Mass spectrometry

Scale up your science

Orbitrap Ascend Tribrid mass spectrometer
With new capabilities for multiplexed quantitative proteomics, PTM analysis, native protein characterization and metabolomics, the Thermo Scientific™ Orbitrap™ Ascend Tribrid mass spectrometer delivers simply the most powerful, flexible BioPharma and Omics platform available. The Orbitrap Ascend Tribrid mass spectrometer can quantify more samples at lower concentrations, achieve greater coverage, and characterize the largest biopharmaceuticals on a single platform, all with less instrument setup to maximize ease-of-use.

**Overcome the challenges in life science mass spectrometry**

**Orbitrap Ascend Tribrid mass spectrometer offers extraordinary sensitivity and versatility**

<table>
<thead>
<tr>
<th>Application</th>
<th>Description</th>
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</thead>
<tbody>
<tr>
<td>General proteomics</td>
<td>Complex samples, wide dynamic range, labile PTMs</td>
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<tr>
<td>Quantitation</td>
<td>Accurate proteomewide quantitation with high throughput</td>
</tr>
<tr>
<td>Structural biology</td>
<td>Low level samples, complex mixtures, high mass range</td>
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<tr>
<td>Biopharmaceutical analysis</td>
<td>Protein-drug structure elucidation, impurity ID, complex</td>
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<tr>
<td>Small molecule analysis</td>
<td>Complex samples, wide dynamic range, labile compounds, isomers</td>
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Thermo Scientific™ Vanquish™ Neo UHPLC system with the Thermo Scientific™ FAIMS Pro™ Interface on the Orbitrap Ascend Tribrid mass spectrometer
What’s new on the Orbitrap Ascend Tribrid mass spectrometer

Key hardware innovations

**QR5 Segmented Quadrupole Mass Filter with Hyperbolic Surfaces**
Improved sensitivity with 0.4 m/z precursor isolation widths

**Advanced Active Ion Beam Guide**
Prevents neutrals and high velocity clusters from entering mass resolving quadrupole

**EASY-IC/ETD/PTCR Ion Source options**
- Based on Townsend discharge
- Reliable and easy to use

**Electrodynamic Ion Funnel**
- Efficient ion transfer
- Broad tuning curves
- Optimized for labile compounds

**Letter-Box Ion Transfer Tube**
Increased ion flux

**Back Ion Routing Multipole**
Enables parallel analysis; performs HCD at MS² stage

**Front Ion Routing Multipole**
Enables parallel analysis: performs HCD at MS³ stage

**OrbitrapMass Analyzer**
Offers resolution > 480K FWHM and acquisition rates up to 45 Hz, TurboTMT

**Auto-Ready ion source**
- Automated and remote calibration
- Fully internal, no need to remove source (nESI, FAIMS)
- Calibration can be scheduled
- Improves data consistency

**Real-Time Database/Library Search**
Database Search/Spectral library directed MSⁿ acquisition

**UVPD option**
Unique fragmentation mode for analyte structure elucidation

**Modified Linear Ion Trap Mass Analyzer**
- Up to 50 Hz MSⁿ and sensitive mass analysis
- Six fragmentation types: CID, HCD, ETD/EThCD/ETciD and UVPD; HMRⁿ+ of up to m/z 8,000
- Extended front section of the high-pressure cell for improved control over ETD and PTCR reactions

**Extended High Mass Range MSⁿ option**
- HMRⁿ+ allows detection of up to m/z 16,000
- Increased MSⁿ fragmentation coverage with low mass range m/z 40

**Options**
- UVPD | ETD | PTCR | HMRⁿ+ | UVPD | FAIMS Pro Duo interface

**Teal**: New on this platform
Quantify more low-level proteins with increased sample throughput using multiplexed quantitative proteomics

Increases in quantitative coverage of lower abundant proteins is critical to detect biologically important molecules. Gaining quantifiable IDs allows scientists to drive more insights into their systems and make more substantiated claims about their data sets. Similarly, improvement in experimental throughput is important, as it reduces the cost of the entire experiment proportionally. Experiments utilizing multiplexing already allow for higher throughput relative to label free, but with the faster scan speeds of the new Orbitrap Ascend Tribrid mass spectrometer, the user can expect equally high protein coverage in about 30% fewer of fractions when using Thermo Scientific™ Tandem Mass Tag™ (TMT)™ multiplexing reagents.

“We need to continue reducing the LC-MS acquisition time even further with a better depth of analysis of smaller numbers of fractionated biological samples. Orbitrap Ascend Tribrid MS enables this solution.”

Steven P. Gygi, Professor of Cell Biology, Harvard Medical School

Twelve high pH fractions were analyzed using 65 min SPS MS³ Real-Time Search method. The data was processed using Harvard pipeline. The results from Thermo Scientific™ Proteome Discoverer™ software agree closely with these results.

Sample courtesy of Steven Shuken, Qing Yu, and Steven Gygi, Harvard Medical School.
Increases phosphopeptide IDs and site localization

Greater coverage of phosphopeptides directly impacts researchers’ ability to measure a biological system, signaling pathway or cellular mechanism of drug activity. Without high confident identification, strong biological conclusions cannot be ascertained. The Orbitrap Ascend Tribrid mass spectrometer gives significantly higher confidence to labile PTM analyses.

“One thing on the Orbitrap Ascend Tribrid MS that is really exciting is the new ion routing multiples, and how by moving the collision cell forward, the speed of the system has gotten even faster from the last model. That means that we can dig deeper in a sample, and we can detect more things in a shorter period of time.”

Joshua Coon, Professor of Chemistry, University of Wisconsin–Madison

![Improved phosphopeptide analysis](image)

<table>
<thead>
<tr>
<th># of PTMs</th>
<th>Phosphopeptides</th>
<th>Modification Sites</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orbitrap Eclipse Tribrid MS</td>
<td>10250</td>
<td>7035</td>
</tr>
<tr>
<td>Orbitrap Ascend Tribrid MS</td>
<td>12050</td>
<td>8821</td>
</tr>
</tbody>
</table>

+18%  +25%

0.5 ug load, 90 min gradient, ES903 column, 15,000 resolution at m/z 200 with 27 ms max injection time.

Sample courtesy of Yuchen He, Prof. Joshua Coon’s lab, University of Wisconsin–Madison.
Native protein measurements allow for probing protein-protein interactions as they occur in the cell. Measuring up to $m/z$ 16,000 enables the analysis of larger and lower charged membrane proteins like GPCRs that are the targets of many therapeutics.

“...The Orbitrap Ascend Tribrid MS combines two fundamental features that make it the perfect instrument for intact protein analysis, working as two high-performance mass spectrometers in one: first, mixtures of large proteoforms can be efficiently interrogated using a refined targeted proton transfer charge reduction (tPTCR) data acquisition. Then, using native electrospray ionization, their multi-proteoform complexes can be detected up to $m/z$ 16,000 thanks to the improved HMR$^n+$ mode. This will enable researchers to gain new insights into proteoform biology to be used in basic and translational research.”

Luca Fornelli,
Assistant Professor,
University of Oklahoma
**Comprehensive native analysis of therapeutic proteins**

Protein therapeutics often generate undecipherable spectra due to their high level of native heterogeneity. By using a combination of PTCR and the HMR+ mode, interpretable spectra can be generated which simplifies the ion population in each scan. This allows for identification of native or low abundant proteoforms, which were indiscernible before, clearly identifiable. The ability to combine HMR+, PTCR, and a multitude of dissociation techniques makes the Orbitrap Ascend Trybrid mass spectrometer the most powerful system for comprehensive characterization of therapeutic proteins.

"The m/z 16,000 range and large ion transmission capabilities of the Orbitrap Ascend Trybrid MS enable full use of PTCR charge reduction for complete characterization of even the most complicated molecules."

Wendy Sandoval, Director, Genentech

Sample courtesy of Wendy Sandoval, Genentech
Automated/remote/pre-scheduled system checks and calibrations

The new Auto-Ready ion source is a fully integrated, standard, easy to use feature which allows the user to increase their productivity. There is no need to remove the source (HESI, nESI, or FAIMS) for calibration, meaning that there is no interruption to the experimental set up between recommended calibrations. The user can automate the calibration to start at a scheduled time every week when there are no experiments planned. The calibration can be run completely remotely, no matter what the last experiment was. Since the calibration can be scheduled regularly and automatically, users can expect improvements in the consistency of their data by maintaining regular calibration without interrupting vital work.

New Auto-Ready ion source
- Separate ion transfer tube
- Dedicated emitter
- Robust delivery system

Automated weekly calibrations
- Calibration can be scheduled to run every Wednesday at 12:00 AM in Check, Calibrate if Required mode.
- Flex VC: full (> 70%)
- Optional Calibration

Learn more at thermofisher.com/OrbitrapAscend