Orbitrap mass analyzer at any stage of MS<sup>n</sup> analysis

### Automatic gain control

Ensures that the ion-routing multipole is always filled with the optimum number of ions for any acquisition type

### Proton transfer charge reduction option

Performs ion-ion reaction to reduce charge states of selected precursors or product ions and can be applied at any stage of  $MS^n$  analysis

### Real-time database search

Searches user-defined database in real time to then select identified/or not identified precursors for further MS<sup>n</sup> experimentation. Example: Selection of identified precursors only for SPS MS<sup>3</sup> quantitation increases the accuracy and proteome coverage of TMT experiments.

### **Real-Time Library Search**

Intelligent acquisition provides higher confidence with real-time spectral matching against a local, customizable spectral library for decision-based triggering of MS<sup>n</sup> acquisition events. It enables library directed MS<sup>n</sup> data acquisition to increase confidence in annotation, including structurally related compounds and improve structured assignment of isomeric species, unknown characterization, and other applications.

### TurboTMT mode

Novel processing mode powered by Phase-Constrained Spectrum Deconvolution Method ( $\phi$ SDM) designed to improve the acquisition rate of TMT experiments

### **Precursor Fit filter**

Allows selection of precursors with defined specificity

### Advanced data-dependent experiments

### TMT SPS MS<sup>3</sup> aided by Real-Time Search

TMT SPS MS<sup>3</sup> experiment with real-time protein database search to perform SPS MS<sup>3</sup> only on identified precursors

### **Universal method**

Powered by Dynamic Maximum Injection Time, ensures the best results from samples with unknown concentrations

### Product-ion triggered-MS<sup>n</sup>

Fragment ion or neutral loss-triggered  $\ensuremath{\mathsf{MS}}^n$  experiment, including at the same  $\ensuremath{\mathsf{MS}}^n$  level

### **Isolation offset**

Custom centering of the precursor isolation window, optimized for broad isotopic distributions

#### Quanfirmation workflow

Synchronous acquisition of HRAM SIM with full scan MS/MS in the linear ion trap for precursor identity confirmation

# Thermo Scientific<sup>™</sup> SureQuant<sup>™</sup> internal standard (IS) targeted protein quantitation workflow

Enhanced PRM experiment that leverages internal standards to guide and automatically maximize outcomes and data quality for real-time targeted proteomics analysis

### **Performance specifications**

Orbitrap mass range	Standard mass range $m/z$ 40–2000, mid-mass range $m/z$ 200–6000, and optional HMR <sup>n</sup> + mass range $m/z$ 500–16,000
Orbitrap resolution	Standard resolution settings range from 7500 to 480,000 (FWHM) at $m/z$ 200, with isotopic fidelity up to 240,000 FWHM
Acquisition rate*	Orbitrap MS <sup>n</sup> acquisition rates up to 45 Hz when measured using a data-dependent experiment with FlexMix calibration solution, MS and HCD MS/MS resolution setting of 7500 @ $m/z$ 200, with a maximum ion fill time of 11 milliseconds; linear ion trap MS <sup>n</sup> acquisition rates up to 50 Hz using a data dependent experiment with FlexMix calibration solution, Orbitrap mass analyzer resolution of 240,000 and HCD MS/MS ion trap detection range of $m/z$ 200–1400, with a MS/MS maximum ion fill time of 10 milliseconds
Orbitrap mass accuracy*	External calibration achieves <3 ppm RMS drift over 24 hours, measured with FlexMix; internal calibration achieves <1 ppm RMS drift over 24 hours, measured with FlexMix
MS/MS Electrospray Ionization (ESI) ion trap sensitivity	2 $\mu$ L of a 50 fg/ $\mu$ L solution of reserpine (100 femtograms total) injected at a flow rate of 500 $\mu$ L/min produces a minimum S/N ratio of 200:1 for the transition of the isolated protonated molecular ions at <i>m</i> / <i>z</i> 609 to the largest two product ions, <i>m</i> / <i>z</i> 397 and <i>m</i> / <i>z</i> 448, when the mass spectrometer is operated at unit resolution in the full scan MS/MS mode, <i>m</i> / <i>z</i> 165–615; the test requires the HESI II probe
Dynamic range	>5000 within a single Orbitrap mass spectrum
MS scan power	Collects MS <sup>n</sup> , for n = 1 through 10, detected in either the Orbitrap or linear ion trap mass analyzer
Synchronous precursor isolation	Samples up to 20 precursors per MS <sup>n</sup> scan
Precursor multiplexing	Up to 20 precursors can be multiplexed using the quadrupole mass filter and front ion-routing multipole to perform selected ion monitoring or tandem MS
Polarity switching	One full experimental cycle acquired in <1.1 seconds where the cycle consists of acquiring one full MS scan in positive and negative polarity at a resolution setting of 30,000

### Performance specifications (continued)

ETD option efficiency	Reaction with ETD anions dissociates the isolated 2 <sup>+</sup> MRFA precursor ( $m/z$ 262) to generate a series of fragments that have a summed relative abundance $\geq$ 15% to the precursor measured in the linear ion trap, using FlexMix solution infused at 6 µL/min
UVPD option efficiency	Exposure of 100 ms dissociates the isolated caffeine molecular ion ( $m/z$ 195) to generate the $m/z$ 138 product ion with a relative abundance of $\geq$ 25% to the precursor measured in the linear ion trap, using FlexMix solution infused at 6 µL/min
PTCR option efficiency	Reaction time of 100 ms causes isolated MRFA 2 <sup>+</sup> molecular ion ( $m/z$ 262) to convert to a charged reduced ion at $m/z$ 524, with a relative abundance of $\geq$ 25% to the precursor measured in the linear ion trap, using FlexMix calibration solution infused at 6 µL/min

\* Under defined conditions

## Installation requirements

### Power

- 230 Vac ±10% single phase, 50/60 Hz, with ground, 3× outlets at 15 A each
- 120 or 230 Vac single phase with earth group for the data system

### Gas

- Ultra-high purity Helium (99.999%) with less than 1 ppm each of water, oxygen, and total hydrocarbons
- High purity Nitrogen (99.5% pure, flow rate 15 L/min) gas supply for the API source and ion routing multipole (IRM)

### Dimensions ( $w \times d \times h$ )

• 1270  $\times$  805  $\times$  703 mm (50  $\times$  31.7  $\times$  27.7 in)

### Weight

• 180 kg (400 lb) without data system, vacuum rough pumps and optional items

### Environment

- System averages 2800 W (10,000 Btu/hr) output when considering air conditioning needs
- Operating environment must be 16–26 °C (59–78 °F) and relative humidity must be 50–80% with no condensation
- Optimum operating temperature is 18–21 °C (65–70 °F)

### EASY-IC/ETD/PTCR ion source options

 Nitrogen supply for EASY-IC/ETD ion source options: Ultra-high purity nitrogen (UHP, 99.999%) with less than 1 ppm each water and oxygen

### FAIMS Pro Duo interface option

• Nitrogen supply for FAIMS Pro Duo interface is 99.5% pure nitrogen at >20 L/min (100 psi)

### Learn more at thermofisher.com/OrbitrapAscend

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