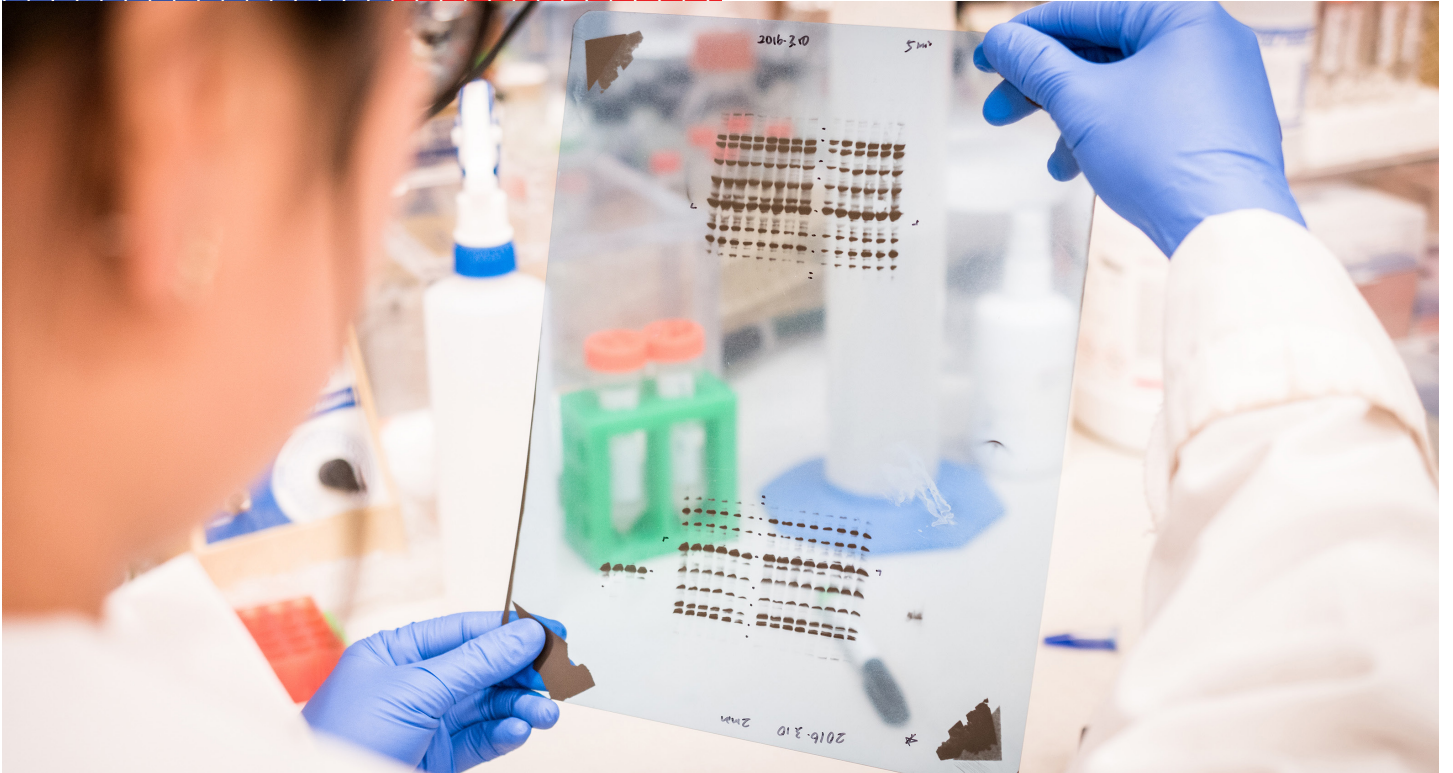
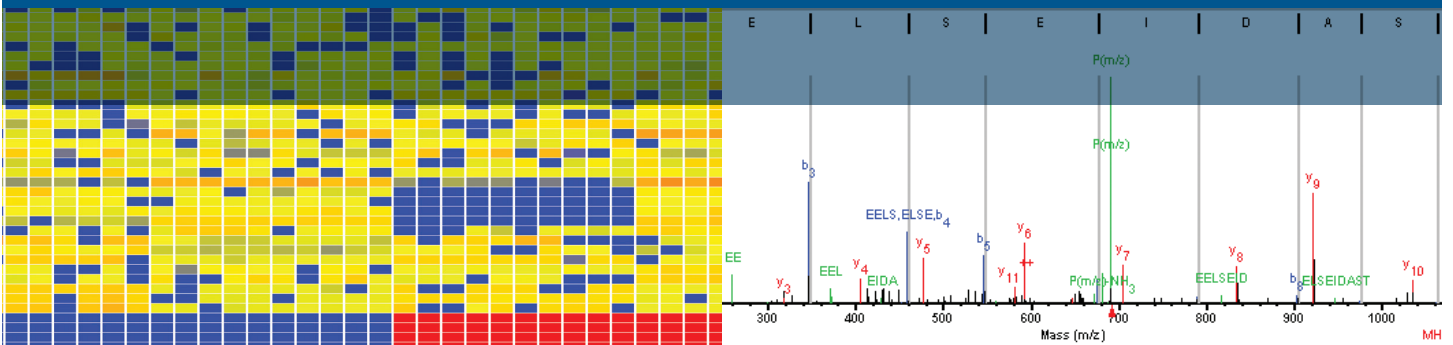


Enhance Your Proteomics Research

Agilent Proteomics



Proteomics Solutions For Your Application

Proteomics is a key tool that can be applied to biological questions across a wide range of disciplines and areas of study. Wherever proteins play a role, proteomics offers the potential for insight that often can be gained no other way.



Basic and clinical research

Identify and verify peptide/protein biomarkers that provide fundamental insights into biology.

Agriculture and crop optimization

Identify and understand protein pathways to optimize crop development, increase yield, and improve pesticide/herbicide resistance.

Food analysis

Identify proteins that correlate with major traits such as food quality, authenticity, immunogenicity, taste, and nutritional value.

Pharmaceutical discovery

Identify proteins as markers of drug-induced toxicity during drug discovery and development.

Biofuels and synthetic biology

Verify protein synthesis to maximize output of the desired end product.

Essential Tools for Discovery and Targeted Proteomics

The field of proteomics has rapidly expanded to cover just about every aspect of protein research from identification and characterization of post-translational modifications, to biomarker discovery and quantitation. Because proteomics analysis presents many distinct challenges, your research goals need to be met by comprehensive, optimized, and practical workflows for accurate and reproducible results.

Proteomics studies can be broadly categorized as:

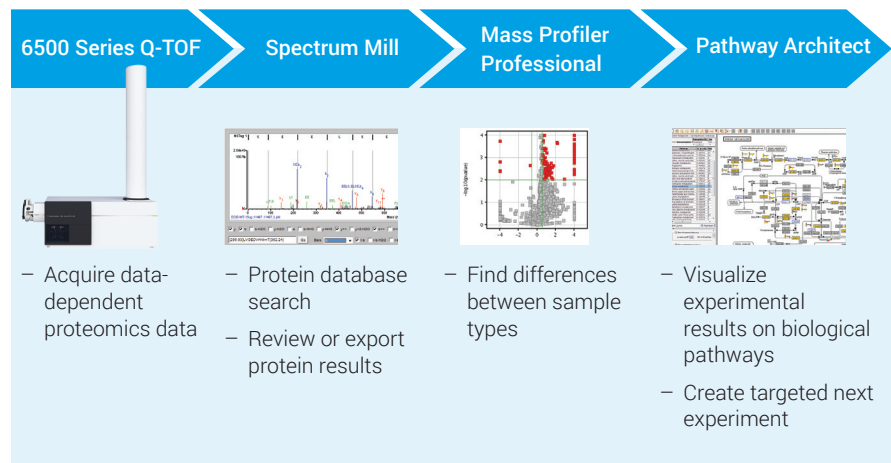
Discovery Proteomics

Identification of proteins without any prior knowledge of what proteins may be present. This is accomplished by searching MS/MS spectra against a protein database. Discovery proteomics can also be used to find differences between sample types, such as control versus treated.

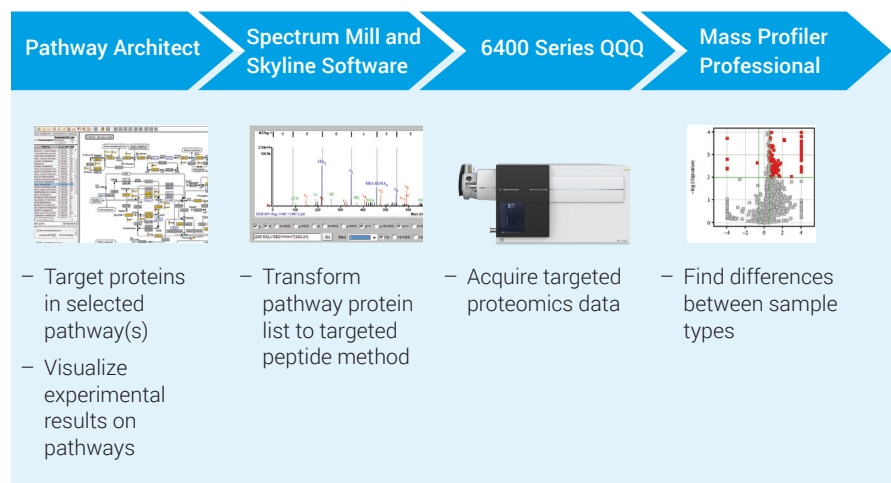
Targeted Proteomics

Confirmation of proteins based on prior knowledge, such as literature, genomic studies, or pathway analysis. Targeted proteomics may also involve accurately quantitating proteins of interest.

Agilent provides the tools you need for both discovery and targeted proteomics research, with a product portfolio that supports nanoflow and standard-flow HPLC and mass spectrometers that deliver accurate protein identification and quantitation.



Discovery proteomics



Targeted proteomics using pathway-directed information to inform the next experiment

Discovery Proteomics

The abundance of proteins in a sample of interest can vary by orders of magnitude, and often the most interesting proteins are present at very low levels. Discovery proteomics places particularly high demands on separation, analytical sensitivity, and informatics.

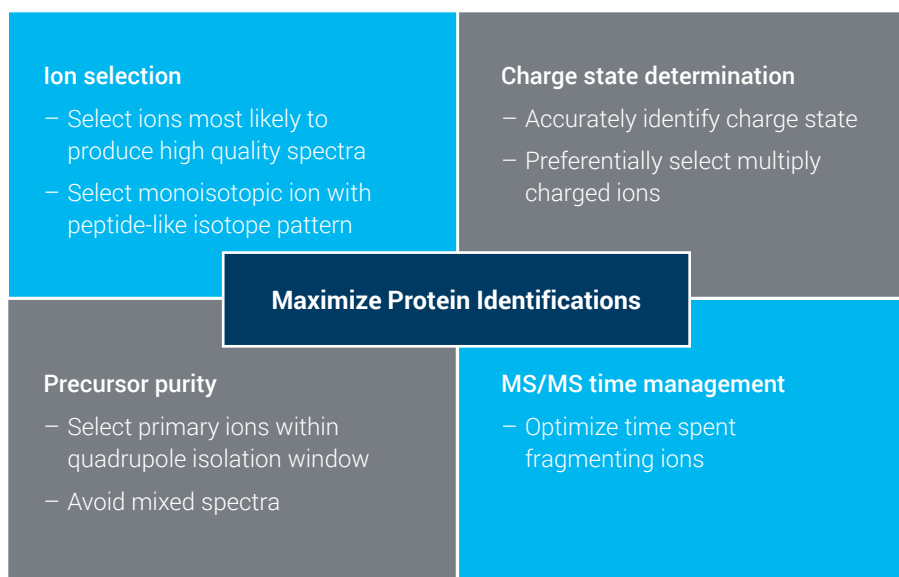
Protein samples are typically analyzed by LC-MS/MS in a data-dependent mode; therefore, a well-designed data-dependent engine (DDE) is an essential feature of the MS instrument. Done well, this approach delivers more high-quality peptide MS/MS spectra, leading to the identification of more peptides and a more complete picture of the proteins present in the sample.

The effectiveness of the DDE depends on the ion selection algorithm to choose the precursor ions most likely to be peptides and to yield high-quality MS/MS spectra. The challenges for effective peptide ion selection are highlighted in the graphic below.

The Agilent 6550 iFunnel Q-TOF LC/MS system dramatically boosts analytical sensitivity, enhancing the detection of low-level peptides.

It also includes an advanced DDE algorithm incorporating all the elements for peptide ion selection, resulting in more confident protein identifications. For comprehensive analysis, the fully-automated iterative MS/MS acquisition mode performs sequential injections with automatic exclusion of precursors previously selected.

The ability of an HPLC to separate peptides can also significantly impact discovery proteomics results. Better separation yields sharper peaks, which simplifies the task of the DDE, leading to more identified proteins.



Data-dependent logic used to maximize protein identification.

Targeted Proteomics

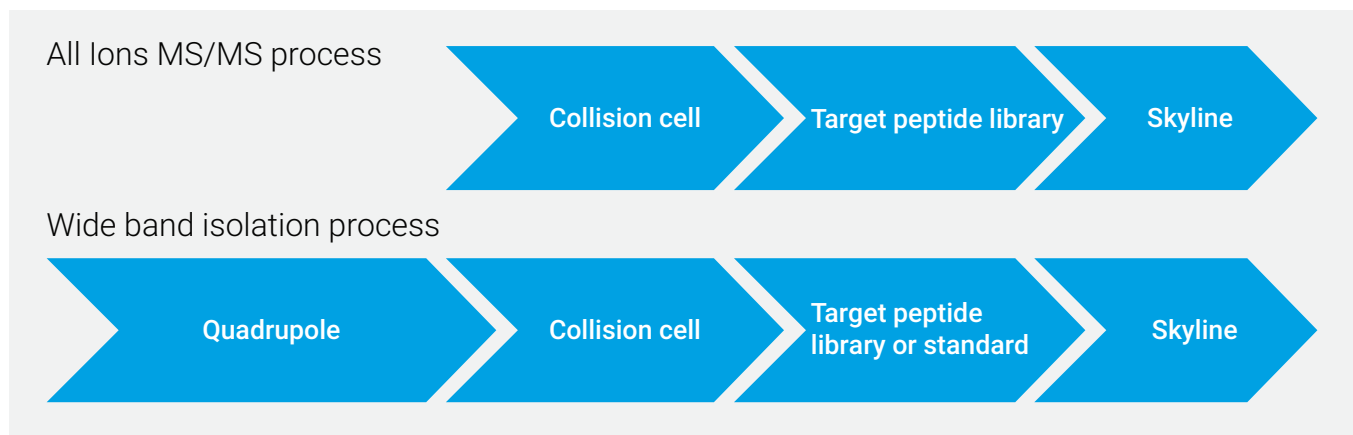
One way that targeted proteomics differs from discovery proteomics is that it screens for peptides from proteins expected to be present based on prior knowledge, such as literature, genomic studies, or pathway analysis.

Accurate mass quadrupole time-of-flight (Q-TOF) instruments are the tools of choice for screening large numbers of proteins simultaneously. Targeted Q-TOF proteomics acquires peptide MS/MS spectra in a data-independent acquisition (DIA) mode. Peptides from specific proteins of interest are identified by screening acquired peptide MS/MS spectra against a peptide MS/MS library.

Data-independent analysis can either use All Ions MS/MS methodology or wide band isolation. All Ions MS/MS does not use quadrupole isolation, and fragments ions in the collision cell with two or more collision energies. Wide band isolation reduces data complexity through isolating and fragmenting a series of narrow mass ranges (~10 Da). These data-independent analysis approaches are only feasible because of the high mass accuracy measurements of the Agilent 6550 iFunnel Q-TOF. Accurate mass peptide MS and MS/MS spectra dramatically reduce incidental false matches when screening a peptide spectral library.

The other approach for targeted proteomics is quantitative analysis of known proteins. The Q-TOF is a very flexible and versatile instrument that can also be used for quantitative proteomics. However, most scientists use triple quadrupole mass spectrometry for routine quantitative analysis because of its superior analytical sensitivity and dynamic range.

Regardless of your approach, data is only the beginning—sophisticated analysis software is key to extracting the maximum knowledge from your experimental results.



Data independent workflows for targeted proteomics.

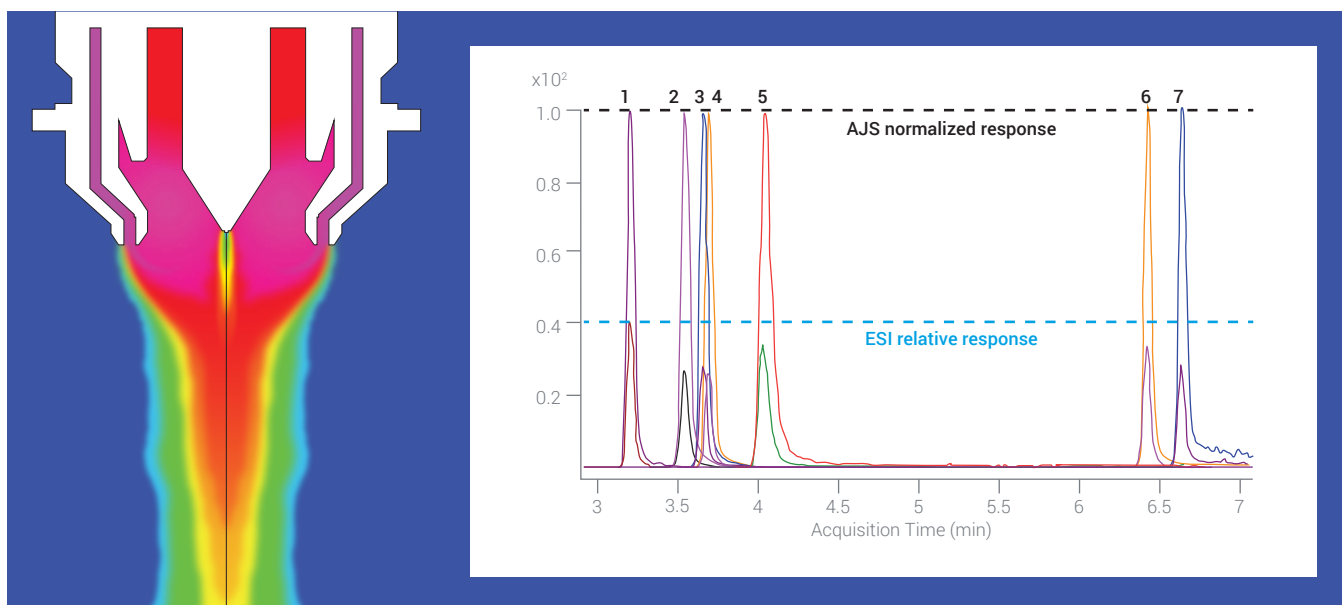
Jet Stream Proteomics

It is now feasible to use UHPLC for high-throughput, robust and reproducible LC/MS analysis of complex proteomics samples for both discovery and targeted workflows. The unique Agilent Jet Stream source technology is at the center of this option.

Historically, proteomics researchers struggled to detect low level proteins using conventional flow chromatography. Due to its enhanced analytical sensitivity, nanoflow LC/MS became widely accepted and routinely used; however, the increased analytical sensitivity of this method comes at the cost of reduced robustness, reproducibility, and ease-of-use.

With innovative Agilent iFunnel technology, which includes the Agilent Jet Stream ion source, you can achieve near-nanoflow HPLC analytical sensitivity using conventional-flow HPLC. The Jet Stream Proteomics solution provides higher speed, greater robustness, and better reproducibility for proteomics analysis.

When sample quantity is not an issue, Jet Stream Proteomics offers the best of both worlds— analytical sensitivity and data quality.



Agilent Jet Stream provides a 3–5X increase in signal for peptides compared to electrospray.

Nanoflow Proteomics

When ultimate analytical sensitivity is required, nanoflow LC/MS using very small bore columns and nanoelectrospray (nanoES) provides the best results. Agilent offers a flexible nanoESI source and Nanodaptor kit to enable nanoflow chromatography using an Agilent 1290 Infinity II System.

Agilent nanoESI

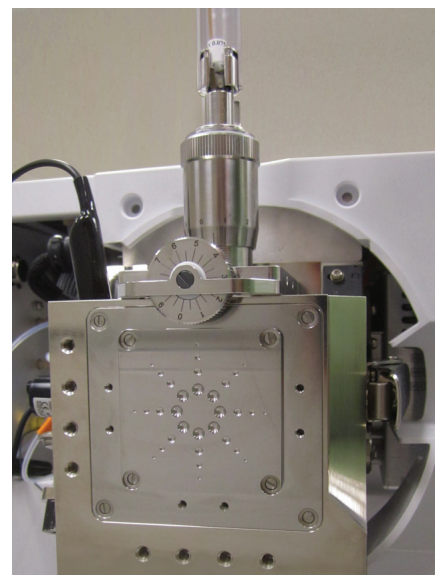
The flexible, easy-to-configure Agilent G1992A nanoESI source is designed to provide the ultimate flexibility for experienced users. The design easily accommodates columns of different lengths and inner diameters as well as multi-dimensional chromatographic configurations to facilitate optimal performance for a wide variety of samples. The enclosed source both enhances safety and reduces background contamination within the analytical laboratory. The Agilent nanoESI source can be used for offline or online nanoES, and provides easy connections for nanocolumns with integral (pulled tips) or separate emitters.

Infinity UHPLC Nanodapter

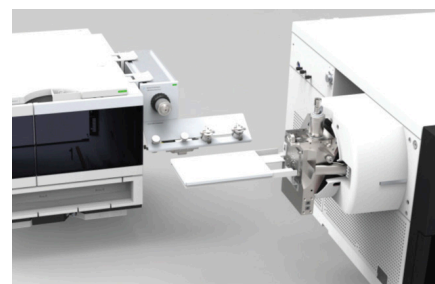
When sample amount is limited, nanoflow LC/MS is the preferred option. The Agilent Infinity UHPLC Nanodapter converts a 1290 Infinity II High Speed Pump to deliver flow rates optimized for nanoflow LC separations. The combination of the Nanodapter with a 1290 Infinity II High Speed Pump precisely delivers optimal flow rates from 100 to 900 nL/min for separations using columns with internal diameters from 75 to 150 μm at pressures up to 1300 bar. This solution is compatible with a wide range of nanoLC columns coupled to the Agilent nanoESI source giving you the flexibility to switch between standard flow Agilent Jet Stream and nanoflow LC/MS depending on sample availability.

Agilent nanoflow solutions

When ultimate analytical sensitivity is required for sample-limited analyses, Agilent offers a flexible nanoflow solution:



Agilent nanoESI source

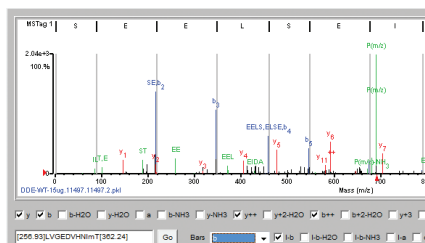


Agilent Infinity UHPLC Nanodapter

Software Tools that Maximize Your Insight

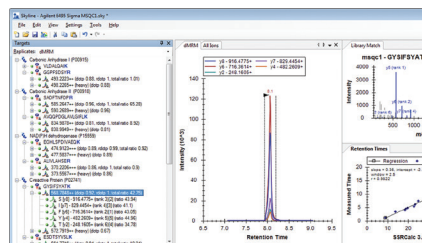
Agilent provides a comprehensive portfolio of software tools supporting both discovery and targeted proteomics workflows.

Agilent Spectrum Mill



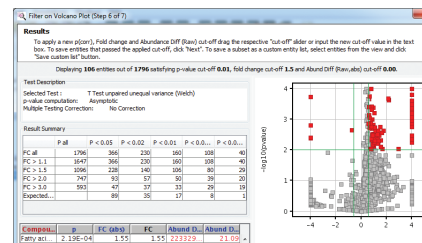
Quickly identify proteins and peptides through fast database searches with automatic or manual match confirmation. Advanced algorithms minimize incorrect matches and calculate false discovery rate. You can also create custom libraries for Skyline software (from the MacCoss group at the University of Washington) for targeted proteomics.

Skyline



Designed specifically for proteomics researchers, the Skyline (from the MacCoss Group at the University of Washington) interface takes you from protein to peptides to precursors and product ions. Tight integration between Agilent and Skyline delivers a seamless user experience. Skyline can be used for Q-TOF analysis in data-independent acquisition mode for targeted proteomics. Peptides are identified by mining acquired MS/MS spectra against a public or custom peptide MS/MS library.

Agilent Mass Profiler Professional (MPP)



A member of the GeneSpring Suite designed specifically for MS data, MPP effectively analyzes and compares proteins or peptides from multiple samples with simple or advanced statistical analysis and visualization tools. MPP facilitates integrated biology analysis by smoothly combining results from genomics, transcriptomics, proteomics, and metabolomics experiments.

LC/MS Instruments and Tools for Proteomics

Chromatographic separation

Agilent 1290 Infinity II System

Offers the industry's best chromatographic performance and flexibility. Taking full advantage of modern sub-2 μm packing material, the 1290 enables outstanding chromatographic separation of complex protein samples. Together with Agilent Jet Stream technology, it delivers fast, reproducible, and sensitive protein results.

Agilent Infinity UHPLC Nanodapter

Switching between standard flow and nanoflow LC has never been easier. Using the Agilent Infinity UHPLC Nanodapter to modify an Agilent 1290 Infinity II High Speed Pump to precisely deliver flow rates from 100 to 900 nL/min provides a robust and easy-to-use nanoflow LC/MS solution for proteomics.

Sample preparation

Agilent AssayMAP Bravo Platform

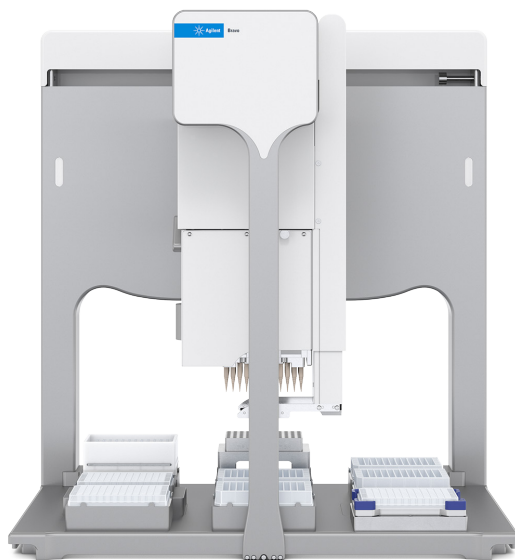
Automates protein/peptide purification, protein digestion, and affinity purification workflows. It is designed for ease of use, and systematically increases reproducibility and throughput.

Agilent Multiple Affinity Removal System

Designed to effectively remove up to 99% of the top 1–14 high-abundance proteins from human or mouse serum samples. It is available in reusable HPLC and spin-filter formats.



Agilent 1290 Infinity II LC System



Agilent AssayMAP Bravo Platform

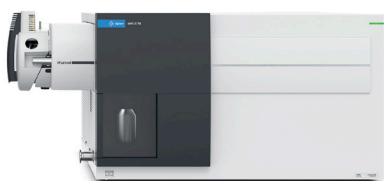
LC/MS analysis

Agilent 6400 Series Triple Quadrupole LC/MS

The proven choice for quantitative applications requiring superior analytical sensitivity, reliability, and overall system robustness. The 6495 with iFunnel Technology incorporating the Agilent Jet Stream source dramatically increases ion sampling, which results in lower detection limits. When sample quantity is not limiting, Jet Stream Proteomics offers the best combination of analytical sensitivity, reproducibility and throughput for peptide quantitation.

Agilent 6500 Series Quadrupole Time-of-Flight LC/MS

A versatile choice for qualitative or quantitative proteomics. These instruments deliver accurate mass measurements with wide linear dynamic range, enabling simultaneous detection of both high- and low-level peptides in complex mixtures. With Agilent iFunnel technology, the 6550 Q-TOF delivers the lowest detection levels of any accurate mass LC/MS/MS instrument.



Agilent 6400 Series Triple Quadrupole LC/MS

Ion sources for proteomics

Agilent Jet Stream Technology

Utilizes thermal gradient focusing technology with super-heated nitrogen sheath gas for greater peptide signal with reduced noise. Agilent Jet Stream technology is designed for standard flow UHPLC separation, and improves peptide sensitivity 3–5X when compared to conventional ESI sources.

Agilent nanoESI Source

A nanoflow ESI source that provides maximum flexibility for experienced users. The design easily accommodates columns of different lengths and inner diameters, facilitating optimal performance for a wide range of proteomics samples.



Agilent 6500 Series Quadrupole Time-of-Flight LC/MS

LC/MS standards

MRM Kits

Developed by MRM Proteomics and available from Cambridge Isotope Laboratories, these kits are designed to evaluate instrument performance for quantitative plasma proteomics. Kits contain tryptic digested plasma samples spiked with isotope-labeled standard peptides of known concentrations; use them to standardize sample preparation workflow or to evaluate day-to-day instrument performance to ensure that you are obtaining consistent results across multi-day experiments. PeptiQuant kits are also available for quantifying large panels of proteins in experimental samples for biomarker discovery and evaluation.

Agilent Complex Proteomics Standard Kit

This kit provides an extract of proteins from Pfu (*Pyrococcus furiosus*). The standard is used for mass spectrometry based proteomics applications to benchmark or evaluate workflow performance and facilitate cross-experiment, cross-instrument, or cross-laboratory data comparisons.

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