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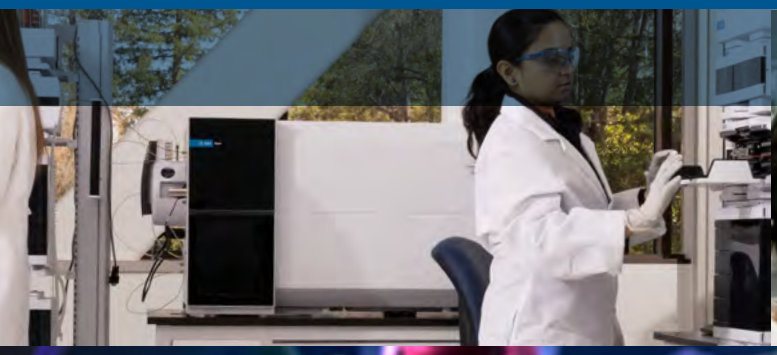
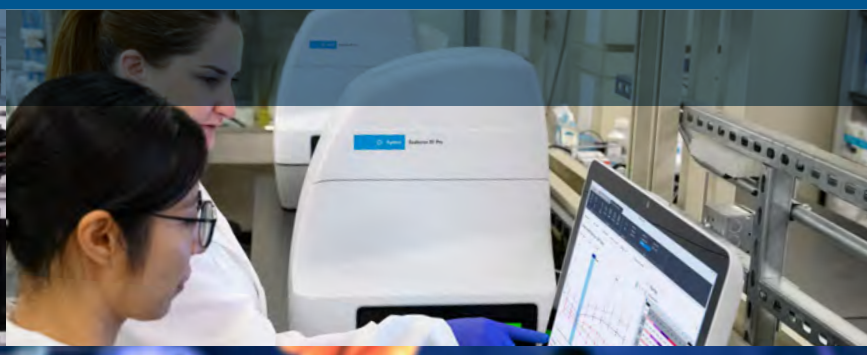
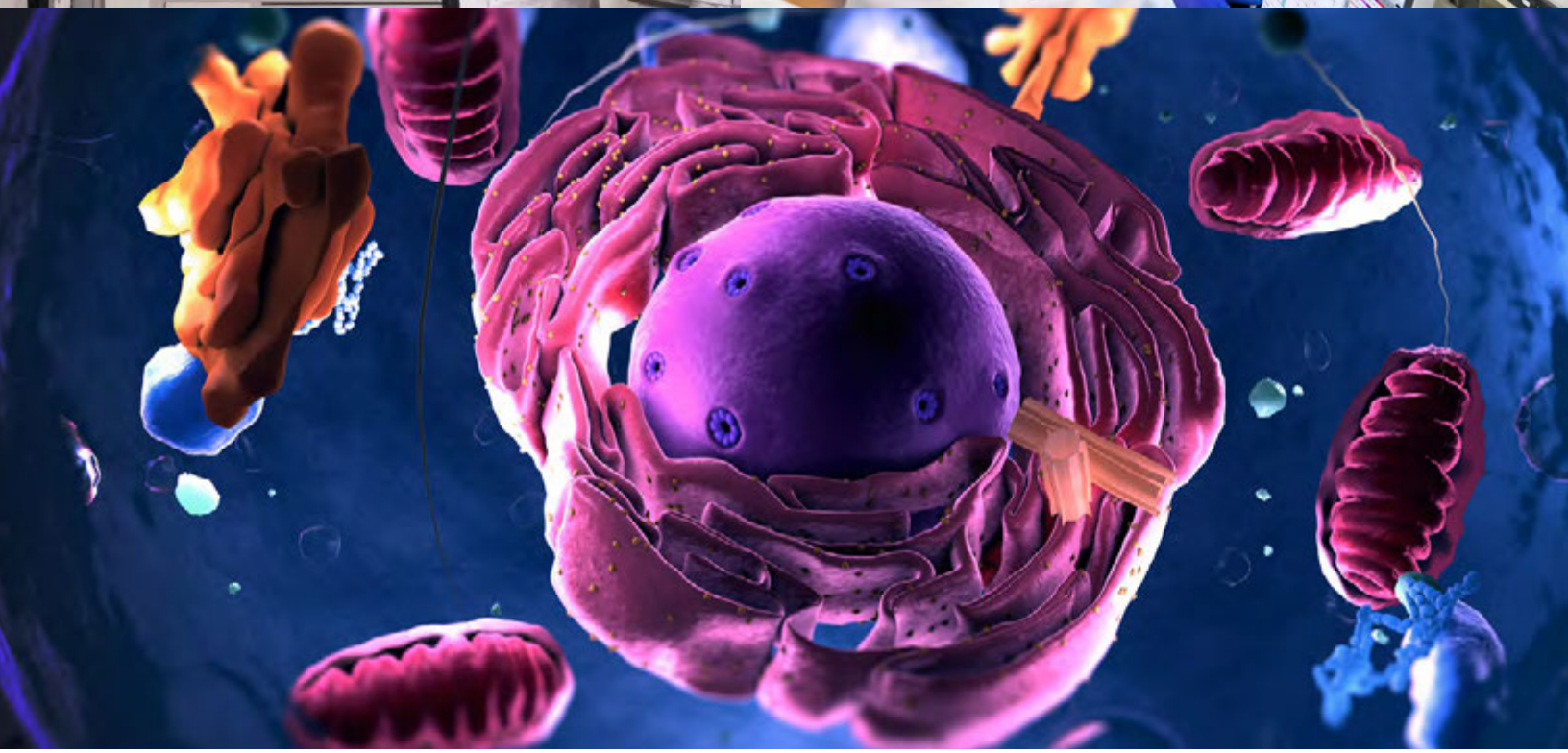
Trusted Answers

Elevate Your Phenotyping Workbench into a Synergistic Workflow

Unleash the power of Agilent Seahorse technology and mass spectrometry







Have You Ever Wondered Why Some Cellular Phenotypes Are Associated With Diseases and Others Are Not?

To help answer that question, researchers must not only observe cells, but also reveal what they do.

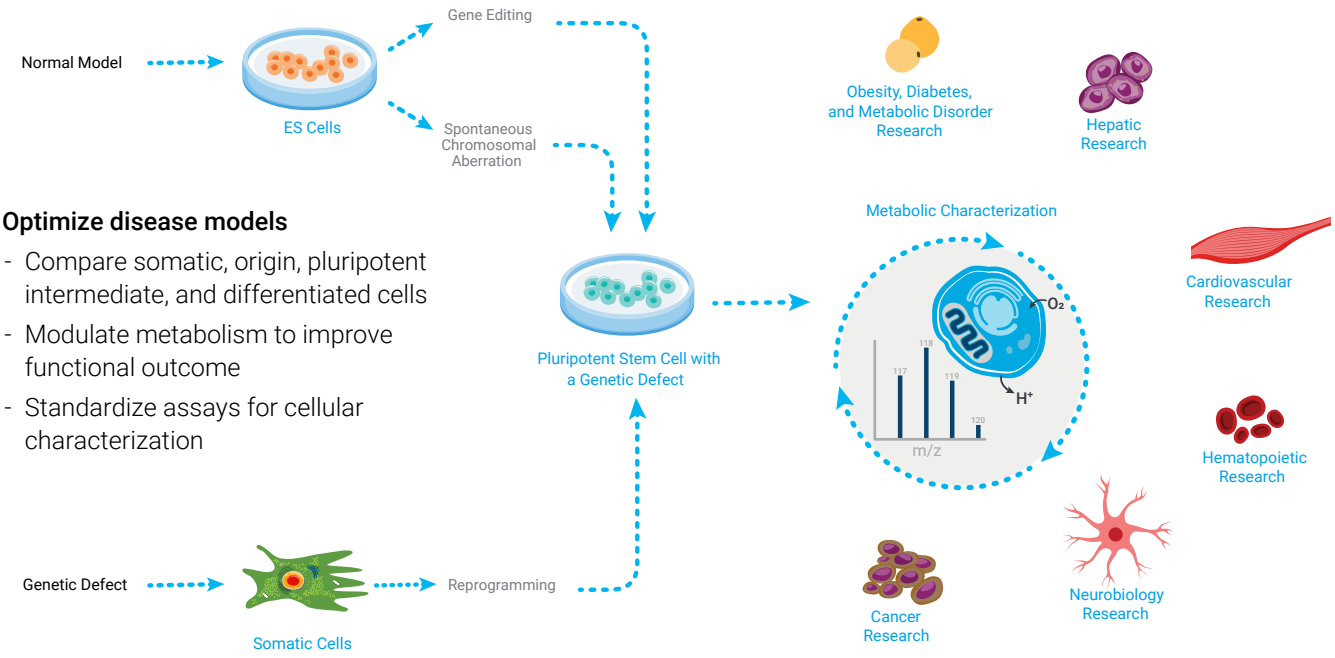
It is important to determine how cellular and metabolic phenotypes impact normal cell function and cellular homeostasis. These insights can provide a mechanism to modulate metabolic intermediates and pathways, to reinstate a healthy phenotype in pursuits of therapeutic discovery and development.

Recent advances in analytical tools allow researchers to explore and harness this link between cellular phenotype and metabolic profile—to reveal biochemical pathways, signaling, and intermediates. Such critical insights enable targeted intervention and modulation to enhance or re-instate healthy phenotypes and to develop safe and effective therapies.

By combining reliable cellular analysis solutions with intelligent mass spectrometry platforms, Agilent can help progress your phenotyping workbench into a synergistic workflow. Used as part of a progressive series of experiments, either in order of progression or orthogonally for complementary results, Agilent Seahorse technology and metabolomics solutions can facilitate a deeper understanding of cellular phenotypes and study their impact on disease. Once you understand the phenotype and its role in disease, this brings opportunities to modulate the phenotype for therapeutic development.

In the following guide, you can learn how the order of operations for these two technologies informs your hypotheses, steers your research questions, and streamlines your experimental workflow. Explore the advantages of using Seahorse analyzers and mass spectrometers collectively in phenotyping experiments. By combining functional metabolic measurements and molecular metabolomics studies, researchers gain valuable insights about the metabolic underpinnings of disease and how they manifest at a cellular level.

Measure Functional Performance and Model Relevance



Optimize disease models

- Compare somatic, origin, pluripotent intermediate, and differentiated cells
- Modulate metabolism to improve functional outcome
- Standardize assays for cellular characterization

This eBook serves as a practical guide for integrating metabolomics and cellular bioenergetics into streamlined workflows, enabling you to gain insights, investigate your hypothesis, and support your research goals. You'll learn about robust, data-driven workflows commonly adopted by researchers using two key technologies that work synergistically for cellular phenotyping research: Seahorse XF technology and mass spectrometry (MS) analysis.

The **Agilent Seahorse XF platform** measures the two primary bioenergetic pathways, mitochondrial respiration and glycolysis, for live cells in real time. This technology provides functional kinetic measurements of cellular bioenergetics that can be quantified for ATP rates and real time energy utilization of bioenergetic pathways. Seahorse measurements deliver critical insights about cellular processes including activation, proliferation, differentiation, cell death, cellular homeostasis, and disease progression. The Seahorse XF platform provides flexibility to customize experiments and target specific research questions. For turnkey solutions in a simplified and streamlined workflow, you can also use Agilent Seahorse XF kits and assays.

With integrated intelligent workflows, **mass spectrometry** can accurately measure individual metabolites and their pool size in cells. This gives an in-depth view of the metabolic pathways active in a cell and how they might change over time or how they might be impacted by a particular intervention. Using stable isotope label incorporation and **Agilent MassHunter VistaFlux software**, you can determine the flux direction through those pathways and understand the metabolic fate of precursors used by cells. This technology can quickly identify and assess metabolites for the specific molecular context about what drives metabolic phenotypes uncovered by Seahorse analysis.

How do Seahorse technology and mass spectrometry complement each other?

In the following pages, you'll learn about specific workflows that demonstrate how these two platforms can be used together to inform and refine your hypotheses.

The value of the combined technologies emerges for:

1. Streamlining your workflow—for example, when an untargeted measurement or screening is used with an exploratory approach to inform a more targeted hypothesis or mechanistic measurement
2. Providing orthogonal or complementary measurements of the same pathways and pathway intermediates—delivering deeper insights and greater confidence in your data

Seahorse analyzers measure the functional metabolic pathways of live cells in real time. Omics methods such as MS can be used to screen for untargeted analytes and assess whether cellular metabolism is impacted in the study. Mechanistic questions can be refined by either method to verify your study's conclusion.

Click the icon to learn more about the workflows covered in this eBook:

Workflow one | page 6
Deriving a functional hypothesis from metabolic screening and isotopic labeling

Workflow two | page 8
Functional confirmation through targeted metabolomics experiments

Workflow three | page 10
Discovery metabolomics leading to functional pathway confirmation and routine studies

Workflow four | page 12
Routine metabolic monitoring and functional confirmation leads to new mechanistic questions

Workflow one:
Deriving a functional hypothesis from metabolic screening and isotopic labeling

The first workflow seeks a deeper functional and mechanistic understanding of metabolic phenotypes by using qualitative stable isotope tracing by MS after metabolic screening with Seahorse. This workflow is appropriate for a researcher comparing a cellular disease model with control cells, to initially establish whether or not a metabolic pathway is driving the disease phenotype. After initially metabolic screening, wherein metabolism is shown to play a role. this workflow requires more refined measurements to narrow down questions about substrates, intermediates, metabolites, and specific drug targets.

"Is metabolism even a driver of my disease model? Once I establish that with a quick phenotyping screen, then I can really dig into mechanisms and potential drug targets."

Step one—phenotyping and screening

Determining how cells use energy and whether they are in oxidative or glycolytic states is often the first place to start when investigating metabolic cellular phenotypes, and for establishing a disease model linked to metabolic phenotype. It is also useful for understanding how drugs might impact energy utilization and dependencies. The Agilent Seahorse XF analyzer can be used to measure the flux through mitochondrial respiration and glycolysis. This information allows you to develop a functional hypothesis on the experimental cell's behavior and metabolic state. The Seahorse data can inform subsequent measurements by indicating whether energetic pathways are impacted in a disease phenotype.

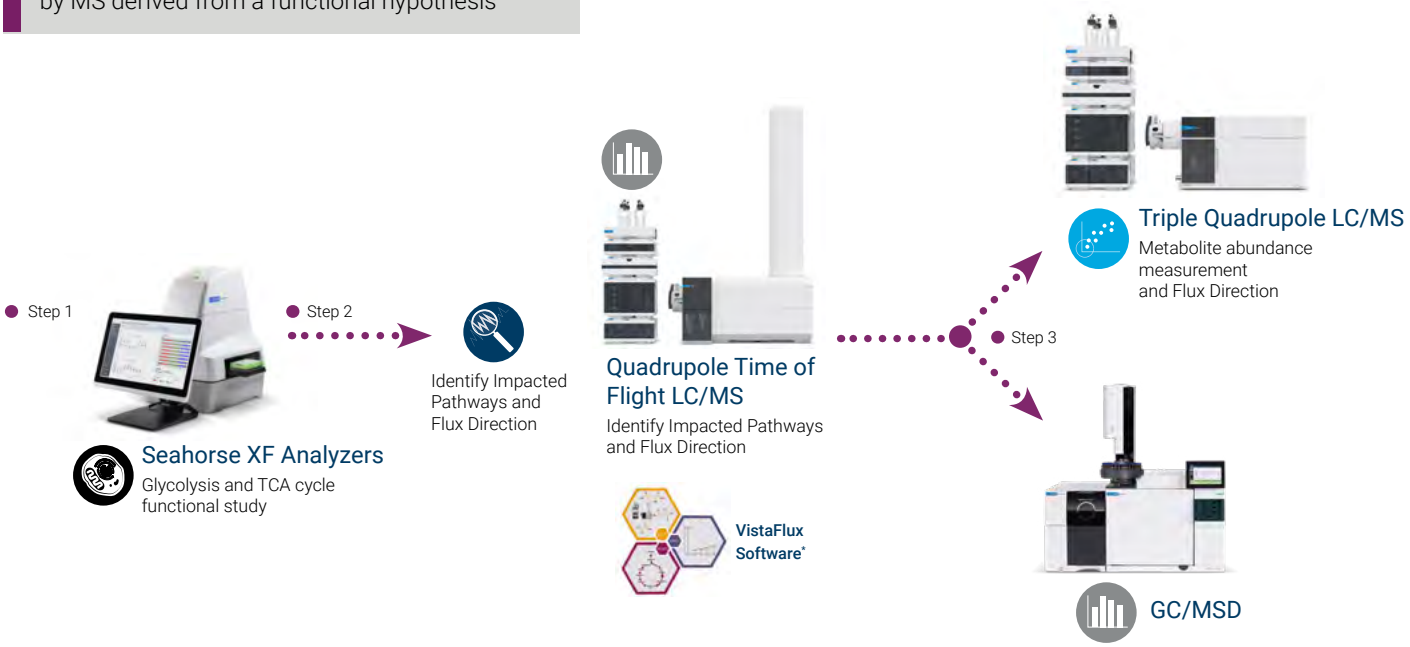
Step two—discovery metabolomics

When Seahorse experiments reveal an impact on energetic pathways in step one, this often leads to mechanistic questions about the broader influence on metabolism. This can be tested by a discovery metabolomics approach. These studies often incorporate stable heavy isotope feeding experiments to directly measure the pathways affected, and flux direction in cells with the Agilent Revident quadrupole time-of-flight LC/MS (LC/Q-TOF) system. By looking at the incorporation of these heavy labels in MassHunter VistaFlux software, you can learn which substrates are being used, the relative activity of active biochemical pathways, flux direction, and metabolic endpoints.

Step three—targeted metabolomics

Taken cooperatively, qualitative stable isotope tracing and Seahorse data allows you to comprehensively determine how cells produce energy and pinpoint specific metabolites with targeted metabolomics studies. With a clearer understanding of the metabolic mechanisms underpinning your experiment, you are well prepared to routinely monitor specific metabolic biomarkers in large cohort studies used in translational or preclinical research. Targeted metabolomics can be achieved with the Agilent 6495D triple quadrupole LC/MS (LC/TQ) system or, alternately, the Agilent 5977C GC/MSD system.

Workflow one: Qualitative stable isotope tracing by MS derived from a functional hypothesis



Workflow one overview:

Cell analysis using Seahorse XF analyzer

- Measure the cellular functional bioenergetics of mitochondrial respiration and glycolysis for live cells in real time
- Determine the metabolic state and phenotype of disease models compared to healthy cells
- Understand the functional impacts of your experiment (target or drug) on cellular metabolism and homeostasis

Quadrupole time-of-flight LC/MS with the Revident LC/Q-TOF system

- Enables a discovery metabolomics approach to global profiling of small metabolites
- Directly measures the incorporation of stable isotopes into metabolites to understand flux direction and metabolic fate
- Uncovers new pathways of interest and further drives your understanding of cellular metabolism with a deeper view of metabolic processes

Triple quadrupole MS with the 6495D LC/TQ system

- Implement metabolic discoveries into routine assessments for large cohort studies in translational or preclinical research
- Target and monitor metabolites in key metabolic pathways
- Enable fully quantitative metabolite measurements with a platform that is highly sensitive and robust

GC/MS with the 5977C GC/MSD system (alternative option)

- Alternative approach to routinely target and monitor GC-amenable metabolites for qualitative or quantitative analysis in large cohort studies
- Reliable platform for monitoring tricarboxylic (TCA) cycle metabolites with trimethylsilyl (TMS) derivatization
- Comprehensive spectral libraries aid in the identification of possible unknowns

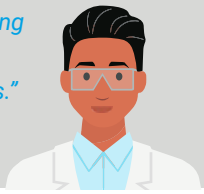
Data analysis with MassHunter VistaFlux software

- Extract stable isotopes from LC/Q-TOF data and perform natural abundance correction
- Understand the relative incorporation of stable isotopes in pathways to determine flux direction
- Study and track additional components of the metabolic network to further understand the impacted metabolic pathways

Workflow two:
Functional confirmation through targeted metabolomics experiments

The second workflow is for confirming the mechanism of action after screening for candidate drugs with metabolic effects. This workflow leverages targeted metabolomics experiments to confirm mechanism of action derived from a Seahorse functional screen in the first step. In this workflow, the effect of cellular modulation or drug efficacy is often already known, and a functional hypothesis may be well understood. However, in some cases these studies may yield questions meriting additional omics studies with a broader focus.

"I do a lot routine screening for candidate drugs that impact metabolism. However, I would really like to confirm mechanism of action and evaluate metabolic switching by monitoring specific metabolites."



Step one—targeted metabolomics

When a change in cellular energetic phenotype is observed in step one, following up with a more targeted metabolomics study measures the individual metabolites and pathways that are impacted. It may help confirm the mechanisms associated with the cellular metabolic response to treatment. For example, targeted metabolomics studies provide specific molecular context to the mechanisms by which cells undergo metabolic switching when primary energy-producing pathways like oxidative phosphorylation are modulated.

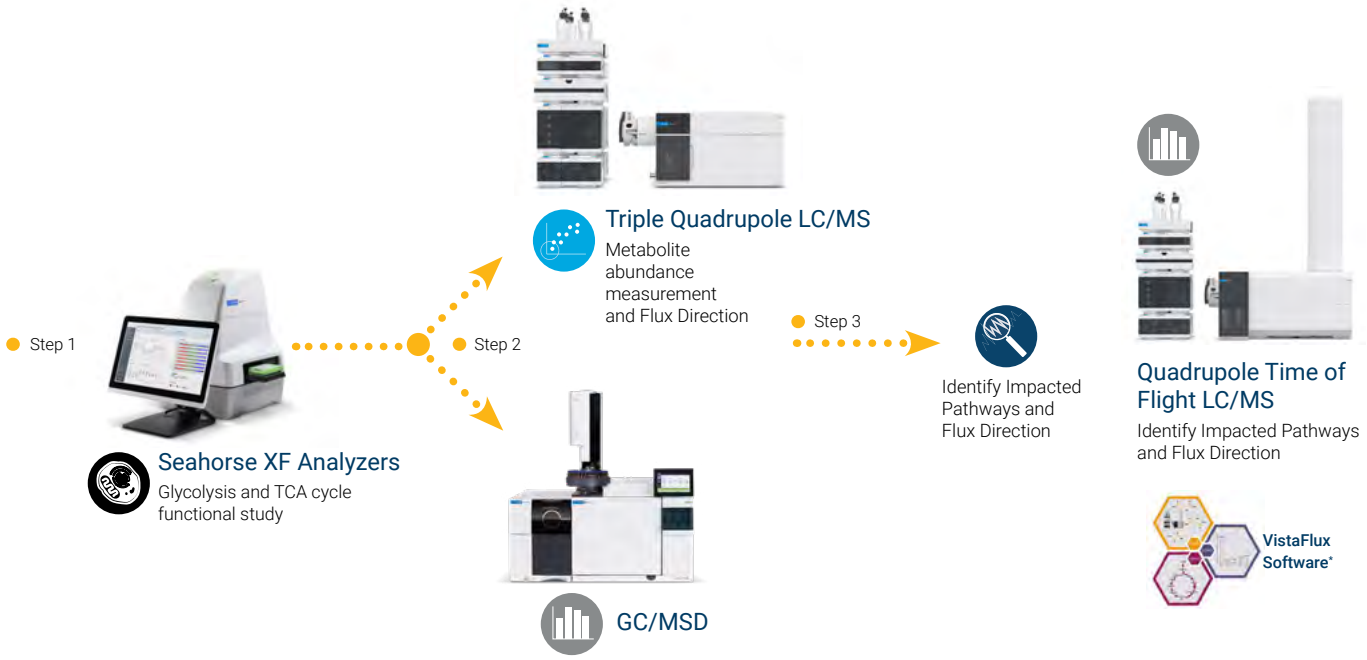
Step two—discovery metabolomics

When Seahorse experiments reveal an impact on energetic pathways in step one, this often leads to mechanistic questions about the broader influence on metabolism. This can be tested by a discovery metabolomics approach. These studies often incorporate stable heavy isotope feeding experiments to directly measure the pathways affected, and flux direction in cells with the Agilent Revident quadrupole time-of-flight LC/MS (LC/Q-TOF) system. By looking at the incorporation of these heavy labels in MassHunter VistaFlux software, you can learn which substrates are being used, the relative activity of active biochemical pathways, flux direction, and metabolic endpoints.

Step three—discovery metabolomics

In some circumstances the data may lead to additional mechanistic questions, requiring discovery metabolomics studies via the Revident LC/Q-TOF system to get a broader view of cellular metabolic pathways. As informed by steps one and two, further refining mechanistic questions delivers deeper insight and provides greater context. A discovery metabolomics approach may also be useful in detecting and characterizing off-target effects, such as toxicity.

Workflow two: Targeted metabolomics experiment derived from a functional hypothesis



Workflow two overview:

Cell analysis using Seahorse XF analyzer

- Routinely screen cellular metabolic response to therapeutic interventions such as new drug candidates or gene edits
- Determine if treatments impact mitochondrial respiration or glycolysis
- Support target identification and validation and mechanism of action in routine studies
- Identify follow-up studies based on promising candidates

Triple quadrupole MS with the 6495D LC/TQ system

- Targeted metabolomics approach to investigate pathways of interest when Seahorse technology identifies a promising therapeutic target or candidate
- Highly accurate and robust platform for routine analysis of metabolites in large cohort studies
- Compare multiple treatments using relative or absolute quantitation

GC/MS with the 5977C GC/MSD system (alternative option)

- Alternative targeted metabolomics approach to investigate pathways of interest when the Seahorse analyzer identifies a promising candidate
- Highly accurate and robust platform for routine analysis of GC-amenable metabolites
- Critical for monitoring volatile organic metabolite biomarkers such as aldehydes, ketones, hydrocarbons, and aromatic compounds produced during metabolism

Quadrupole time-of-flight LC/MS with the Revident LC/Q-TOF system

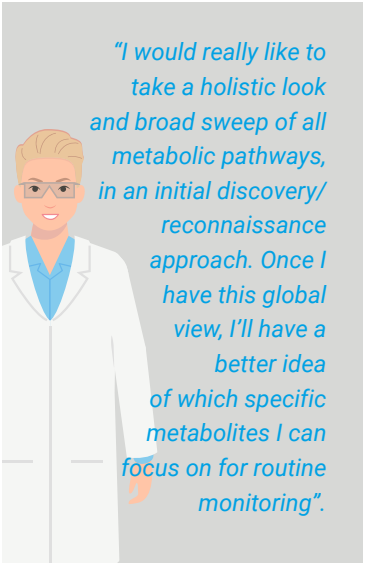
- Discovery metabolomics approach to further contextualize results from Seahorse technology and targeted metabolomics studies
- Detect off-target effects and characterize them with stable isotope studies

Data analysis with MassHunter VistaFlux software

- Extract stable isotopes from LC/Q-TOF data and perform natural abundance correction
- Understand the relative incorporation of stable isotopes in pathways to determine if flux size or direction changes during treatment
- Characterize off-target effects at the pathway level

Workflow three:
Discovery metabolomics leading to functional pathway confirmation and routine studies

The third workflow is a discovery metabolomics workflow that leads to routine pathway confirmation in larger studies. In this workflow researchers have taken a global snapshot of the cells' metabolic pathways. Researchers can provide valuable context to their measurements by synergistically using Seahorse analysis to obtain a deeper understand the cells' functional metabolic state and their total capacity to produce energy. With mechanisms understood, this leads to development of routine monitoring of specific metabolites in larger studies.



Step one—discovery metabolomics analysis

Researchers may first seek a broader untargeted investigation, starting directly with a discovery metabolomics experiment to measure the relative pool size changes in the metabolic network between multiple cell lines or treatments. This enables a holistic view of metabolism and pathway changes, providing insights into the fundamental metabolic and mechanistic drivers behind disease states. Metabolomics screening provides a comprehensive view of the effects of gene edits or cellular modulations, enabling further hypothesis development and confirmation through orthogonal techniques such as Seahorse analysis.

Step two—cellular metabolic phenotyping

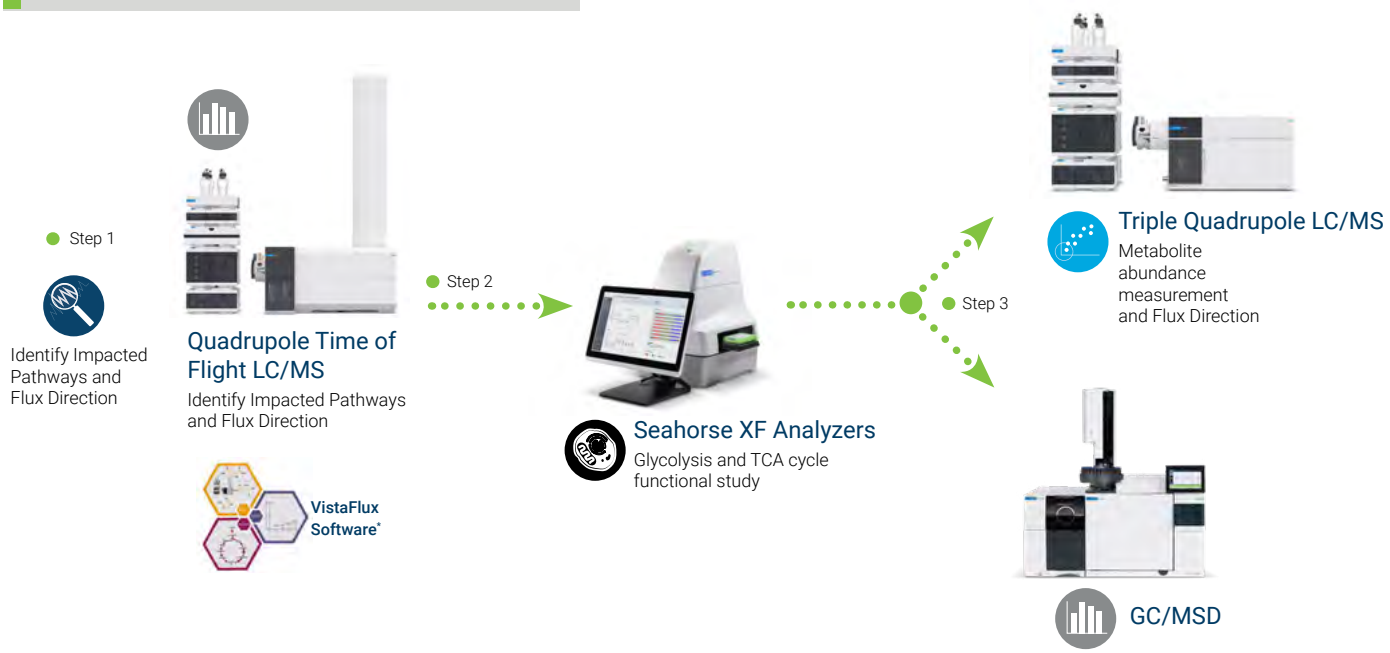
The discovery metabolomics steps may then suggest that bioenergetic pathways are impacted, warranting further cellular metabolic phenotyping studies to provide additional context on the cellular functional state. To provide this additional characterization and context for the initial omics measurements, Seahorse metabolic measurements can provide further information by measuring metabolic pathways that are actively being used by live cells in real time. Additionally, Seahorse kits and assays allow the investigation of more targeted and mechanistic questions driving these metabolic changes, such as analyzing capacity for total ATP production or changes in substrate utilization.

Step three—streamlining metabolic measurements

The first two steps in this workflow provide complimentary measures of metabolism, permitting the streamlining of the metabolomics workflow in future studies. Having correlated isotope tracing experiments with Seahorse studies, the two complementary datasets reveal trends seen in individual pathways that can be viewed in the context of the overall energetic state of the cell. Further, researchers can explore plasticity and the metabolic switch between mitochondrial respiration and glycolysis.

With mechanistic insights previously provided in previous steps, researchers can then alternate between steps two and three to routinely monitor key metabolic biomarkers and pathways as part of a large cohort study or drug candidate screen.

Workflow three: Discovery metabolomics leading to functional pathway confirmation



Workflow three overview:

Quadrupole time-of-flight LC/MS with the Revident LC/Q-TOF system

- Directly determine relative metabolite pool sizes
- Identify pathways impacted by experiment
- Determine flux direction with stable isotope studies

Data analysis with MassHunter VistaFlux software

- Extract stable isotopes from LC/Q-TOF data and perform natural abundance correction
- Understand the relative incorporation of stable isotopes in pathways to determine if flux size or direction changes during treatment
- Understand the metabolic origin of metabolites via label incorporation

Cell analysis using Seahorse XF analyzer

- Contextualize results with the bioenergetic state of the cells
- Determine if cells undergo metabolic switching during the experiment
- Monitor mitochondrial respiration and glycolysis, and bioenergetic capacity and reserves

Triple quadrupole MS with the 6495D LC/TQ system

- Targeted metabolomics approach to routinely monitor metabolic markers and key pathways
- Highly accurate and robust platform for routine analysis of metabolites
- Compare multiple interventions using relative or absolute quantitation

GC/MS with the 5977C GC/MSD system (alternative option)

- Alternative targeted metabolomics approach to routinely monitor volatile metabolic markers and key pathways
- Highly accurate and robust platform for routine analysis of GC-amenable metabolites
- Critical for monitoring volatile organic metabolite biomarkers such as aldehydes, ketones, hydrocarbons, and aromatic compounds produced during metabolism

Workflow four:
Routine metabolic monitoring and functional confirmation leads to new mechanistic questions

The fourth workflow routinely monitors key metabolites in a targeted metabolomics experiment. In this workflow, a routine targeted metabolomics panel focused on key metabolite classes and pathways is assessed using varying interventions (for example, drug candidates). These routine panel tests may elucidate trends that warrant deeper metabolism exploration to refine the mechanism of action.

Step one—targeted metabolomics

The first step is to run a routine panel of tested metabolites using a targeted approach to screen for impacts at the molecular level. This assessment allows you to identify which interventions are generating a phenotypic response by measuring relative pool sizes of key targeted metabolites and pathways.

Step two—cellular phenotyping

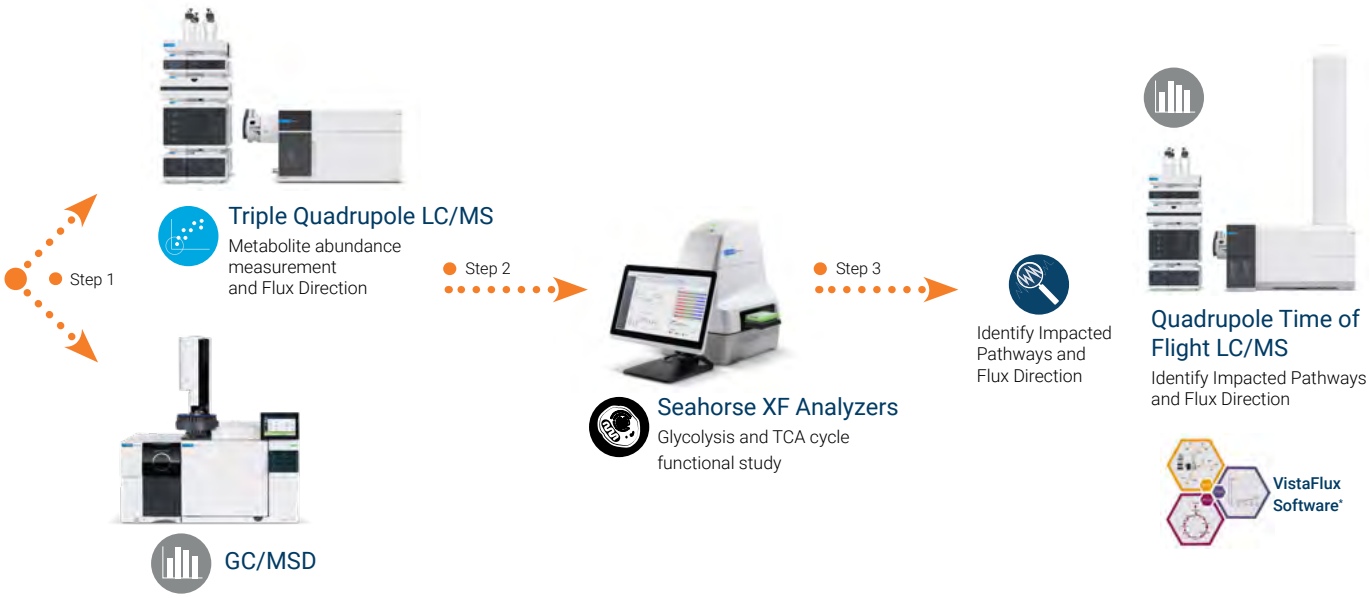
Once impacts on the relevant metabolites and metabolic pathways are identified from the targeted experiment, Seahorse measurements provides confirmational phenotyping at the cellular level. By comparing the test to the control, you can assess whether metabolic responses observed during the experiment occurred due to the drug intervention.

Step three—discovery metabolomics

In some cases the metabolic phenotype characterized on the Seahorse analyzer may yield novel and interesting new results from the routine screening, indicating the need to gain additional context and insights to mechanism of action. Using a discovery metabolomics approach, you'll be able to determine what additional pathways are impacted. With this expanded view, you can use stable isotope studies to explore flux direction and metabolic fate to provide further insights into the intervention's mechanism of action.

"I would really like to establish a routine workflow for screening how different drug candidates impact metabolites. This way, I could easily flag which drug candidates I might need to dig deeper into—to better understand the mechanism of action."

Workflow four: Funtional hypothesis derived from a targeted metabolomics experiment



Workflow four overview:

Triple quadrupole MS with the 6495D LC/TQ system

- Targeted metabolomics approach to routinely monitor metabolic biomarkers and key pathways
- High sensitivity to target metabolite pool sizes for comparative analysis
- Compare multiple treatments using relative or absolute quantitation

GC/MS with the 5977C GC/MSD system (alternative option)

- Alternative targeted metabolomics approach to routinely monitor volatile metabolic markers and key pathways
- Reliable platform for monitoring tricarboxylic cycle (TCA) metabolites with trimethylsilyl (TMS) derivatization
- Comprehensive spectral libraries aid in the identification of possible unknowns

Cell analysis using the Seahorse XF analyzer

- Confirm metabolic phenotypes by measuring the energetic state of the cells
- Determine if treatment affects mitochondrial respiration or glycolysis
- Determine whether cells undergo metabolic switching during the experiment
- Support or inform mechanism of action in routine screening studies

Quadrupole time-of-flight LC/MS with the Revident LC/Q-TOF system

- Discovery metabolomics identifies additional pathways impacted by your experiment
- Determine flux direction and metabolic fate with stable isotope studies
- Discover and identify unknown metabolites responding to the experiment

Data analysis with MassHunter VistaFlux software

- Extract stable isotopes from LC/Q-TOF data and perform natural abundance correction
- Understand the relative incorporation of stable isotopes in pathways to determine if flux size or direction changes during treatment
- Understand the metabolic origin and fate of metabolites via label incorporation

Products for Agilent workflow solutions

Leverage Seahorse XF analyzers to:

- Measure functional metabolism in live cells
- Determine metabolic phenotype and measure cellular bioenergetics
- Assess mitochondrial respiration and glycolysis in live cells, in real time

Learn more: **Agilent Seahorse XF Applications in Cell Metabolism**

Revident LC/Q-TOF system can be used to:

- Perform global profiling of metabolites using high resolution mass spectrometry
- Identify unknown analytes to gain deeper biological insight
- Enable a discovery metabolomics strategy to identify new pathways of interest

Explore the system: **Intelligence that inspires**

Use MassHunter VistaFlux software to:

- Perform qualitative flux analysis based on high resolution mass spectrometry data
- Interpret stable isotope incorporation within pathways
- Study and track metabolic flux networks using an integrated software workflow

Discover more software capabilities: **Flux analysis mass spectrometry software, VistaFlux Software**

The 6495 LC/TQ system enables:

- Perform quantitative measurement for GC-amenable compounds
- Compare measurements for volatile compounds produced by metabolism
- Identify unknown volatile analytes via spectral libraries to gain deeper biological insight

Discover how: **Triple quadrupole LC/MS system, 6495D mass spectrometer**

Use the 5977C GC/MSD system to:

- Perform quantitative measurement for GC-amenable compounds
- Compare measurements for volatile compounds produced by metabolism
- Identify unknown volatile analytes via spectral libraries to gain deeper biological insight

Read more: **Discover the Possibilities with GC/MS solutions**

Additional resources:

- **Instrument selection guide**
- **Guide to the kits and assays**
- **Applications in Cell Metabolism**
- **Combining Cellular Bioenergetics with Metabolomics**
- **An Automated Dual Metabolite + Lipid Sample Preparation Workflow for Mammalian Cell Samples**
- **Seahorse Microplate selection brochure**

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