GenNext® TECHNOLOGIES

Agilent Technologies

Agilent & GenNext Technology Integration

Synergy for Actionable, High-Resolution Structural Biology Insights

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Combining Protein Footprinting with LC/Q-TOF Technology for Advanced HOS Studies

The fusion of **GenNext's AutoFox® Protein Footprinting Technology** with **Agilent's 6500 Series of Quadrupole Time of Flight LC/MS** platforms delivers actionable, high-resolution structural biology insights into protein surface topology, conformational dynamics, and structural perturbations.

GenNext AutoFox®
Protein Footprinting System

Agilent 6560 Ion Mobility LC/Q-TOF

When pairing the automated radical generation and labeling capabilities of the AutoFox System with the sensitivity and resolution of Agilent's LC/Q-TOF systems, researchers can not only detect changes in biotherapeutic quality and polydispersity, but also quantify and localize specific structural alterations—providing actionable data for formulation, comparability, and stability studies.

Cutting-Edge Technology Synergy

The advanced workflow starts with Hydroxyl Radical Protein Footprinting (HRPF) performed by the GenNext AutoFox System, followed by detection and quantification on an Agilent LC/Q-TOF System, and then finishes with data analysis using GenNext's FoxWare® and Agilent's MassHunter IM-MS software.

GenNext's AutoFox Protein Footprinting System with FoxWare Software is trusted for delivering high-resolution protein footprinting data, enabling researchers to gain profound insights into protein structures and conformational changes. This system is highly regarded for its precision, reproducibility, and efficiency in mapping protein-ligand interactions, therapeutic-target interactions, and protein conformations in their native environments.

Engineered for sensitive and accurate analysis of complex samples, Agilent's 6500 Series of LC/Q-TOF platforms offer a wide range of capabilities and analysis power for every research challenge. For example, **Agilent's 6560 Ion Mobility LC/Q-TOF** system, with its advanced ion mobility separation technology, allows for the precise analysis of complex biological samples. Its ability to separate ions based on their shape and charge provides an added dimension of structural information crucial for comprehensive protein analysis. This powerful instrument uncovers structural information missed with traditional LC/MS systems, including separation of in-class isomers via high-resolution demultiplexing (HRdm).

The GenNext-Agilent workflow fusion facilitates the collection of more comprehensive and high-resolution data, deepening our understanding of protein functions and mechanisms.

TECHNOLOGY WORKFLOW

Phase One:

Protein Footprinting using the GenNext AutoFox® System

In the AutoFox System, the protein of interest is mixed with hydrogen peroxide and then flowed past a pulsing light source which photolyzes the hydrogen peroxide into two •OH and modifies solvent exposed amino acids. Following labeling, the sample is deposited into a quench solution of DMTU and methionine. After quench, oxidation is detected and then quantified using bottom-up proteomics.

Phase Two:

Detection & Quantification on an Agilent LC/Q-TOF System

Labeled protein digests are analyzed using an Agilent LC/Q-TOF system in the 6500 Series (such as the 6530, 6540, 6550, and 6560). The labeled proteins are proteolytically digested into peptides, which are then analyzed to identify and quantify site-specific modifications for comparison of solvent accessibility and conformational changes between the protein samples.

Phase Three:

Data Analysis using GenNext FoxWare Software and Agilent MassHunter IM-MS Software

FoxWare Software's intuitive algorithms easily and efficiently generate qualitative and quantitative comparative studies of Higher Order Structure (HOS). MassHunter IM-MS software provides a platform for interactive browsing and visualization of data from single LC-IM-MS data files.

TECHNOLOGY ADVANTAGES

The fusion of these two state-of-the-art technologies allows researchers to:

- **Achieve Higher Resolution Structural Insights**
  The integrated system offers unparalleled resolution in mapping protein structures and interactions, providing deeper insights that are essential for understanding complex biological mechanisms.
- **Make Faster, High-Value Decisions**
  By significantly reducing the time required for protein structure and drug-target interaction analysis, researchers can obtain rapid and accurate results, enabling faster decision making in drug discovery and development.
- **Gather Comprehensive Data**
  The combination of protein footprinting and ion mobility mass spectrometry delivers a wealth of detailed data, enhancing the ability to make informed decisions.
- **Enhance Drug Design**
  Detailed structural insights facilitate the design of more effective and targeted therapeutics, accelerating development pipelines and improving drug efficacy.