

# Discover the Drivers of Cell Fate, Function, and Fitness

Agilent Seahorse XF analysis platform





# Are You Measuring What's Really Important to Your Cells?

To fully understand what drives cell phenotype and function, you must consider the influences of energy metabolism.

Examining energy metabolism has led to new insights into biological function. In fact, some of the decade's most significant discoveries have hinged upon elucidating the role of energy metabolism in cellular processes.

**Live-cell, functional metabolic data is impacting therapeutic research and discovery in areas like these:**

## Immunology

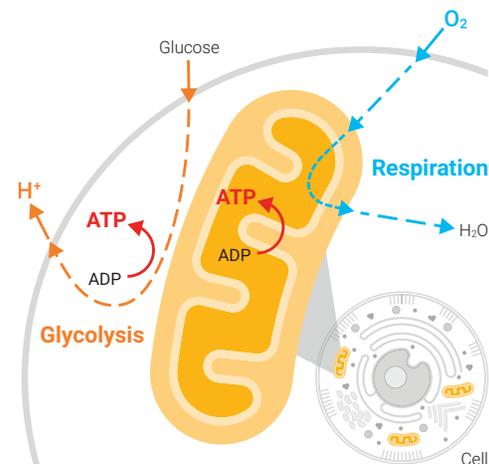
- Determining metabolic fitness of immune cells
- Measuring and modulating immune cell activation in real time
- Targeting metabolic pathways to improve immunotherapy design and performance

## Cancer

- Rewiring metabolism to support rapid growth
- Understanding which nutrients fuel cancer cells
- Uncovering how metabolism impacts the tumor microenvironment

## Toxicology

- Detecting mitochondrial liabilities among potential therapeutics
- Assessing the risk of mitochondrial toxicity *in vitro* with high specificity and sensitivity
- Determining the mechanism of action of mitochondrial toxicants

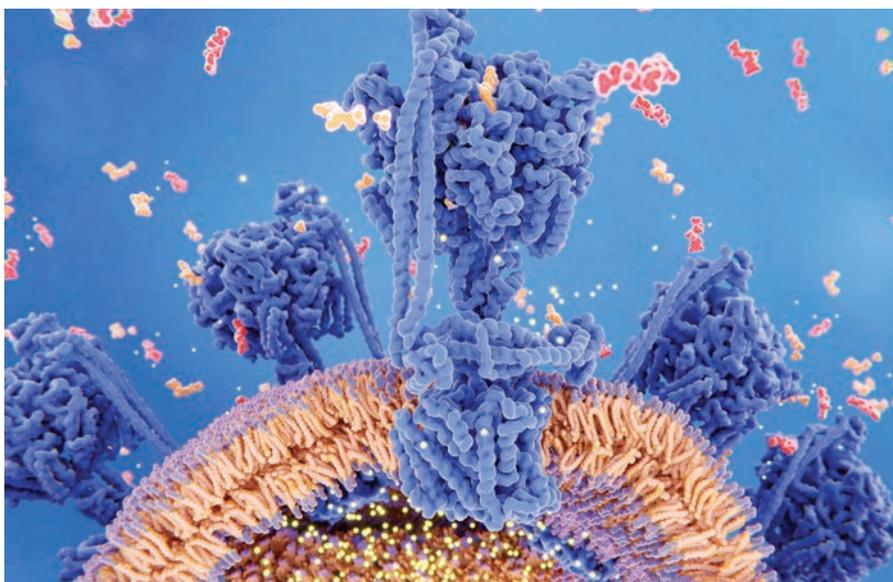


## Obesity, diabetes, and metabolic disorders

- Measuring functional effects of genetic changes to metabolic pathway components
- Examining nutrient use in healthy and diseased cell models
- Assessing fatty acid oxidation and glycolysis in different cell types

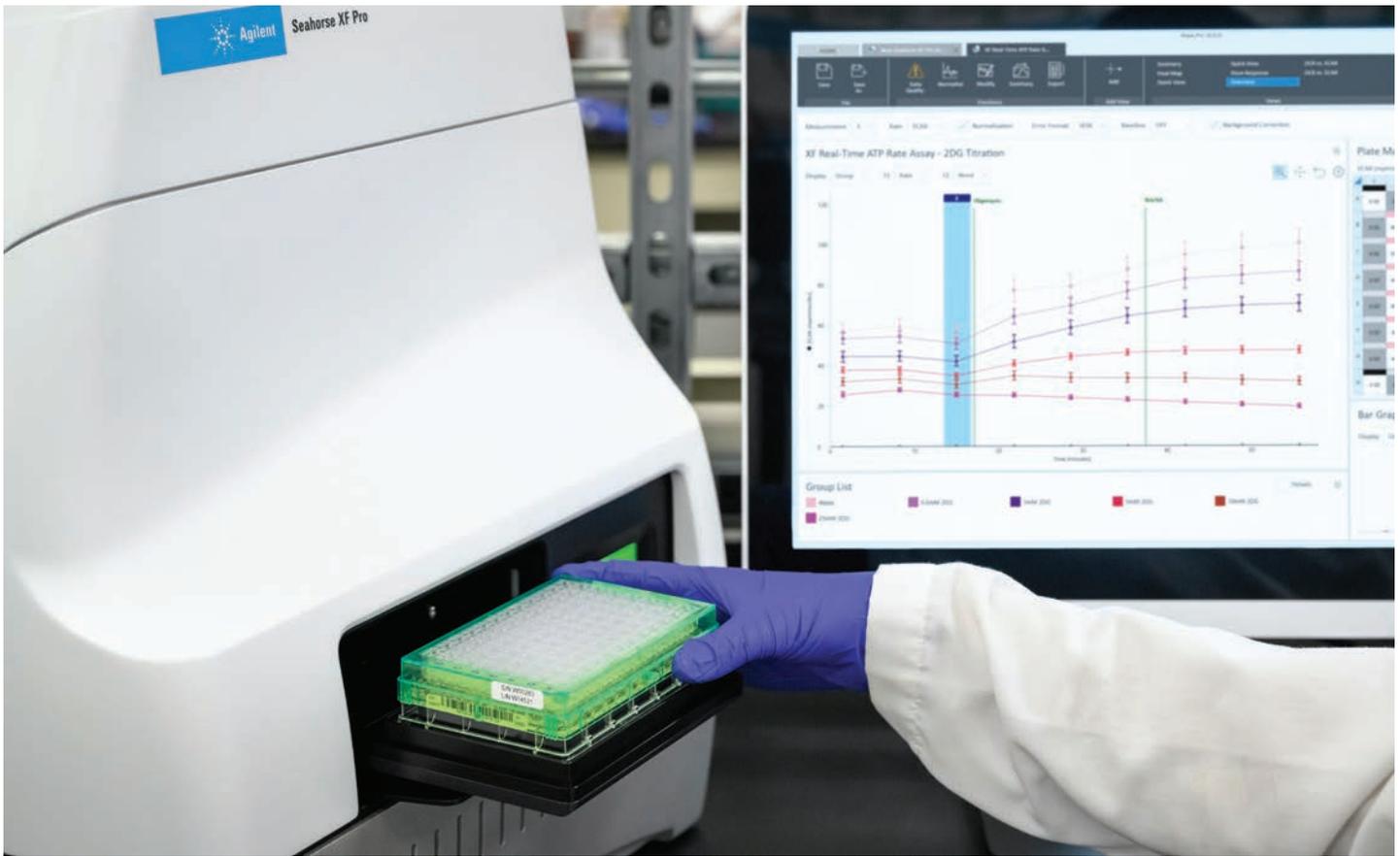
# Complete Platform for Live-Cell Metabolic Assays

Comprising instruments, assay kits, software, and consumables, Agilent Seahorse XF platforms are designed to support you from sample to insight.



## Move beyond analyzing what your cells are and get a clear picture of what they do

- Instrumentation for real-time extracellular flux (XF) measurements in live cells
- Validated kits, media, and reagents for standardized XF assays
- Patented sensors, plastics, and consumables for reliable XF data
- Powerful analytics to help interpret and share your results



## Agilent Seahorse XF analyzers: The standard for measuring energy metabolism in live cells

Seahorse XF analyzers enable robust measurements of mitochondrial activity, glycolysis, and ATP production rates in a microplate format.

- Automatic calculation of oxygen consumption and proton efflux rates
- Label-free detection of discrete bioenergetic changes in live cells—in real time
- Compatibility with both adherent and suspension cells, as well as isolated mitochondria
- Instrument-controlled gentle mixing, measuring, and compound injections
- High sensitivity to analyze as few as 5,000 cells per well
- Temperature controlled to maintain cell health and kinetics



### Agilent Seahorse XF Pro analyzer

Maximize your capacity for XF assays and minimize cost per-sample with high-sensitivity capability and consistent measurements. The Seahorse XF Pro (96-well format) enables many experimental groups per assay, maximizing design flexibility and sample throughput. It comes with state-of-the-art software and streamlined workflows that will greatly enhance user experience.

#### Key applications:

- Phenotypic screening
- Testing many conditions at one time
- Dose-response studies
- OMICS functional confirmation
- Spheroids



### Agilent Seahorse XF HS Mini analyzer

High-sensitivity capabilities—combined with an easy-to-use interface—make this eight-well analyzer accessible for every lab while delivering robust XF analysis on limited cell numbers. It also allows you to use fewer cells per well, compared with other XF platforms.

#### Key applications:

- Optimization of experimental conditions before scaling up
- Rare, sorted, and/or primary immune cells
- Quiescent and low-respiring cell types
- Cell lines and *ex vivo* samples



### Agilent Seahorse XFe24 analyzer

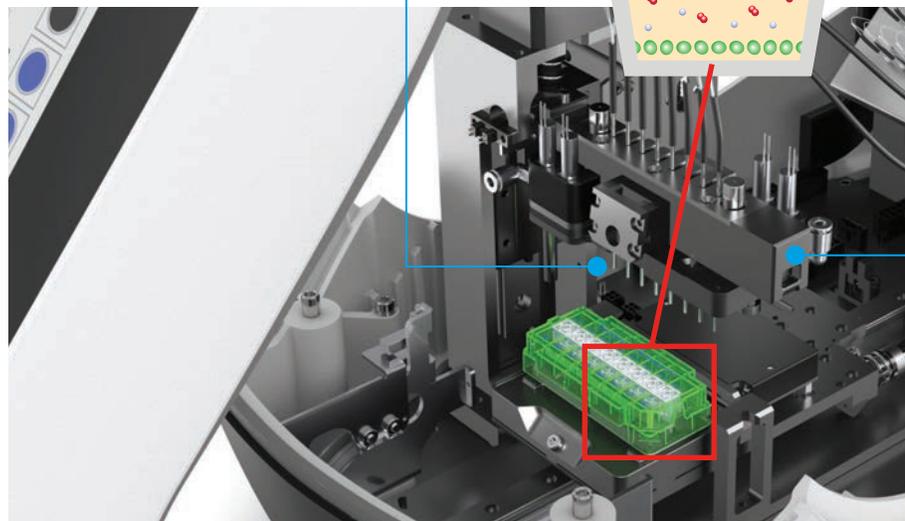
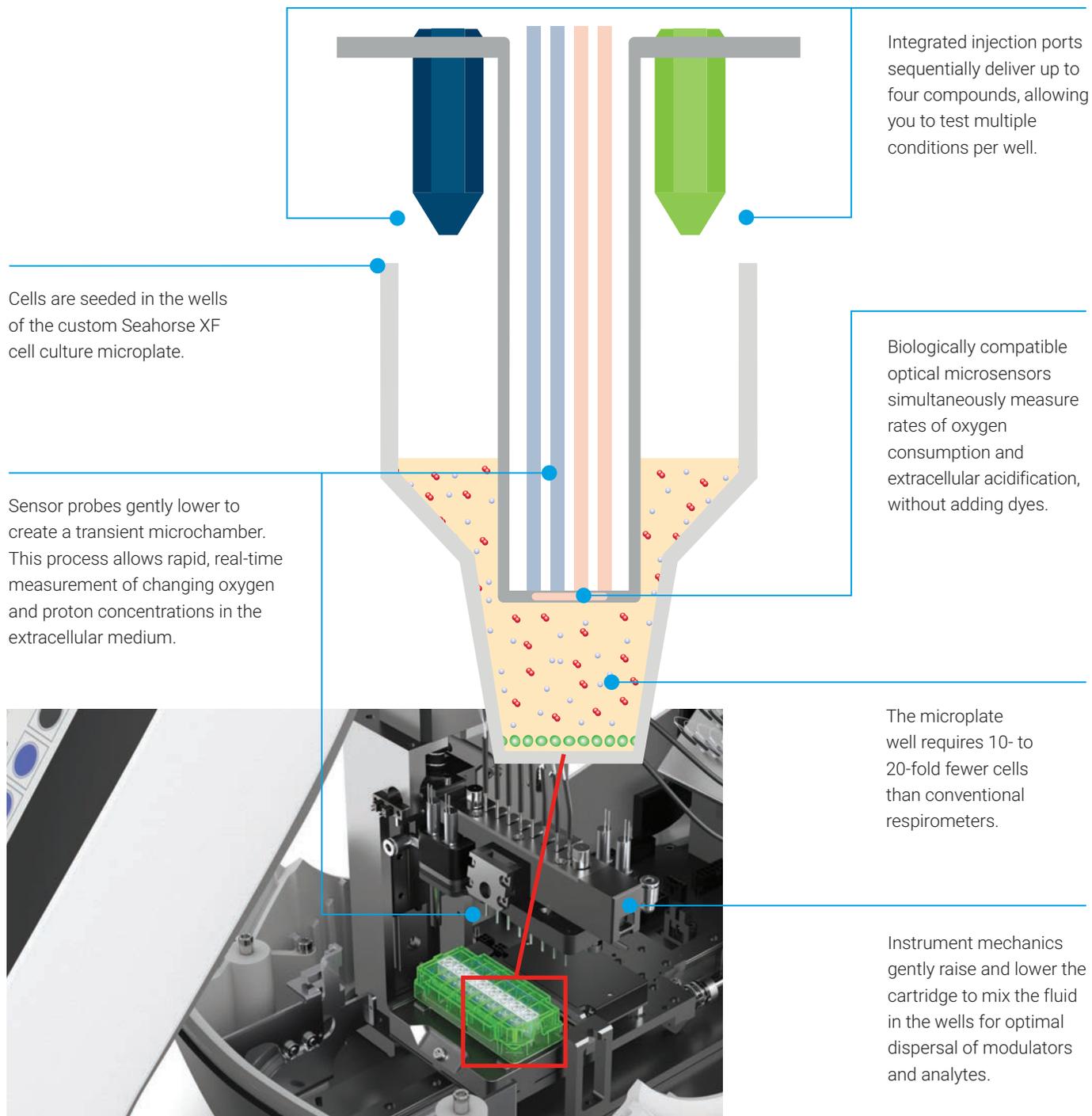
Featured in hundreds of peer-reviewed publications, the Seahorse XFe24 uses a larger well format suitable for islets and other specialty samples.

#### Key applications:

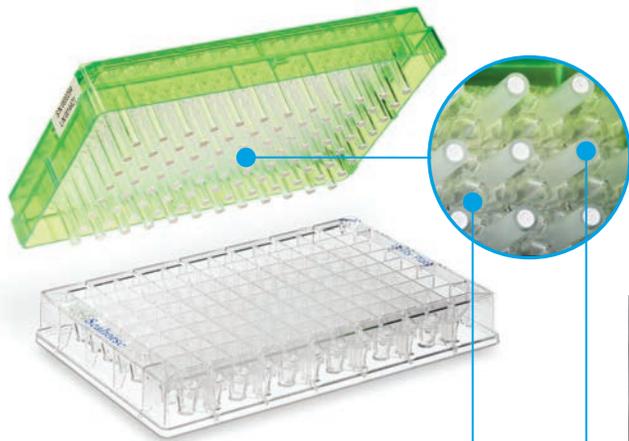
- Islets
- Small-model organisms

## Take a Closer Look at Seahorse XF Technology

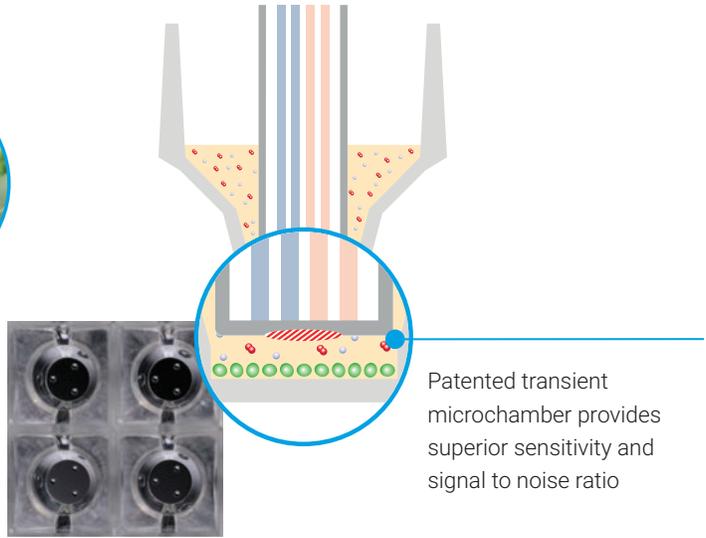
The patented Agilent Seahorse XF platform lets you measure both metabolic energy pathways simultaneously with speed and precision.



## Smart plastic technology makes it all possible



Patented integrated injection ports (4 per well) for adding compounds, stimulators, inhibitors, substrates, and beads conjugated with antigens, let you test multiple conditions per well.



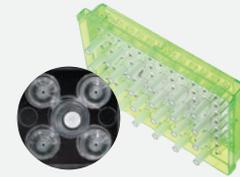
Patented transient microchamber provides superior sensitivity and signal to noise ratio

Solid state sensor probes contain polymer embedded fluorophores that are inspected through a machine vision process, allowing detection of OCR and PER simultaneously

## Quality XF sensor cartridges and cell plates are integral to the Seahorse XF measurement system

### XF sensor cartridges: Essential to sensitive, simultaneous measurement of bioenergetic pathways

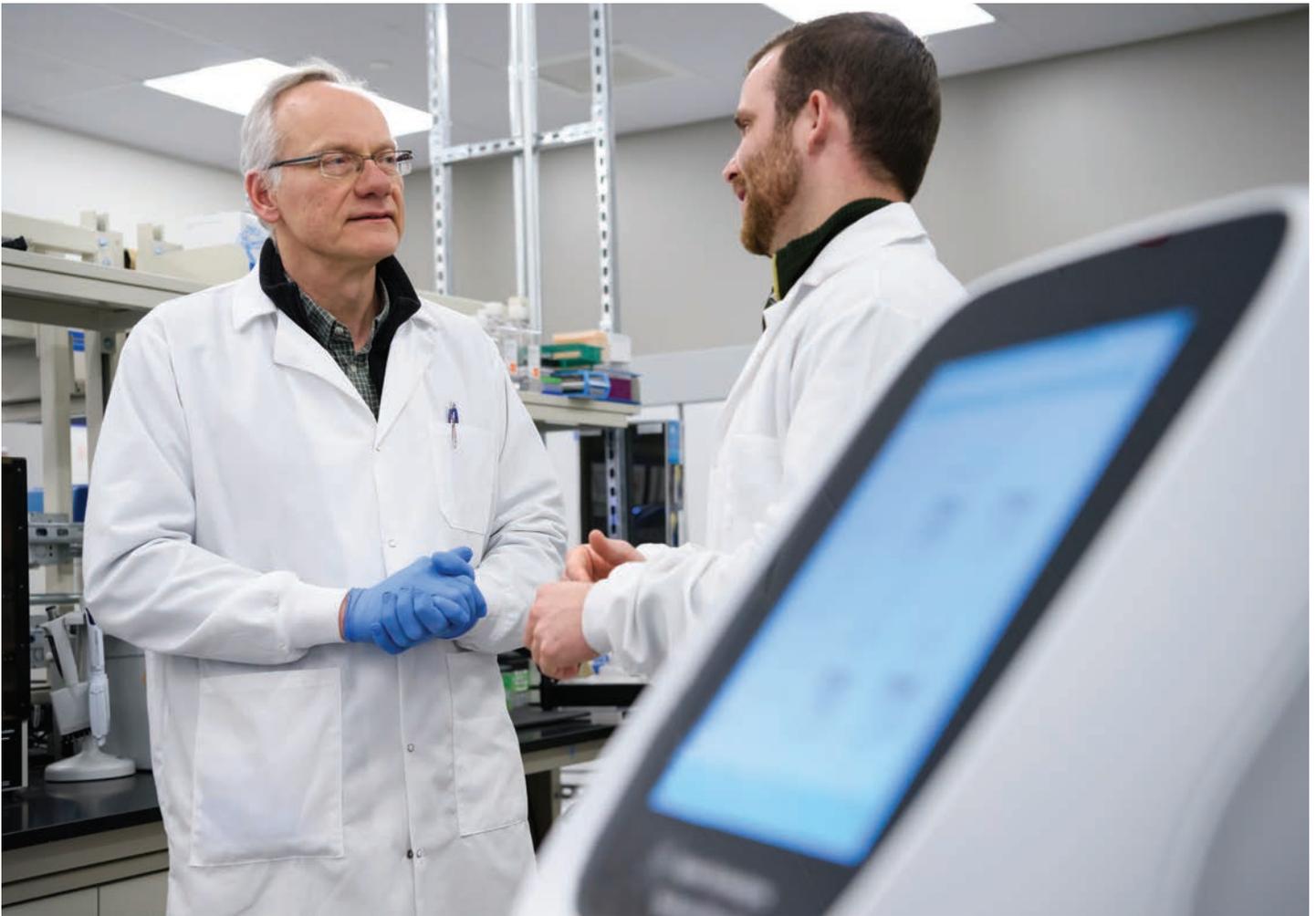
- Non-invasive sensors measure dissolved oxygen and free protons.
- Built-in injection ports deliver compounds at preprogrammed intervals.
- Precision manufacturing process ensures consistent lot-to-lot performance.



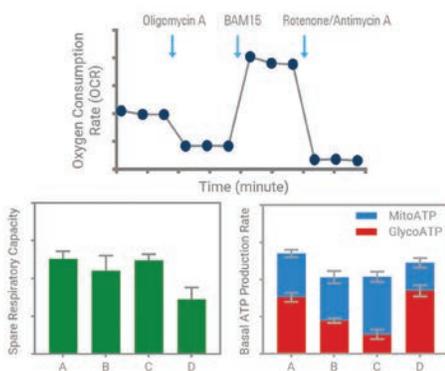
### XF cell culture microplates: Specific to application and instrument type

- Plates are tissue-culture treated and irradiated for cell culture operations.
- Unique well geometry enables rapid, sensitive XF measurements.
- Adheres to SBS standards for plate reader compatibility.
- Poly-D-Lysine coated plates ensure more consistent suspension cell workflows.
- XF HS miniplate accommodates fewer cells per well when sample amount is limited.





## Customized assays for cell therapeutics development

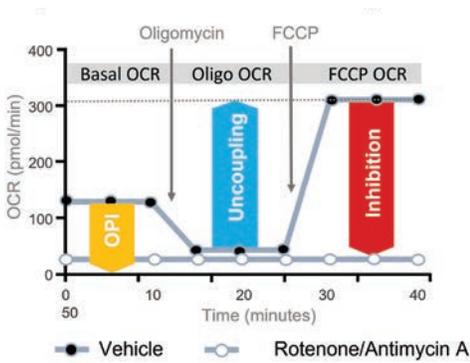


### XF T Cell Metabolic Profiling Assays (Kit part number 103772-100)

With optimized reagents for different T cell populations, these assays provide robust bioenergetic parameters linked to two critical attributes for antitumor properties: T cell persistence and T cell metabolic fitness.

- Suitable for evaluation of construct design, engineering strategies, starting material selection, or metabolic conditioning during *in vitro* cell expansion
- Applicable for use in assessing the capacity of T cells to maintain metabolic fitness in tumor microenvironments
- Can be used for NK cell metabolic profiling

## Turnkey solution for preclinical safety studies



### XF Mito Tox Assay (Kit part number 103595-100)

This assay solution integrates the Agilent Seahorse XF Pro analyzer, a streamlined workflow, and enhanced software tools, enabling you to easily identify drug-induced mitochondrial toxicity.

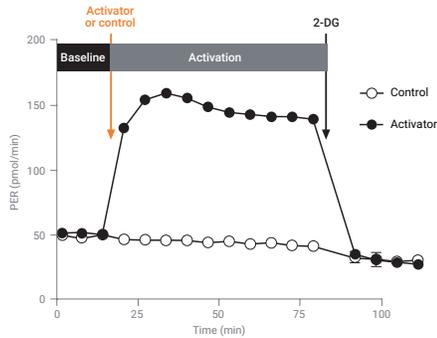
- A direct functional assay to provide high sensitivity and specificity.
- Simplified assay design with quantitative and binary MTI parameters to quickly identify the type and magnitude of mitochondrial toxicity.
- Enhanced software tools to deliver an end-to-end solution that reduces your time to conclusion.

## Immune-cell activation assays for general research applications

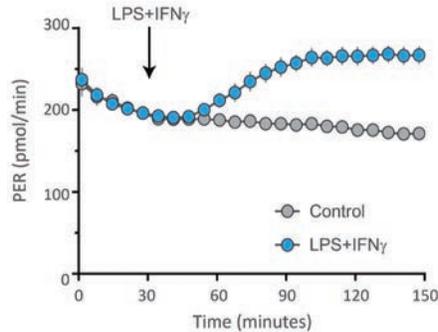
Detect activation responses in minutes upon stimulation, providing an early window for studying immune cell activation, modulation, and related metabolic reprogramming.

### T Cell Activation Assay

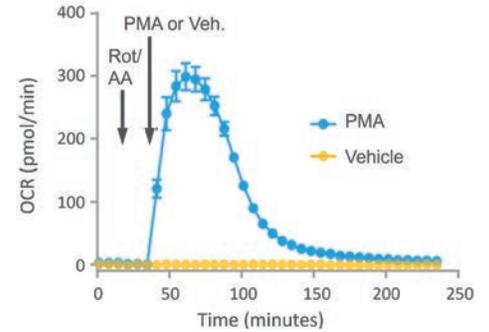
(Kit part number 103759-100)



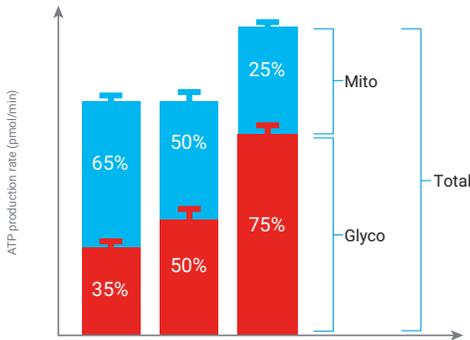
### Macrophage Activation Assay



### Neutrophil Activation Assay



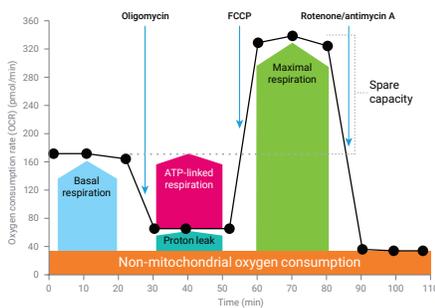
## Core assays for therapeutic discovery and research



### XF Real-Time ATP Rate Assay (Kit part number 103592-100)

This quantitative method measures the production rates of adenosine triphosphate (ATP) from mitochondrial respiration and glycolysis simultaneously in live cells. It can be used to:

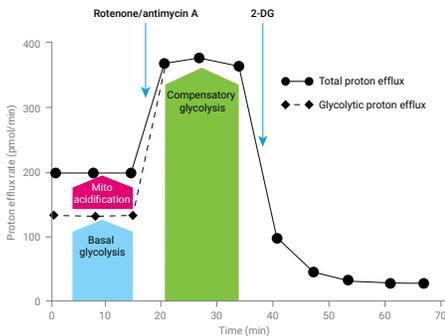
- Assess metabolic phenotype changes during cell activation, proliferation, and differentiation.
- Identify pathway liabilities.
- Screen for compound effect on cellular ATP production.



### XF Cell Mito Stress Test (Kit part number 103015-100)

Use this widely recognized test to gain a comprehensive understanding of mitochondrial (dys)function.

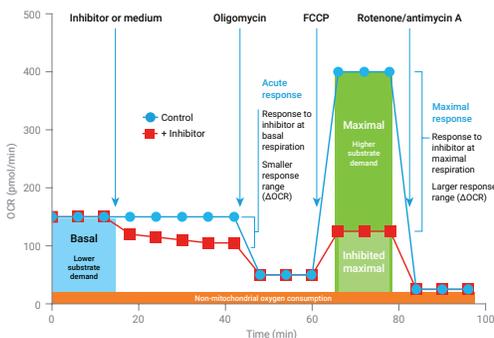
- Report on multiple parameters—including basal and ATP-linked respiration, maximal and spare respiratory capacity, and proton leak.
- Investigate functional differences between cell types and the impact of genetic or pharmaceutical interventions.



### XF Glycolytic Rate Assay (Kit part number 103344-100)

Accurately measure glycolysis in live cells by quantifying proton efflux rate (PER) specific to glycolysis.

- Detect transient and rapid metabolic switches (such as the Warburg effect) in minutes.
- Measure the effects of metabolic modulators on glycolytic rates.
- Report on multiple key parameters: basal and compensatory glycolysis.



### XF Substrate Oxidation Stress Tests

(Kit part numbers 103572-100, 103573-100, 103574-100)

With these optimized kits, you can dive deeper to study the impact of three primary substrates on mitochondrial and cellular function.

- Explore which substrates are relevant or required for a specific cellular function and/or phenotype.
- Discover how cell phenotype can be controlled via manipulation of substrate oxidation activities.

# Enhance data quality and transform data into insights

## Integrate imaging and normalization into your Seahorse XF analysis for more consistent and interpretable results

The BioTek Cytation, an automated digital imaging system, can be seamlessly integrated into XF assay workflow when interfaced to a single XF controller software. This system allows users to:

- Easily normalize signal by cell count to compare across treatment groups
- Examine cell culture integrity/health before and after XF assays
- Improve data quality and interpretability



## Intuitive, streamlined experimental setup and data analysis experience empowered by the state-of-the-art XF software and analytics platform

Wave Pro Controller software, created for the XF Pro analyzer, includes preloaded templates, advanced template import features, and dose-response assay setup for quick design of experiments.

Web-based Seahorse Analytics offers exclusive cutting-edge data analysis features (such as multi-file analysis, hit identification, dose-response curves, Z-prime) allowing for customizable analysis views/reports that can turn data to insights quickly. It also provides secure data storage. Data can be exported into third-party graphing and statistical software like GraphPad Prism and Microsoft Excel.

## Turn raw metabolic data into publishable results

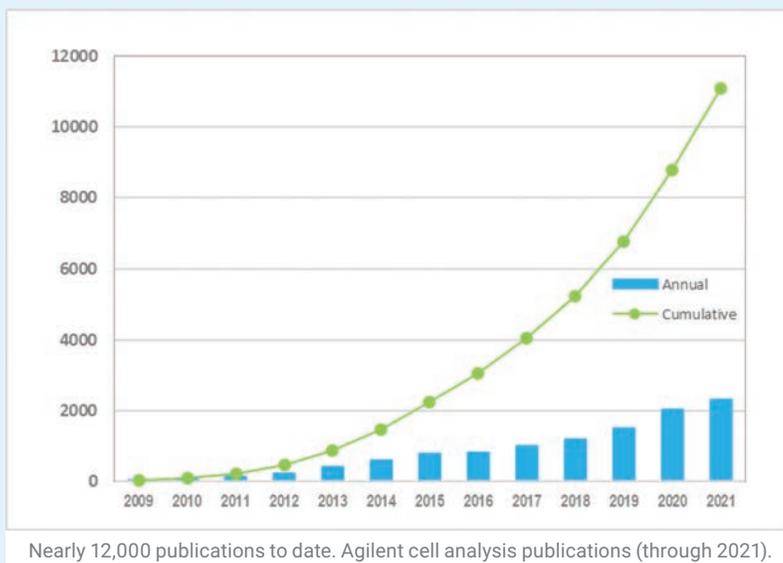
Agilent Seahorse Analytics, the next generation of XF data analysis, provides secure data storage and intuitive data analytics in the cloud. Features include:

- Desktop-like interactivity for accessibility from anywhere on both PC and Mac
- Built-in formulas for automatic calculations and summaries of Seahorse XF assay parameters
- Customizable analysis views for turning data to insight—or for easy data sharing
- Flexible tools for easily exporting your data into third-party graphing and statistical software applications, such as GraphPad Prism and Microsoft Excel



### Did you know that having papers with functional data improves your chances of getting published in a top-tier journal?

Observing intact cell behavior in real time provides much-needed context and confirmation of omics data. No wonder it's playing a growing role in peer-reviewed literature.





## Discover how XF Pro technology can advance your drug discovery studies

Dysfunctional metabolism is associated with many different disease states and is a rich source for bolstering target discovery programs. By incorporating live-cell metabolic measurements, early drug discovery scientists are gaining deeper understanding of therapeutic target effects and the impact of therapeutic agents to alleviate disease states.



Provide cellular attributes linked to antitumor potential of T cell therapeutic products



Identify novel drug targets



Validate target effect on cell function



Determine drug safety



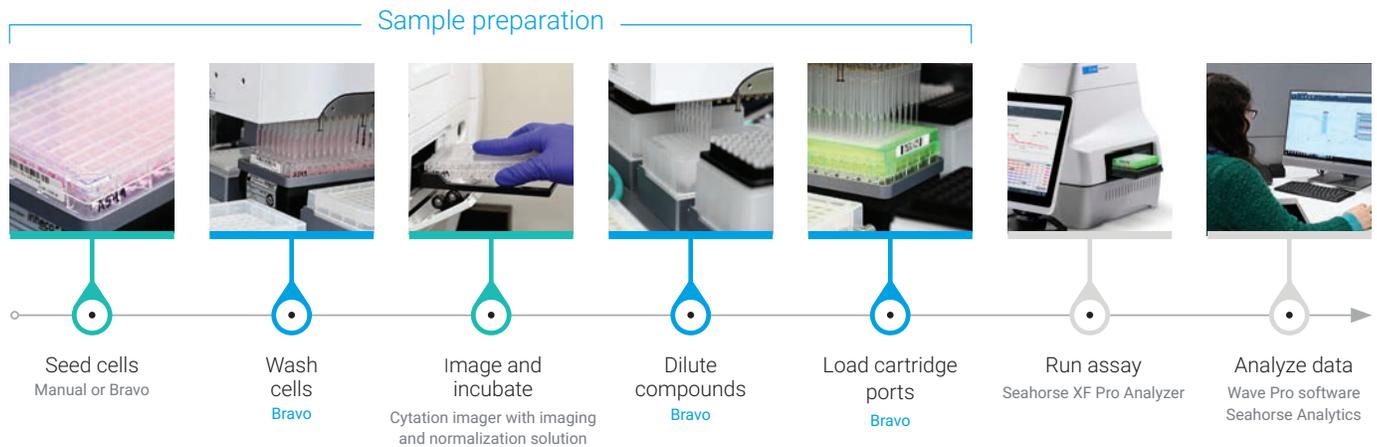
Confirm function post genetic modification



Characterize *in vitro* disease model

# Complete Your Workflow—and Advance Your Live-Cell Metabolic Analysis

Agilent offers a complete workflow for live-cell metabolic analysis, including cell metabolism analyzers, liquid handling automation, imaging plate readers, and high-quality consumables. All work together to help you achieve consistent, reproducible data.



## Consistent data starts with consistent sample preparation

Getting reliable data from live cell-based assays can be challenging if your assay prep methods are not reliable. Automated sample preparation can maximize productivity and minimize human error.

The Bravo Seahorse Assay Workbench allows users of all experience levels to:

- Standardize sample preparation. Intuitive software ensures consistent results between users.
- Increase walk-away time, improve the efficiency of your lab personnel.
- Minimize variability, generating consistent data no matter who is running the protocol.



## Agilent Value Promise

We guarantee you at least 10 years of instrument use from your date of purchase. Otherwise, we will credit you with the residual value of the system toward an upgraded model.

Learn more about Agilent Seahorse XF technology:

[www.agilent.com/chem/discoverXF](http://www.agilent.com/chem/discoverXF)

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[cellanalysis.support@agilent.com](mailto:cellanalysis.support@agilent.com)

Europe

UK: **0800 096 7632**

Germany: **0800 180 66 78**

Netherlands: **0800 022 7243**

Other EU: **+45 3136 9878**

[cellanalysis.support@agilent.com](mailto:cellanalysis.support@agilent.com)

Asia Pacific

[cellanalysis.support@agilent.com](mailto:cellanalysis.support@agilent.com)

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