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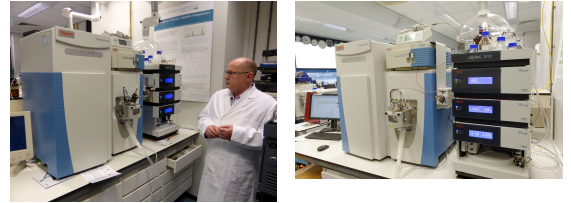
## Q ExactivePlus Orbitrap LC-MS/MS

<https://labfacilities.wur.nl/SearchDetail.aspx?deviceid=844ee816-b824-4fe6-8142-6746790053dd>

### **Brand**

Thermo Scientific

### **Type**



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### **Organisation**

Plant Sciences Group

### **Department**

Bioscience

## Description

This analytical platform is designed for use in a wide range of both metabolomics and proteomics analyses. With its high sensitivity, fast scan speed and multiplexing capabilities at high mass resolution, the Q Exactive Orbitrap FTMS mass spectrometer is an outstanding detector for fast and high-throughput separation and mass peak annotation techniques required for metabolomics and proteomics analyses.

The Q Exactive is unique in its ability for fast ionization mode switching at high mass resolution, providing alternating positive/negative scans of accurate mass ions during fast chromatographic separation. In addition, the high resolution MS/MS data, generated by using the quadrupole (Q) option with high collision dissociation (HCD), enables metabolite and peptide identification and quantitation of even more compounds with greater confidence. Attached to a Dionex UltiMate 3000 U-HPLC system for fast chromatographic separation, a photodiode array detector and the online (+/-) switching capability at high mass resolution allows the most comprehensive metabolomics profiling of compounds present in complex samples and saves considerable time during experiments in which screening in both ionization modes is necessary or desired.

Alternatively, for proteomics applications the system can be connected to a nanoUPLC system for maximal sensitivity in detection and identification while peptides are separated either in 1D or 2D mode. This set-up is optimal for proteomics analysis of complex peptide mixtures. The precursor ion trapping option (C-trap) and fast switching Orbitrap MS/MS guarantee a very high sensitivity in detection and identification providing accurate mass MS/MS spectra.

### Principle

Compounds or peptides in crude or more purified extracts are firstly separated by LC and then ionized at the source of the Q Exactive Orbitrap FTMS. The S-lens at the source filters the ions from non-charged compounds and impurities. Subsequently, the Quadrupole can be activated to filter for only one specific ion of interest up to a wide range of ions that are transferred to the C-trap. Here ions can be sent to the Orbitrap mass analyser with or without high collision fragmentation in the HCD cell, depending upon the users demands. Within the Orbitrap, the  $m/z$  values of the entering ions are accurately determined at high mass resolution, based on Fourier transformation of the mass-dependent ion oscillation frequency. The high mass resolution of all ions in combination with indicative MS/MS fragments ensures sensitive and accurate detection and quantification of a large number of target molecules present in the extracts. In the untargeted mode, fast scanning at both a wide  $m/z$  range and a high mass resolution enables the detection and relative quantification of hundreds to thousands of molecules.

### Metabolomics

For metabolomics analyses, crude plant extracts are prepared using a suitable general extraction fluid (e.g. methanol / methanol-water), sonication and filtration. If needed samples are concentrated by including a freeze drying step. Extracts are then injected one-by-one into the HPLC or UPLC and pass through a suitable column to separate the individual constituents before they enter the photodiode array detector and finally the Q Exactive mass spectrometer. For targeted analyses, the Quadrupole mass filter in combination with HCD fragmentation at high mass resolution enables sensitive and highly specific detection of both negative and positively charged molecular ions of compounds of interest. For untargeted approaches, the Q Exactive enables the simultaneous detection of hundreds to even thousands metabolites within a wide  $m/z$  window at high mass resolution, with both the ability for scan-to scan polarity switching to cover as many metabolites as possible and simultaneously collecting HCD fragments for compound confirmation and (partial) characterization. Using dedicated software for untargeted data analyses, large series of samples can be compared for differences and similarities in the relative abundance of all metabolites detected, both known and yet unknown compounds.

### Proteomics

For proteomics analyses protein extracts from diverse samples can be analysed. Protein mixtures are converted to peptides, using a site-specific protease (trypsin in most cases). After purification of these peptides, the highly complex peptide mixture (up to a hundred thousand peptides) is injected onto a nanoLC reversed phase column. Peptides can be separated in one gradient (in 1D mode), or for even higher separation resolution in 2 orthogonal LC gradients (2D mode). The nanoLC system is on-line directly coupled to the nanospray inlet of the mass spectrometer. The peptide ions are detected in MS mode, resulting in very high resolution accurate mass spectra. Subsequently, multiple peptides are individually selected by the Quadrupole filter, collected in the C-trap, transferred to the HCD cell for fragmentation, and redirected into the C-trap and finally injected into the OrbiTrap for a fast (50-100 millisecond) MS/MS spectrum. A typical analysis of a complex mixture in a 1 hour gradient will result in more than 60.000 MS/MS spectra, matching to 10- to 20.000 peptides derived from more than 1000 proteins. For all these peptides an abundance value is available, which enables a relative quantitative comparison of peptide abundances between multiple samples (untargeted analysis).

## ***Technical Details***

Supplier: Thermo Scientific Instruments Specifications: Q Exactive MS fitted with a Dionex UltiMate 3000 Liquid Chromatography system or with EASY NanoLC. The Thermo Scientific Q ExactivePlus benchtop LC-MS/MS combines high-performance quadrupole precursor selection with very high resolution, accurate-mass Orbitrap FTMS detection to deliver high performance and tremendous versatility. All features are essential for the envisaged applications and specifications are greatly advanced compared to the existing old instruments. The key features are:

- Resolving power up to 280,000 at  $m/z$  200
- General mass range 100 – 1,200 Da up to 500 – 6,000 Da
- Maximum scan speed 12Hz
- Intra-scan dynamic range > 5000:1
- Quadrupole mass filter
- C-trap precursor ion trapping
- High-energy collisional dissociation (HCD)
- Spectral multiplexing for enhanced duty cycle
- S-Lens ion source for increased sensitivity
- Both ESI and APCI sources
- Both UPLC and nanoUPLC upfront separation options
- Combined with Diode Array detector

## ***Applications***

- Untargeted metabolomics: global profiling and comparison of hundreds of known and yet unknown metabolites
- Untargeted proteomics: identification and quantification of thousands of proteins
- Targeted analyses natural and chemical compounds, like plant (secondary) metabolites, pesticides, drugs etcetera
- Compound identification using MS/MS at ultra-high mass resolution
- Multiplexed targeted (semi)quantitation of hundreds of peptides or compounds in complex samples