Agilent

Trusted Answers

# Your Path to Success

## Agilent Solutions for Metabolomics

# Understanding Metabolomics

Agilent is the leading global solution provider for measuring metabolism, offering you a broad array of cutting-edge instrumentation and innovative informatics solutions.

## What is metabolomics?

Metabolomics is the study of endogenous metabolites, called the metabolome, which is a collection of low molecular weight (50 to 1,500 Da) compounds with a wide range of physiochemical properties. Measuring the metabolome provides important information about the functional status of a biological system. Its close proximity to the phenotype of an organism provides complementary information to genomics and proteomics.

## What is qualitative flux analysis?

Metabolomics is a powerful technique for understanding biological systems by measuring the abundance of metabolites, however, understanding is often complicated by a lack of dynamic information. Significant changes in flux through a pathway may not result in altered abundance of metabolite intermediates. Changes in flux are the result of either differences in the amount of enzyme present (for example, transcriptional levels) or activity of the enzyme (for example, inhibitors or mutations). Qualitative flux analysis highlights the relative rate of reactions using stable isotope tracing (typically containing $^{13}C$, $^{15}N$, or $^{2}H$) and results in changes in the natural isotopic pattern of downstream metabolites.

## Agilent metabolomics solutions

Innovative metabolomics solutions from Agilent provide a powerful portfolio of instruments and informatics tools. A common software platform combines the results from multiple analytical techniques, helping answer challenging biological questions faster. Agilent collaborates with leading metabolomics scientists to develop next-generation solutions and workflows to accelerate your metabolomics research.

# Metabolomics is Integral to a Variety of Research Areas

### Basic and clinical research

Identify and verify metabolite biomarkers that correlate with disease states as well as provide fundamental insights into biology.

### Agriculture

Identify and understand metabolic pathways to optimize crop development, yield improvement, and pesticide/herbicide resistance.

### Food and nutrition

Identify the presence or absence of metabolites that correlate with major traits such as food quality, authenticity, taste, and nutritional value, and aid in the development of nutraceuticals.

### Pharmaceutical

Identify metabolites and markers of toxicity for drug discovery and development.

### Environmental

Identify metabolites that relate to the effects of chemicals and other stressors in the environment on a biological system.

### Biofuels and synthetic biology

Identify metabolite profiles to optimize fermentation processes and biofuel production.

### Systems toxicology

Find predictive signatures of toxicity in plasma and urine that can act as surrogates for evaluating the level of exposure to drugs and environmental pollutants.

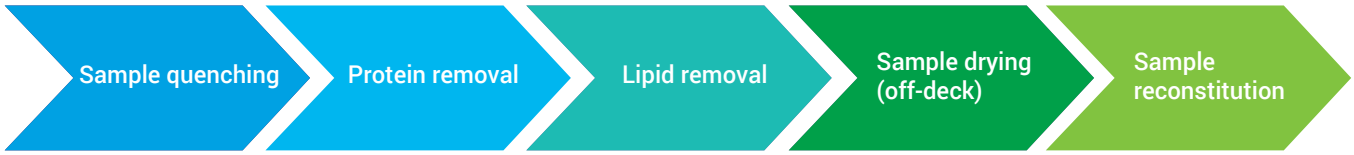
*"Agilent's mass spectrometry systems combine the high acquisition speed and wide dynamic range necessary to identify and quantitate a diverse range of cellular metabolites. We can simultaneously find needles in a haystack and measure the haystack.""*

**– Amy A. Caudy, Ph.D.,**
The Donnelly Centre for Cellular and Biomedical Research
University of Toronto

# Agilent Solutions for Metabolomics

A good sample preparation strategy is vital to generating quality results. The strategy must address quenching metabolism, lysing cells where required, and effectively extracting metabolites. This process must be tailored for the metabolites of interest, compatible with the analytical method, and reproducible.

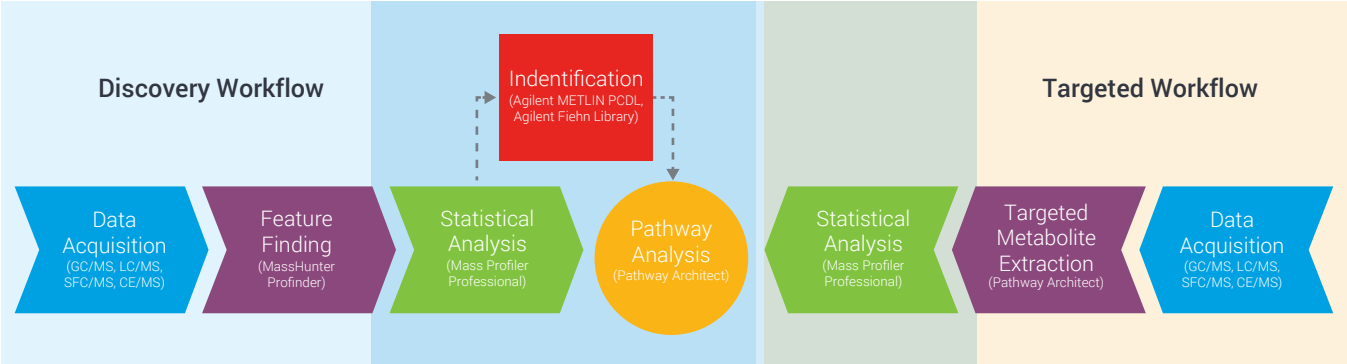
The sample matrix may present challenges in the analysis, including irreproducible chromatography, system fouling, and ionization suppression. Proteins and lipids are two common classes of biomolecules that should be removed from the sample extract. Proteins are precipitated during quenching, and lipids are generally removed by liquid-liquid extraction. Agilent Captiva EMR—Lipid technology offers an alternative approach that both filters the protein precipitate and performs efficient lipid removal in one step. Lipids are removed based on a combination of steric hinderance and hydrophobic interaction. Effective lipid removal significantly improves method reliability and ruggedness while reducing ion suppression of target metabolites.




Manual sample preparation is time-consuming and prone to error, which can impact a metabolomics study. Automation can achieve consistent and reproducible results that are operator independent. Based on the Agilent Bravo automated liquid handler, the Bravo Metabolomics Sample Prep Platform is designed for extracting metabolites from plasma samples.

This standardized plasma sample preparation solution incorporates room temperature quenching and Captiva EMR—Lipid removal technology. After preparation, samples are dried off-deck and then reconstituted on-deck prior to LC/MS analysis.

Agilent Bravo Metabolomics Workbench software uses a form-based interface and requires no complex programming, allowing any user to walk up and start the automated sample preparation. This workbench provides greater batch-to-batch consistency, more precise pipetting, and reduced variability between users.




Agilent workflow for discovery and targeted metabolomics research

## Discovery Metabolomics

Discovery metabolomics is the global profiling of metabolites by hyphenated MS techniques. Following separation and detection of compounds, features are found across all data files. The results are statistically analyzed, differential features are found and then identified.

Agilent has developed robust workflows for performing global metabolite profiling by GC/MS, LC/MS, CE/MS, and SFC/MS, including metabolomics-specific software. MassHunter Profinder is uniquely designed to find and visualize features in a sample batch. Results are then imported into Mass Profiler Professional (MPP) software for visualization and statistical analysis. MPP processing methods can be stored and used to automate analysis. MPP contains powerful statistical algorithms, mathematical models, numerous visualizations, pathway analysis, metabolite identification, and R-scripting.

## Targeted Metabolomics

Targeted metabolomics studies focus on quantifying specific metabolites of interest. Triple quadrupole mass spectrometers are best suited for this task because of their broad dynamic range, high sensitivity, and selectivity for compound confirmation. The results are statistically analyzed and differential features are found.

The Agilent Metabolomics dMRM Database and Method enables straightforward implementation of an optimized LC/MS/MS analysis of 219 central carbon metabolites, including organic acids, sugars, sugar phosphates, and nucleotides. The analytical method was developed in collaboration with Adam Rosebrock, PhD, from Stony Brook University. This solution includes a curated database with retention times, optimized MS/MS acquisition parameters, and a data acquisition and analysis method. Exported results include metabolite names, integrated peak abundances, and CAS chemical identifiers for easy import into MPP for multivariate sample comparisons and pathway analysis.

# Agilent Solutions for Metabolomics

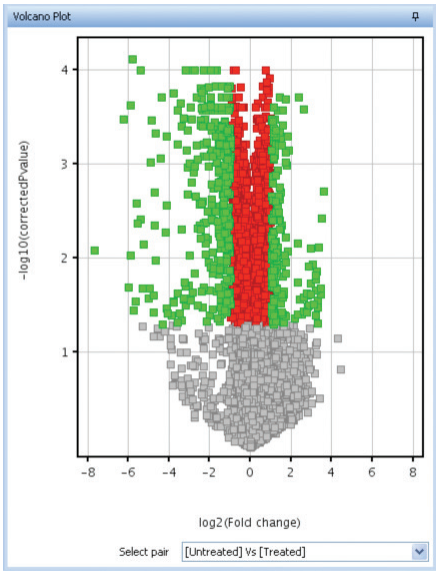
## Software tailored to your metabolomics research needs

Metabolomics researchers face challenges as studies become larger and more complex. Multivariate statistics are used to find differences between study groups. However, it's not enough to know what metabolites are differential; understanding the biological context is critical. Visualizing processed study results onto metabolic pathways facilitates biological understanding. Agilent offers advanced analysis software for processing and interpreting complex metabolomics data.

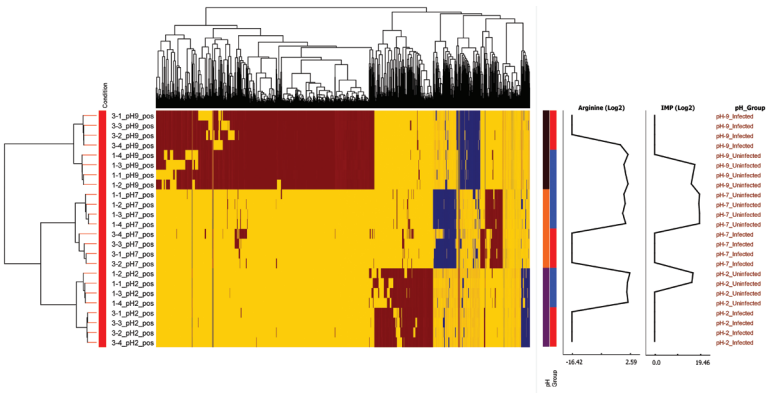
In discovery metabolomics, feature extraction is the first step in the software workflow. MassHunter Profinder provides targeted and untargeted batch feature extraction for mass spectrometric data. It supports MS-only data from Agilent's broad portfolio of mass spectrometers, including time-of-flight instruments as well as nominal mass GC/MS systems. Profinder offers grouping of replicate data files, binning and alignment, chromatogram overlay, manual re-integration of compounds, and easy export of results to MPP for subsequent statistical analysis.

MPP software includes principal component analysis, ANOVA, clustering algorithms, correlation analysis, and class prediction, to efficiently turn large sample sets into meaningful information. Metadata can be added to the analysis to help find relationships in complex sample data. Features can be annotated using the built-in ID browser to match based on retention time and spectra against the highly-curated Agilent METLIN LC/MS database or the Agilent Fiehn GC/MS library. These metabolomics-specific databases include compound identifiers for subsequent mapping to pathways.

MPP has an intuitive visual tool for creating simple or complex processing methods that can be stored and used to automate analysis. The ability to create methods is particularly useful when the same type of analysis will be performed routinely.




Mass Profiler Professional software includes volcano plot functionality to simultaneously calculate the fold change in abundance for each mass entity as well as p-value significance. These settings can be changed interactively and the results can be viewed in both graphical and table formats.




Mass Profiler Professional software includes correlation analysis tools, which can measure the strength and directionality of pair-wise relationships between any two variables. This heat map shows mass spectrometry abundance data after hierarchical clustering with the correlated metabolites (Arg and IMP) on the right.

*"Compound identification is a major bottleneck in metabolomics. To address this challenge, I am pleased to collaborate with Agilent to help develop the METLIN Personal compound database with MS/MS spectral library."*

– **Gary Siuzdak, Ph.D.,**
Senior Director, Scripps Center for Mass Spectrometry

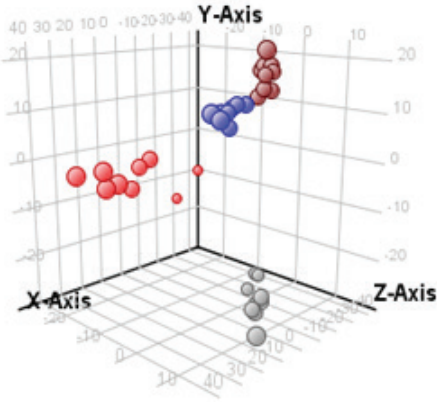
## Gain deeper biological insight

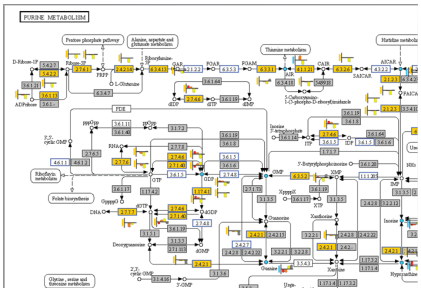
By incorporating a pathway-centric workflow into omics experiments, scientists can concurrently focus on the analysis, and potentially accelerate the process of discovery to biological insight. MPP's Pathway Architect module interactively filters, maps, and visualizes data on biological pathways using publicly available pathway databases such as KEGG, BioCyc, and Wikipathways. The software maps metabolites, proteins, and genes onto curated pathways, graphically projecting data onto pathways for interactive analysis.

The experimental results from MPP are projected onto pathways where you can filter, zoom, or select data. Pathways can be selected and a list of metabolites, proteins, transcripts and genes can be exported and used by other programs to create new "Pathway Directed Experiments." For example, the protein identifiers for a given pathway can be exported to create a targeted peptide analysis. This pathway-centric workflow speeds the route from discovery to insight and enables efficient planning and execution of the next series of experiments.

## Integrate metabolomics with other omics

While genomics, transcriptomics, proteomics, and metabolomics are in wide use in both industry and academia, these experiments—performed alone—are often insufficient to uncover meaningful correlations amid the high level of noise omics experiments typically generate. Integration of data from multiple omics can provide enough constraints to greatly improve the signal-to-noise of the analysis. The Pathway Architect module of Mass Profiler Professional allows either single omics analysis or joint analysis of multiple omics, enabling you to discover commonly affected pathways and aid in your ability to find reliable answers more quickly.



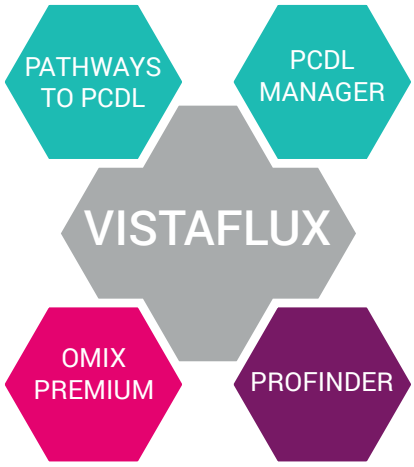

The KEGG pathway for purine metabolism shows nodes (teal) that represent metabolites; adjacent to the nodes are heat strips summarizing the average differential abundance values for the different conditions. A teal bar along the heat strip indicates metabolite; a yellow bar indicates a result for gene expression.

## Extend metabolomics with qualitative flux analysis

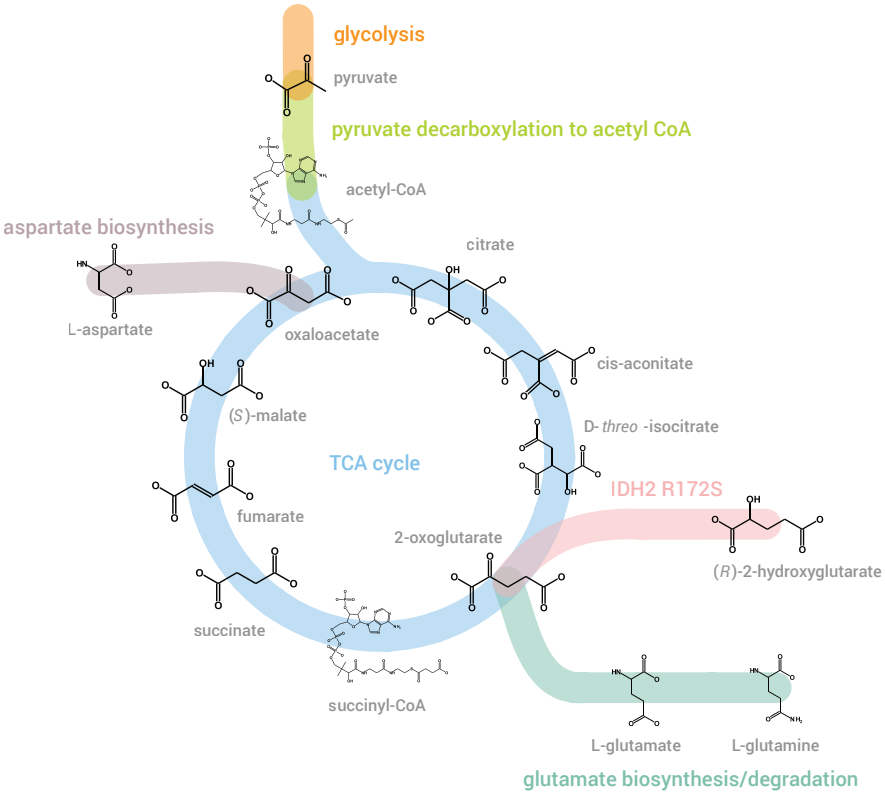
Agilent VistaFlux allows you to quickly obtain information about selected pathways and fluxes using stable isotope label tracing with mass spectrometric analysis. Qualitative flux analysis presents multiple analytical challenges such as mining the target metabolites, accounting for isotopologues, correcting for naturally occurring isotopes, and visualizing the results in a biological context. MassHunter VistaFlux is designed to meet these challenges and provides a solution for MS-only data from Agilent TOF-based high resolution LC/MS systems.

In qualitative flux analysis, a stable isotope labeled tracer ($^{13}C$, $^{15}N$, or $^{2}H$) is introduced into the biological system and results in changes in the natural isotopic pattern of downstream metabolites. Following analysis by LC/MS, the data is mined in Profinder using a target list derived from known metabolic pathways. Metabolites differing only in isotopic composition (isotopologues) are measured for each target compound and this information is used to track metabolic flux.

Profinder easily exports batch results (.pfa format) to Omix Premium for pathway visualization and biological interpretation. Using both static and animated pathway visualizations, experimental results are easy to communicate and it is simple to export figures for use in publication and presentations.




VistaFlux is composed of four software packages that facilitate the workflow: create and edit a target metabolite list (Pathways to PCDL and PCDL Manager), extract metabolite isotopologue data (Profinder), and visualize results on pathways (Omix Premium).




Omix Premium visualization of the TCA cycle modified for use in a IDH2 mutant cell line study.

## GC/MS Analysis

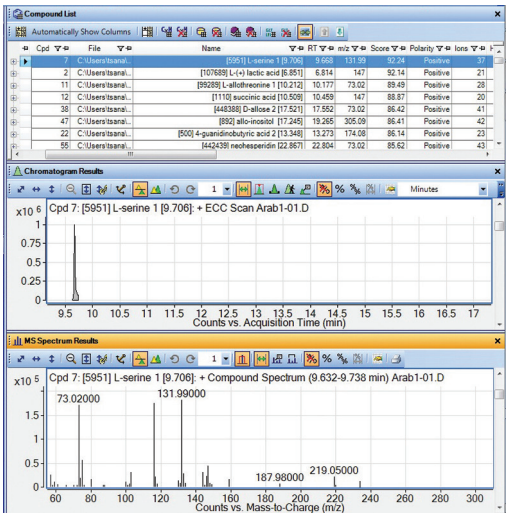
The Agilent 5977B high-efficiency source GC/MSD system incorporates an ultra-efficient electron ionization source to maximize the number of ions created and transferred into the analyzer, revolutionizing single quadrupole performance.

Agilent 7000 and 7010 Series triple quadrupole GC/MS systems provide low detection limits, robust performance, and software tools that make it easy to optimize your methods.

The Agilent 7200B GC/Q-TOF system delivers high sensitivity and selectivity with the added value of accurate mass and high-resolution data for structural confirmation, unknown compound identification and superior untargeted screening capabilities.

### Agilent Fiehn GC/MS metabolomics library

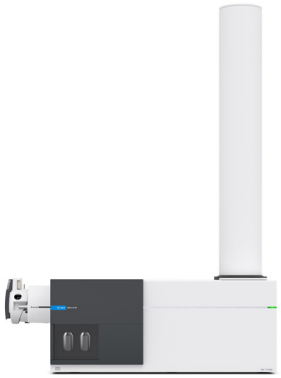
Developed with Dr. Oliver Fiehn, this is the largest commercially available and growing metabolomics-specific library, containing searchable GC/MS EI spectra and retention-time indices from approximately 1,437 common metabolites. The library comes with complete, preprogrammed GC/MS methods, and documents for GC/MS metabolomic analysis to maximize research success.




MassHunter Qual supports feature extraction of Agilent GC/MSD files using the "Find by Chromatographic Deconvolution" algorithm. Feature extracted peaks are matched to the Agilent Fiehn GC/MS Metabolomics Library for identification.

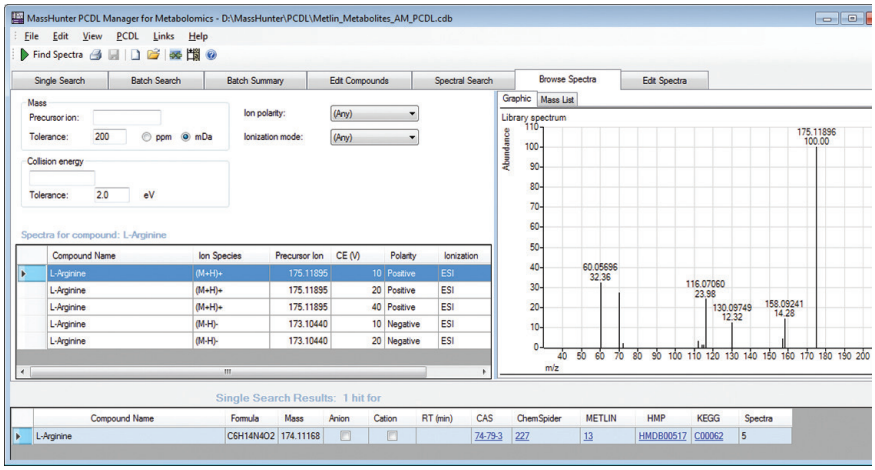
## LC/MS Analysis

Agilent 6200 Series accurate-mass TOF LC/MS systems provide the ability to identify both small molecules and large biologic compounds with high resolution, accurate mass analyses data for maximum value in qualitative assays.



The Agilent 6500 Series accurate-mass Q-TOF LC/MS brings the power of accurate mass MS/MS to provide strong confidence in identifying, screening, profiling, or quantitation in complex samples.

Agilent 6400 Series triple quadrupole LC/MS systems provide triple quadrupole performance for superior sensitivity, renowned reliability, and overall system robustness.




Agilent METLIN Personal Compound Database and Library contain approximately 80,000 compounds, including 39,000 lipids and 11,800 metabolites with curated MS/MS spectra. Used with TOF and Q-TOF data, identification is enabled using accurate mass and/or retention time database searching. Matching MS/MS data to the spectral library provides more confident metabolite identification.


Agilent InfinityLab Poroshell 120 HILIC-Z columns allow retention of polar metabolites using MS compatible solvents. The innovative column chemistry provides superior retention of polar metabolites and is stable at both low and high pH. For metal-sensitive metabolites, InfinityLab Deactivator Additive can be used to improve detection and peak shape.

## A new window into cell metabolism

Measuring functional metabolism in live cells and determining the metabolic phenotype provides a powerful guide for metabolomics experimental design.

Agilent Seahorse XF Analyzers provide a downstream approach to measuring cellular bioenergetics, enabling rapid assessment of metabolic function in live cells under multiple conditions.

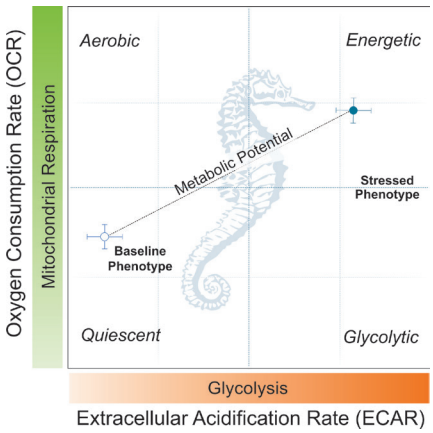
Determining the effects of chemical or genetic manipulations on cellular metabolic function quickly and easily yields complementary data that adds efficiency and direction to metabolomic research.

### Metabolic rates in minutes

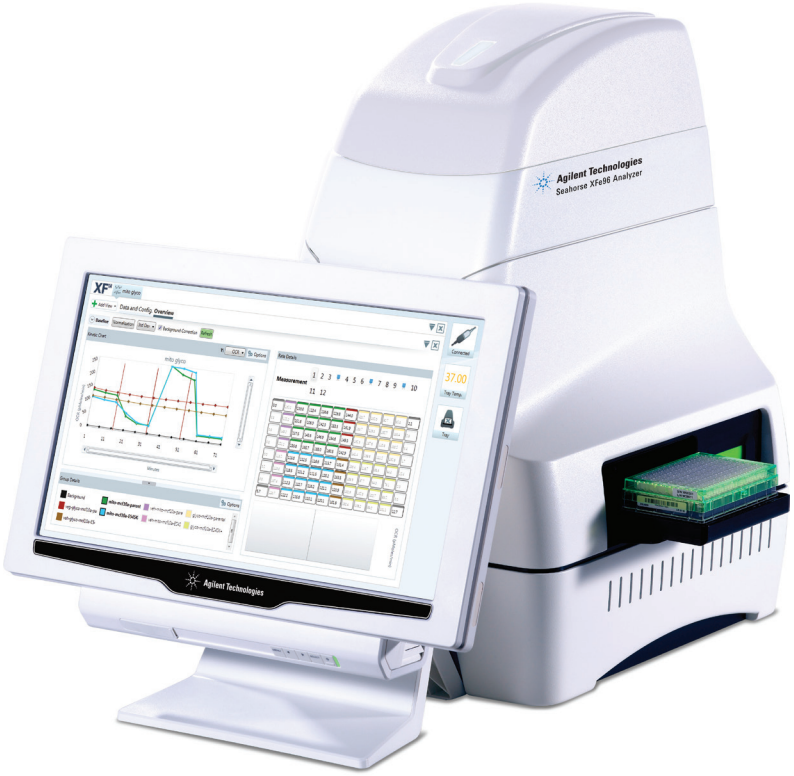
Seahorse XF Analyzers measure the activity of the two major energy-producing pathways of the cell – mitochondrial respiration and glycolysis – in live cells, in real time.

Seahorse XF Analyzers feature automated compound addition, solid-state fluorescence sensors in a disposable microplate cartridge, and software that automatically calculates and analyzes metabolic parameters.

Seahorse XF assay kits and reagents specifically developed for pathways, such as oxidative phosphorylation, glycolysis, and fatty acid oxidation, enable you to further interrogate and analyze cell function with same-day results.




The Seahorse XF Cell Energy Phenotype Test provides a high-level assessment of metabolic state and pathway preference, delivering results in under an hour.




Seahorse XF analyzers are available in 96, 24, and 8-well formats to accommodate a range of throughput and sample needs: adherent and suspension cells, cultured or *ex vivo* samples, model organisms, and isolated mitochondria.

Learn more:
**www.agilent.com/chem/metabolomics**

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