

Product Focus: Proteomics

Accelerating Protein Identification and Quantification with Novel Technologies

The applications of proteomics span an increasingly broad range of fields, including in the clinical laboratory, for the discovery of protein biomarkers, and in medical research. The key technologies used, such as mass spectrometry (MS) and Western Blotting, have seen significant advancements in recent years and also rely on sophisticated sample preparation methods and software to obtain rapid, reliable results.

Advancing automation

There is a growing need to automate upfront sample preparation for proteomic workflows, to keep pace with cutting-edge analytical instruments. The **Agilent AssayMAP Bravo Platform** is an easy to use yet flexible automation solution for preparing protein and peptide samples for liquid chromatography MS (LC/MS) analysis. The AssayMAP platform automates LC/MS sample preparation workflows that include one or more of the following applications: affinity purification from complex biological matrices, on-cartridge and in-solution enzymatic digestions, reversed-phase peptide/protein cleanup, phosphopeptide enrichment, and peptide fractionation for comprehensive protein characterization.

The AssayMAP Bravo Platform is a state-of-the-art Bravo liquid handler enhanced with a Bravo AM Head containing precision flow syringes. Liquid flow is precisely controlled to accommodate quantitative binding and elution in a single pass. Recoveries approach 100% with reproducibility similar to HPLC (<5% CV). A simple user interface allows scientists to easily set key assay parameters for error-free, push-button routine operation. The platform is based on three key elements: (1) Microchromatography cartridges with a wide range of standard chemistries; (2) The Bravo automated liquid handler equipped with proprietary, positive displacement probe syringes; and (3) Intuitive software designed for simplicity in an open access environment.

Agilent AssayMAP Bravo Platform



The new automated liquid handling system from **Eppendorf**, the **epMotion® 5075vt**, provides reliable protein purification, and includes a vacuum unit for filter plates. With this integrated vacuum station and Eppendorf ThermoMixer®, the epMotion 5075vt allows true walk-away automation of purification protocols that rely on vacuum filtration or magnetic beads.

The epMotion 5075vt is all about productivity and flexibility. Loaded and unloaded with the gripper, the vacuum station adapts automatically to any filter plate type. Silent operation of internal pump with no need for extra tubes, wiring, or reservoir maintenance. The Eppendorf silicone mat accessory helps to save time and money by allowing you to use only portions of a 96-well plate when working with smaller sample numbers. The VAC thermo lid accessory also reduces the extraction time substantially, by accelerating the ethanol evaporation step.

Mass spectrometry-based proteomics

The popularity of MS for the identification and quantification of proteins has grown exponentially in recent years, and novel methods are continuously being developed to delve deeper into the proteome. The **timsTOF Pro from Bruker** is a state-of-the-art quadrupole time-of-flight (QTOF) mass spectrometer, the first instrument of its kind to be powered by the unique combination of trapped ion mobility separation (TIMS) and parallel accumulation serial fragmentation (PASEF), for high speed and high sensitivity proteomics. The timsTOF Pro can now be integrated with the novel LC system, the Evosep One, for robust, high-throughput clinical proteomics. The novel pairing of these two technologies enables biomarker research and validation for large sample cohorts, on over 200 samples per day, which is revolutionary for clinical proteomics.

TIMS coupled with TOF-MS and novel chromatographic separation offers increased peak capacity, with peak widths of two seconds, and the speed to handle the increased complexity when using short gradients, with superior reproducibility of results.

The unparalleled combination of separation power, speed and sensitivity of the timsTOF Pro reduces overhead-time and maximizes the analytical depth of coverage in high throughput shotgun proteomics workflows.

timsTOF Pro from Bruker



Sophisticated software

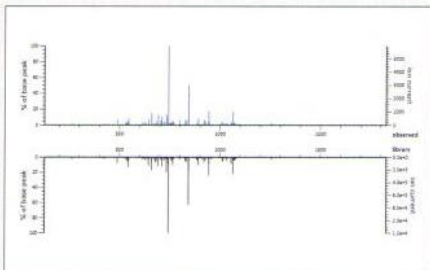
There is more information hidden in raw mass spectral data than current instrument vendor or 3rd party software can recover. **PeakInvestigator® (PI) 2.1** from **Veritomyx Inc.** automatically extracts this additional information from mass spectral profile data, delivers improved mass resolution, optimizes signal-to-noise, and adds confidence intervals to each mass spectrum. PI is the only commercial centroiding software that delivers confidence intervals for both the m/z (mass) and abundance values within spectra. PI lowers the cost and time of discovery proteomic studies by increasing the quality of the MS data generated – whether it be from a single mass spectrum or an entire set of runs. In peptide-centric or bottom-up proteomic studies, these benefits translate into more peptide identifications and higher confidence assignments. PI can be applied to data generated from any mass spectrometer and because it has no user-definable parameters, it automatically delivers information-rich data while eliminating operator variability.

Material compiled by The Scott Partnership



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MS-MS Fragmentation with Mascot from Matrix Science



MassWorks (5.0) calibration and analysis software from **Cerno Bioscience** allows for the rapid quantitation of deamidation/deamination impurities in oligos, peptides, and proteins from direct infusion or fast chromatography MS, using its patented TrueFit MX mixture analysis method. By using raw profile mode MS data and a unique calibration that corrects the MS peak shape, a mathematically exact solution of the overlapping spectra is calculated that can quantify the relative amounts of both the native and the degradation impurity down to levels of 1% or less.

In the development and production of large molecule drugs, sensitive and accurate quantitation of deamidation/deamination impurities is of high importance during both the R&D phase and the manufacturing and distribution process. This process can be dramatically improved using MassWorks, reducing measurement time from hours to minutes while maintaining high sensitivity and accuracy.

Mascot software, from **Matrix Science**, enables researchers to identify, characterize and quantify proteins from mass spectrometry data. The latest version of **Mascot Server (2.6)** has added support for searching spectral libraries, with a single search against any combination of amino acid Fasta files, nucleic acid Fasta files, and spectral library files. When the search is complete, the results can be viewed and interrogated using the unique features of the protein family summary. This provides the advantages of rigorous protein inference together with grouping of related proteins into families. Mascot Server 2.6 has added functionality to enable easy creation of spectral libraries from traditional Fasta search results.

Akoya Biosciences has introduced a novel system for highly multiplexed quantitative protein analysis in tissues.

The CODEX™ System (CO-Detection by indEXing) technology uses a proprietary barcoding technology for simultaneous analysis of up to 50 protein markers at single-cell resolution in FFPE and fresh frozen tissues.

The CODEX™ System integrates with researchers' existing hardware to economically convert their standard fluorescent microscopes into highly multiplexed imaging systems. Akoya's offering is currently in early access sites and is comprised of CODEX Barcoded-Antibodies, reagents, a fluidics instrument and a software analysis suite for image processing through interpretation.

Identifying specific proteins

Western Blotting is a key technique for detecting the presence of specific proteins in a complex mixture.

Milo™ from **ProteinSimple** is the world's first Single-Cell Western platform. It measures protein expression in thousands of single cells in one run (approximately four to six hours), so you can profile heterogeneity in your samples. Milo is a versatile platform that uses conventional Western antibodies for detection, giving researchers access to 10-100x more antibodies than flow cytometry or mass cytometry. Milo can resolve protein isoforms in individual cells, identify target expression heterogeneity, and count and quantitative at the same time. Single-Cell Westerns with Milo reveal cell subpopulations that cannot be measured by conventional Western blotting, which pools thousands or millions of cells to give averaged measurements. Milo brings the power of Westerns to the single-cell level so scientists can study rare cell subpopulations, simplify their phospho-flow studies and get protein validation of their single-cell RNA-seq data.

Expedeon has introduced **2view™**, an innovative double labelled secondary antibody that enables extended detection within the widely used Western blot application. The product offers two modes of detection from a single antibody, and consists of a ternary complex developed using the company's InnovaCoat and Lightning-Link technology.

Milo from ProteinSimple



2view secondary antibodies are labelled simultaneously with a visible (InnovaCoat GOLD nanoparticles) and proprietary chemi-luminescent enzymatic detection system, for two levels of detection.

When performing a Western blot, the amount of protein of interest is usually unknown: **2view** solves this problem by enabling two detections on the same blotted membrane: if the analyte is present down to nanogram level a red band will be visible; for lower amounts of analyte (picogram), the membrane can be developed using a chemi-luminescent substrate via horseradish peroxidase (HRP) and enhanced chemi-luminescent (ECL) reagents. **2view** is easy to use with a single incubation and has a high signal-to-noise ratio, ideal for researchers in the proteomic and immunology fields.

The **Agile R100 Assay Troubleshooting Tool** by **Nanomediical Diagnostics** is a novel, optics-free binding assay platform that enables quick validation of small molecules and protein interactions. Don't send false positives to secondary screening - validate hits with a direct measurement tool that eliminates the confounding interactions that occur in labeled biochemical or cell-based assays. As a compact, fast, and affordable label-free device, Agile R100 provides options to measure weak and tight binders or unstable membrane proteins, and allows for the capture of elusive binding data, including kinetics, for all compounds.

Companies mentioned in this Product Focus

Agilent Technologies	www.agilent.com
Akoya Biosciences	www.akoyabio.com
Bruker Daltonics	www.bruker.com
Cerno Bioscience	www.cernobioscience.com
Eppendorf	www.eppendorf.com
Matrix Science	www.matrixscience.com
Nanomediical Diagnostics	www.nanomedicaldiagnostics.com
ProteinSimple	www.proteinsimple.com
Expedeon	www.expedeon.com
Veritomyx Inc	www.veritomyx.com

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