# Анализаторы Seahorse XF

## Технические характеристики


**По вопросам продаж и поддержки обращайтесь:**

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Архангельск (8182)63-90-72
Астрахань (8512)99-46-04
Барнаул (3852)73-04-60
Белгород (4722)40-23-64
Брянск (4832)59-03-52
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Волгоград (844)278-03-48
Вологда (8172)26-41-59
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Новосибирск (383)227-86-73
Омск (3812)21-46-40
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Оренбург (3532)37-68-04
Пенза (8412)22-31-16
Пермь (342)205-81-47
Ростов-на-Дону (863)308-18-15
Рязань (4912)46-61-64
Самара (846)206-03-16
Санкт-Петербург (812)309-46-40
Саратов (845)249-38-78
Севастополь (8692)22-31-93
Симферополь (3652)67-13-56
Смоленск (4812)29-41-54
Сочи (862)225-72-31
Ставрополь (8652)20-65-13
Сургут (3462)77-98-35
Тверь (4822)63-31-35
Томск (3822)98-41-53
Тула (4872)74-02-29
Тюмень (3452)66-21-18
Ульяновск (8422)24-23-59
Уфа (347)229-48-12
Хабаровск (4212)92-98-04
Челябинск (351)202-03-61
Череповец (8202)49-02-64
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# 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 research and discovery in areas like these:

**Immunology**

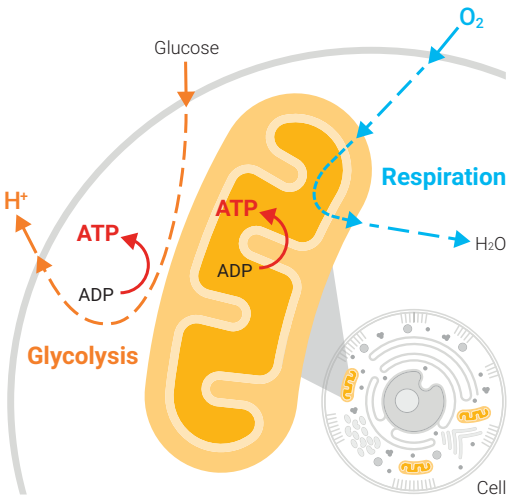
- Determining metabolic fitness of immune cells
- Measuring and modulating immune cell activation in real time
- Targeting metabolic pathways to improve immunotherapy design and performance

**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 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 XFe96 Analyzer

Maximize your capacity for XF assays and minimize cost per sample. The Seahorse XFe96 enables many experimental groups per assay, maximizing design flexibility and sample throughput.

Key applications:

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

### Agilent Seahorse XFe24 Analyzer

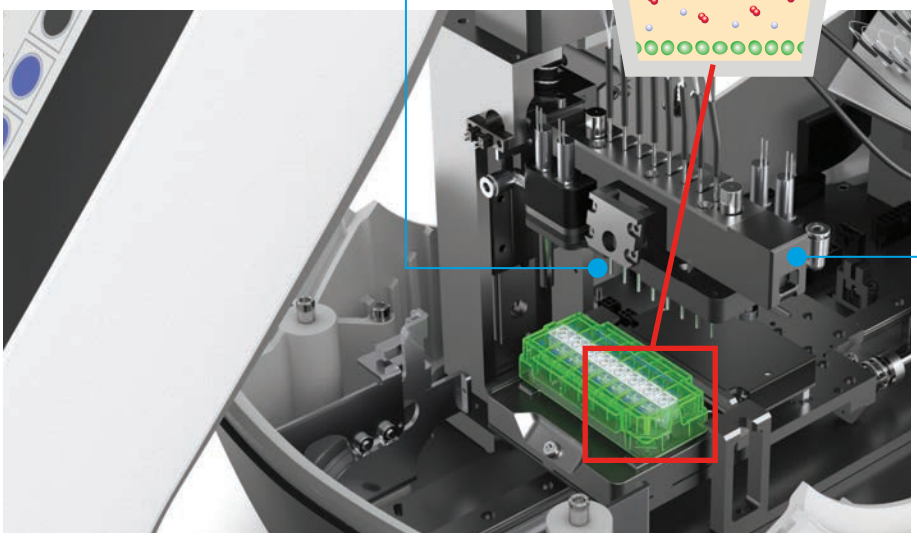
Featured in hundreds of peer-reviewed publications, the Seahorse XFe24 uses a larger well format suitable for islets and other specialty samples.

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.



Integrated injection ports sequentially deliver up to four compounds, allowing you to test multiple conditions per well.

Cells are seeded in the wells of the custom Seahorse XF cell culture microplate.

Biologically compatible optical microsensors simultaneously measure rates of oxygen consumption and extracellular acidification, without adding dyes.

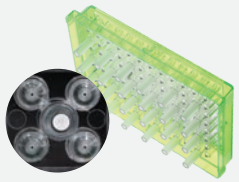
Sensor probes gently lower to create a transient microchamber. This process allows rapid, real-time measurement of changing oxygen and proton concentrations in the extracellular medium.

The microplate well requires 10- to 20-fold fewer cells than conventional respirometers.

Instrument mechanics gently raise and lower the cartridge to mix the fluid in the wells for optimal dispersal of modulators and analytes.

## 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

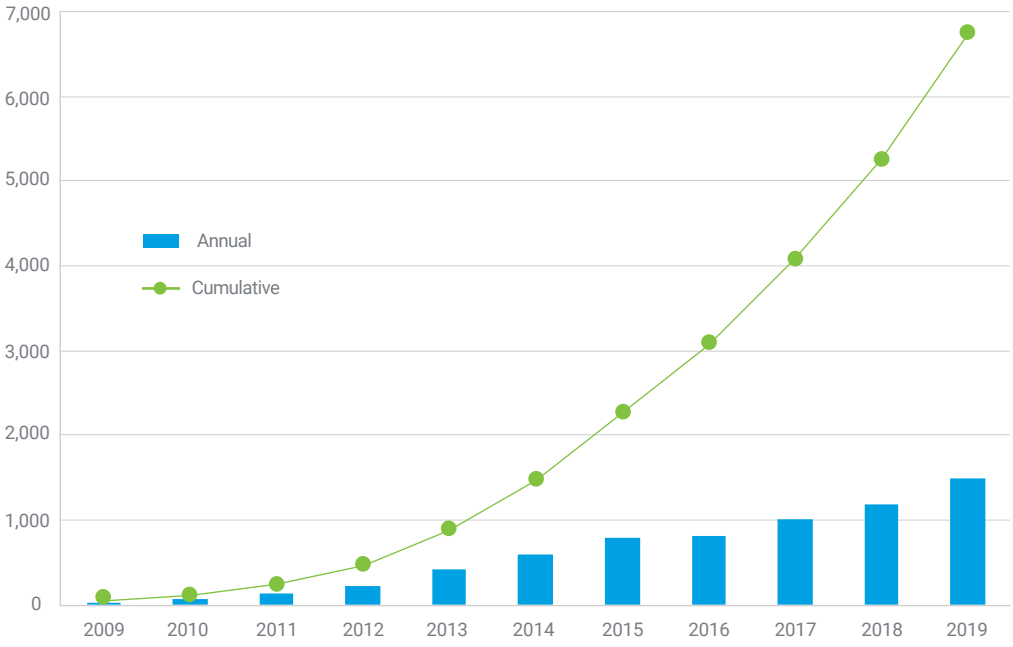
- Plates are tissue-culture treated and irradiated for cell culture operations.
- Unique well geometry enables rapid, sensitive XF measurements.
- Adheres to SBS standards for plate reader compatibility.
- Poly-D-Lysine coated plates ensure more consistent suspension cell workflows.
- XF HS miniplate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

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

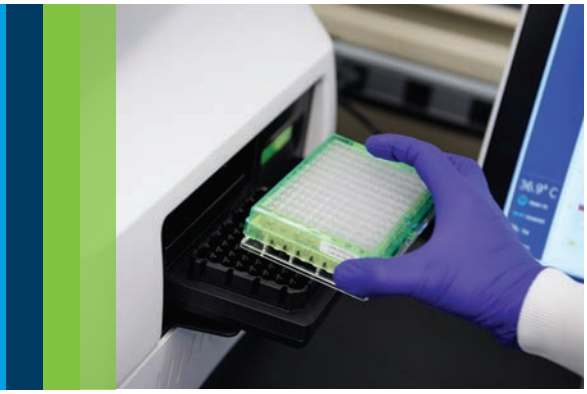
Observing intact cell behavior in real time provides much-needed context and confirmation of omics data. No wonder it's playing a growing role in peer-reviewed literature.

**Nearly 7,000 publications to date**

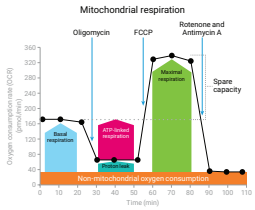
Agilent cell analysis publications (through 2019)



# Gain Deeper Insight into Your Cell Function with Agilent Seahorse XF Assay Kits

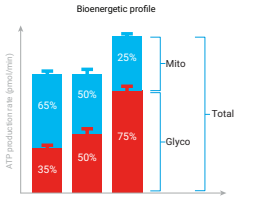


How are the cells generating energy? Has my treatment changed the phenotype? Agilent Seahorse XF Assay kits—built on validated biology—help you answer these questions and more.



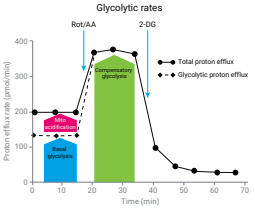

## Agilent Seahorse XF Cell Mito Stress Test Kit

This assay is well-recognized for gaining insight into the components of mitochondrial function. Investigate functional differences among cell types and drug candidates, as well as genetic or biochemical interventions.



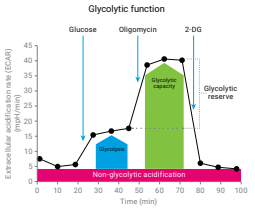

## Agilent Seahorse XF Real-Time ATP Rate Assay Kit

The only assay that measures the rates of ATP production from glycolysis and mitochondrial respiration simultaneously. Quantification of ATP production rate delivers a dynamic picture of cellular bioenergetics, giving you a unique understanding of cellular phenotype and function.



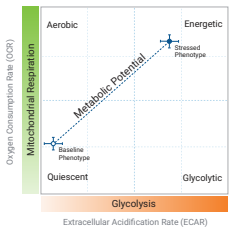

## Agilent Seahorse XF Glycolytic Rate Assay Kit

Precisely measures glycolysis in live cells, revealing transient responses and rapid metabolic switches not discernible in endpoint lactate assays. You can also quantify proton efflux specific to glycolysis.



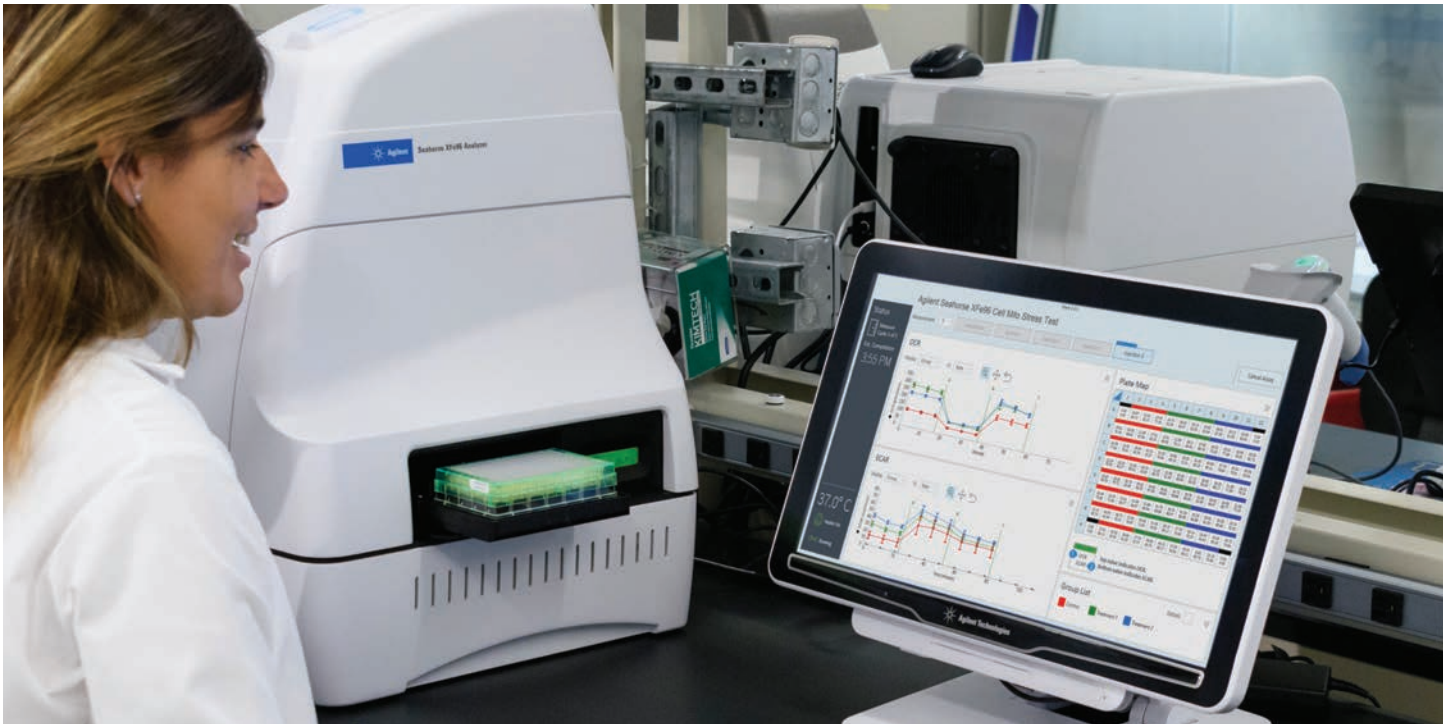

## Agilent Seahorse XF Substrate Oxidation Kits

A suite of optimized kits for rapidly measuring cellular substrate oxidation by assessing changes in oxygen consumption (OCR) in live cells. These kits facilitate investigation of how cells alter or shift oxidation of specific substrates to perform key cellular functions, as well as respond to genetic or pharmaceutical interventions.




## Agilent Seahorse XF Cell Energy Phenotype Test Kit

This simple assay simultaneously measures mitochondrial respiration and glycolysis with just a single injection, so you can rapidly determine energy phenotypes of cells and investigate metabolic switching. It's also a useful assay for screening compounds that can modulate mitochondrial and/or glycolytic function.



# Transform Data into Insights

## Control without the complexity

The Agilent Seahorse XFe Analyzer and XFp Analyzer software interfaces provide an intuitive, streamlined experimental setup and data acquisition experience.

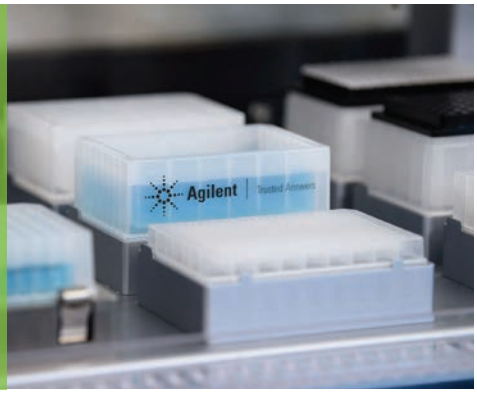
- Predefined templates designed for each Seahorse XF Assay Kit ensure accurate, consistent experiment setup.
- Kinetic rate data is automatically calculated and displayed in real time, allowing you to observe time-dependent biological responses throughout your assay.

## 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:

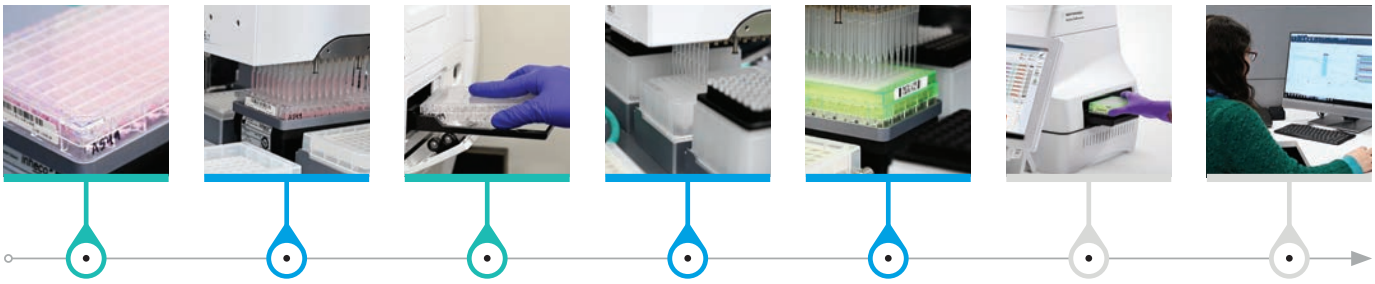
- Desktop-like interactivity for accessibility from anywhere on both PC and Mac
- Built-in formulas for automatic calculations and summaries of Seahorse XF assay parameters
- Customizable analysis views for turning data to insight—or for easy data sharing
- Flexible tools for easily exporting your data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

# 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.

Sample preparation



Seed cells
Manual or Bravo

Wash cells
Bravo

Image and incubate
Cytation imager with imaging and normalization solution

Dilute compounds
Bravo

Load cartridge ports
Bravo

Run assay
Seahorse XFe96 Analyzer

Analyze data
Wave software
Seahorse analytics

## 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.

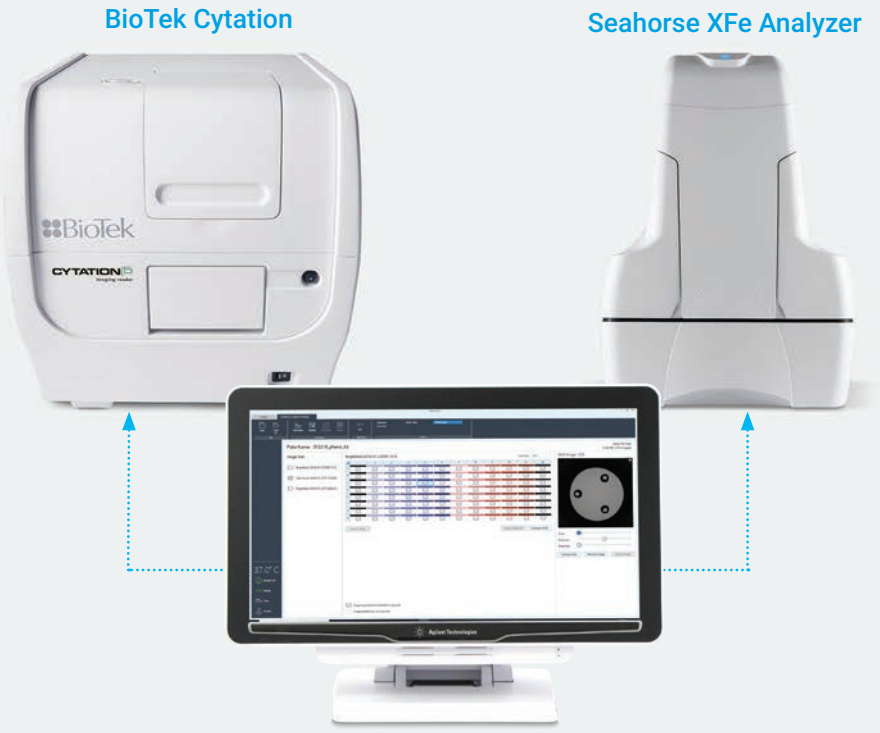
The Bravo Seahorse Assay Workbench allows users of all experience levels to:

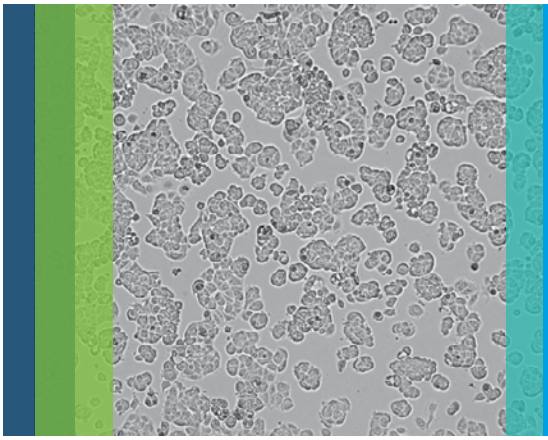
- Standardize sample preparation. Intuitive software ensures consistent results between users.
- Increase walk-away time, improve the efficiency of your lab personnel.
- Minimize variability, generating consistent data no matter who is running the protocol.

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

The BioTek Cytation combines automated digital microscopy and conventional microplate detection in a configurable, upgradable platform.

- Take advantage of powerful imaging, data capture, and data analysis.
- Count cellular objects *in situ*.
- Easily document wells of interest.
- Normalize cell count to compare across treatment groups.
- Seamlessly integrate normalization data and well images into your XF assay result file.





# Agilent Seahorse XF Technology Imaging and Data Normalization Solution

**Agilent Seahorse XFe Analyzers:**
Metabolic Analysis in Live Cells

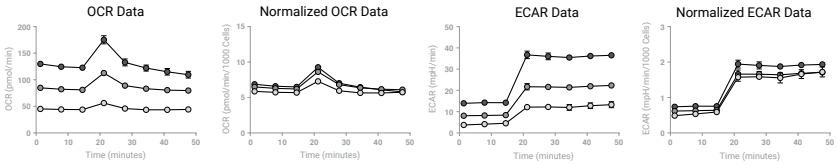
**BioTek Instruments Cytation 1 and Cytation 5:**
Cell Imaging for Normalization

- Agilent Seahorse XF Analyzers simultaneously measure the two major energy pathways of the cell - mitochondrial respiration and glycolysis - in live cells, in real time.
- BioTek Cytation 1/5 Imagers automate digital quantitative microscopy with fluorescence and high contrast brightfield cellular visualization from 1.25 - 60x without complexity.

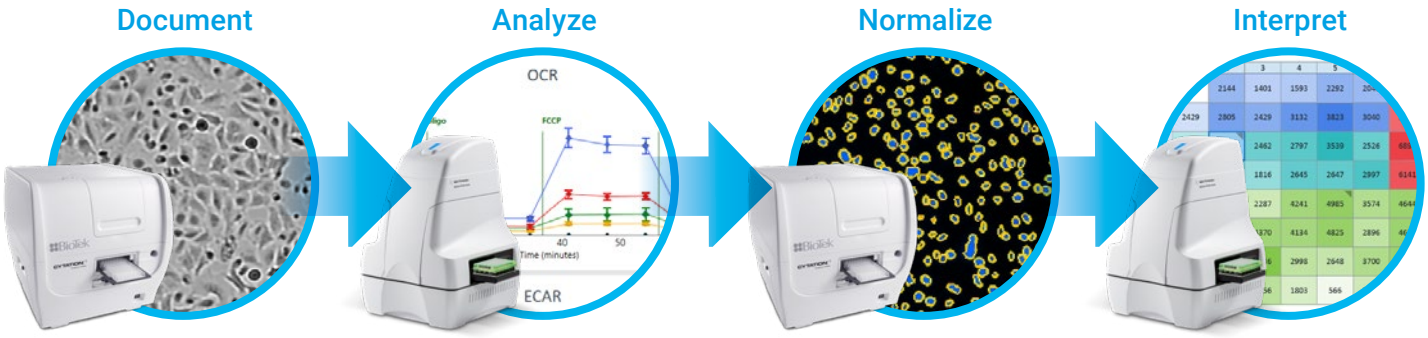

## Standardized Normalization Method

## XF Data Interpretation Made Easier

- Perform XF analysis with an easy-to-use, reliable, validated, and supported cell count based normalization solution
- Improve XF data interpretation by applying cell count numbers directly to your XF data enabling plate-plate, experiment-experiment, and well-well comparisons
- Document the cell culture condition throughout the XF assay to quality control assay readiness and assist with finding outliers
- Associate normalization values, brightfield and fluorescence images in WAVE
- Enhance live cell assay reproducibility
- Simplify normalization workflow with uncomplicated software and single controller operation to communicate data between both devices




SKOV3 cells were plated at 10, 20, and 30K cells per well. Raw OCR and ECAR change with injection of oligomycin + FCCP.

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## BioTek Cytation 1 Technology

Cytation 1 and Cytation 5 Cell Imaging Multi-Mode Readers offers fluorescence and high contrast brightfield imaging up to 60x magnification without the expense and complexity commonly associated with other digital microscopy systems. XY stage, focus, exposure, image capture and LED intensity are all fully automated for ease of use. BioTek's powerful Gen5 software enables seamless capture to quantitative publication ready data with no extensive training required. Temperature control to 45°C and shaking are standard, and both $CO_2$/$O_2$ control and reagent injectors are optional. An available multi-mode detection module includes high sensitivity filter-based fluorescence and a monochromator system for UV-Vis absorbance. Cytation's unique, patented design provides both quantitative phenotypic cellular information with well-based quantitative data.

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## Agilent Seahorse XF Technology

The role of metabolism in cellular and physiological processes is well established, with many diseases now linked to metabolic dysfunction or reprogramming. Agilent Seahorse XF technology simplifies cell energy metabolic analysis. It uses label-free technology to measure changes in oxygen consumption rate (OCR) and extracellular acidification rate (ECAR). Cells are seeded into wells and up to four drugs such as inhibitors or stimulators may be added to the cells automatically. In the sensor cartridge each sensor tip is sourrounded by four integrated drug injection ports. Drugs are released sequentially into the well and mixed into the media by the fiber optic probe. Changes in cellular metabolism induced by the drugs are measured kinetically and in real-time.

## Other Features

- **Real-time Results** – this integrated system reports metabolic rates in just minutes, without sample extraction or labeling. Wave software controls the instrument and performs rate measurements on the fly for same day results.
- **Live-Cell Responses** – detect responses to substrates, inhibitors, and other compounds in real time via the 4-port injection system and automated mixing while maintaining physiologic temperature (37ºC).
- **Flexible Assay Design** – the 96-well or 24-well plate formats accommodate many conditions in a single run and are best for dose-response studies and compound screening.
- **High Sensitivity** – analyze as few as 5000 cells per well in the custom 96-well plate.
- Easily create assay protocols and analyze data with Seahorse Wave software.
- Measure mitochondrial function with the Seahorse XF Cell Mito Stress Test.
- Generate a metabolic phenotype within one hour with the Seahorse XF Cell Energy Phenotype Test.
- Determine the ability of cells to use the glycolytic pathway to meet energy demand with the Seahorse XF Glycolysis Stress Test.
- Quickly determine dependency of cellular energy production on mitochondrial substrates with the Seahorse XF Mito Fuel Flex Test.

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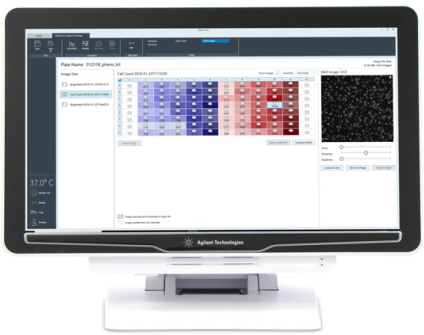
# Get the Best in Usability and Processing Speed with the Agilent Seahorse XFe Controller Upgrade



The Seahorse XFe Controller Upgrade is an IT-friendly system upgrade including a Windows 10 (64-bit) computer and 64-bit hardware for Agilent Seahorse XFe Analyzers that provides modern Windows and touchscreen technology with the same small benchtop footprint.

| Benefit | The NEW XFe Controller | The Previous XFe Controller |
|---|---|---|
| **Latest Windows OS** | **Windows 10 (64-bit) OS** provides faster system responsiveness, improved file management, and security. | **Windows 7 (32-bit) OS**, cannot be upgraded. Security and feature updates discontinued after Microsoft's "extended support" period (Jan 2020). |
| **Power to Run Your Longest Assays** | Robust computer performance via the **Intel Core i5 processor** and **16 GB RAM** provides greater operational reliability. | XFe assays can be resource intensive; the **Intel Core i3 processor** and **4 GB RAM** support basic assay performance. Refer to XFe Controller Best Practices to avoid performance issues. |
| **Customizable System Security** | Enhanced default Windows security with Windows Defender. Supports installation of preferred antivirus software, disk encryption, and Windows Updates. | Default Windows security is Microsoft Security Essentials. Alternative antivirus software and disk encryption is **not supported**. |
| **IT-Friendly Hardware** | Built-in Wireless (802.11 b/g/n/ac) and Ethernet Port simplify integrating the XFe Analyzer into your institutions network infrastructure. | Ethernet Port only, less flexibility for configuring network access, especially for more stringent network security requirements. |

Additional benefits:

- Better touchscreen performance
- Faster data processing and analysis in Wave
- Compatible with the Agilent Seahorse XF Imaging and Normalization system

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| Specifications | NEW XFe Controller | Previous XFe Controller |
|---|---|---|
| **Operating System** | Windows 10 | Windows 7 |
| **Processor** | Intel i5 | Intel i3 |
| **Memory (RAM)** | 16 GB | 4 GB |
| **Hard Disk Drive** | 2 TB SSD | 125 GB |
| **USB** | USB 3.0 & 2.0 | USB 2.0 |
| **Ethernet Port** | LAN RJ45 (Gigabit) | LAN RJ45 (Gigabit) |
| **Wireless LAN** | 802.11 b/g/n/ac | None |
| **Bluetooth** | BT 4.2 | None |
| **Display Size** | 21-inch widescreen | 18.5-inch diagonal |
| **Resolution** | 1920 x 1080 | 1366 x 768 |
| **Touch Screen** | Projected Capacitive | S.A.W. |
| **Imaging & Normalization Ready** | Yes | No |
| **Part Numbers** | S7807A (XFe96), S7807B (XFe24) | |

Upgrade Includes: new controller computer, internal 64-bit hard drive, installation and transfer of all instrument system data to the new controller.

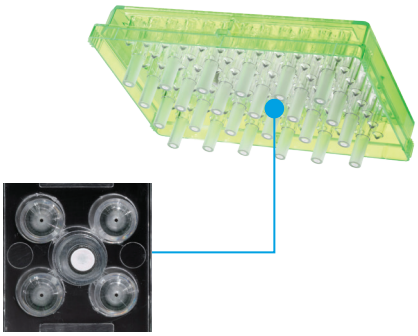
# Agilent Seahorse XF FluxPaks Take You from Sample to Insight

Convenient FluxPaks contain the essential consumables you need to perform XF assays.

## Sensor cartridges

The essential component for simultaneously measuring bioenergetic pathways.

- Non-invasive sensors to measure dissolved oxygen and free protons
- Four injection ports
- Lot-to-lot consistency
- High-precision manufacturing process
- One-year shelf life from manufacturing date
- Patented design



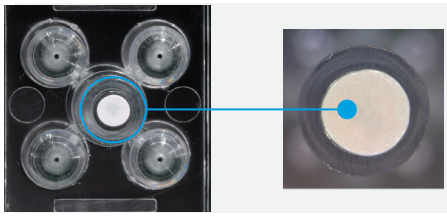
## Cell culture microplates

Choose from many plates to suit your application and sample type. See page 8 for a complete list.

- Tissue culture treated and irradiated for sterility
- Tapered walls to facilitate cartridge alignment, especially at low volumes
- Well “bumps” define microchamber height and volume
- Conforms to ANSI/SLAS Microplate Standards for footprint dimensions and well positions
- Polystyrene material
- Living cells, isolated mitochondria, islets, and spheroids
- Poly-D-Lysine 96- and 8-well formats

## Calibrant and calibration plates

- Premixed calibrant: ready to use and optimized for XF sensor cartridge calibration
- Calibration plates: suitable for calibration and hydration

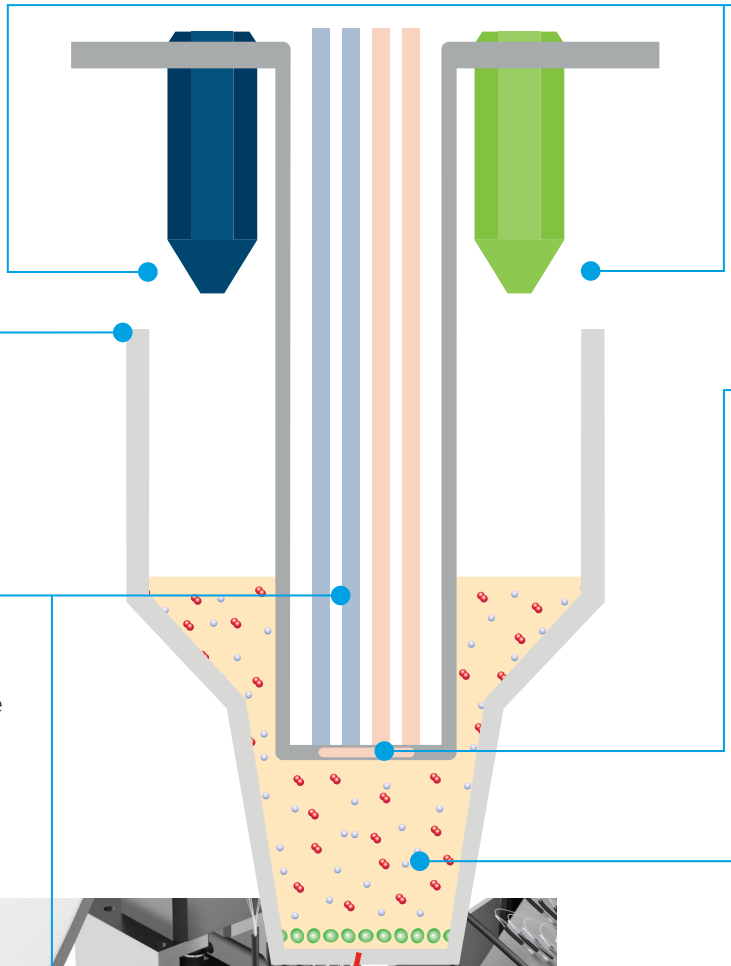



### Precise sensor cartridge spotting

High-resolution machine vision imaging inspects the spot quality on every cartridge.

# Seahorse XF Technology: A Closer Look

The integrated drug delivery system lets you sequentially add up to four compounds per well, so you can perform a complete mitochondrial or glycolytic kinetic assessment.



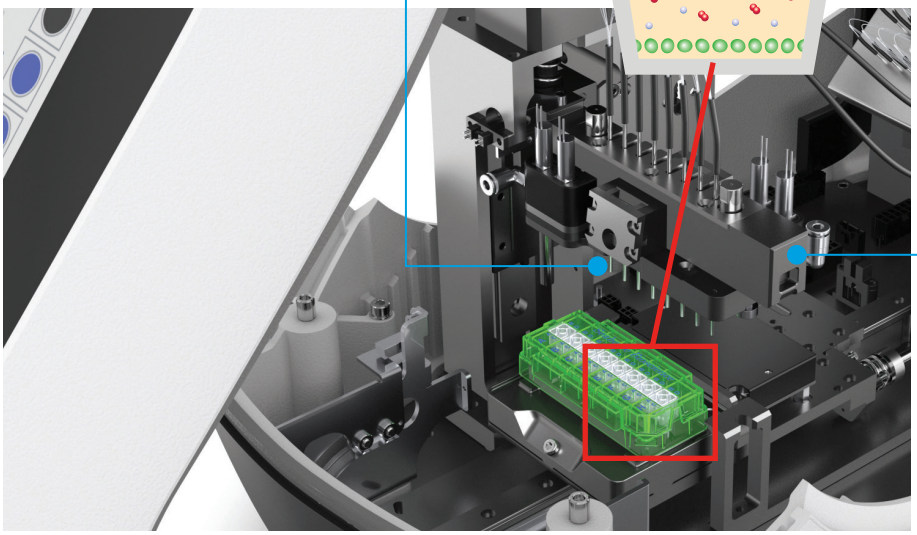
Integrated injection ports sequentially deliver up to four compounds, allowing you to test multiple conditions per well.

Cells are seeded in the wells of the custom Seahorse XF Cell Culture Microplate.

Biologically compatible optical microsensors simultaneously measure rates of oxygen consumption and extracellular acidification, without adding dyes.

Sensor probes gently lower to create a transient microchamber. This process allows rapid, real-time measurement of changing oxygen and proton concentrations in the extracellular medium.

The microplate well requires 10- to 20-fold fewer cells than conventional respirometers.



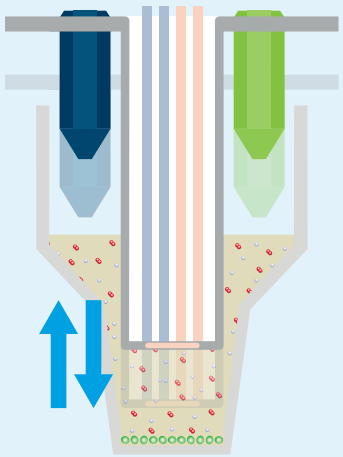
Instrument mechanics gently raise and lower the cartridge to mix the fluid in the wells for optimal dispersal of modulators and analytes.

## What happens during a measurement cycle?

Each measurement cycle amounts to one time point; over time, the kinetic data is plotted and displayed.

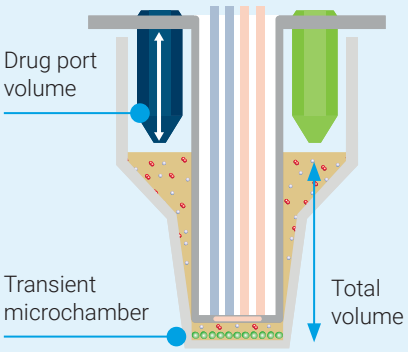
### Mix

When the assay starts, the sensor cartridge probes lower into each well and repeatedly mix the culture medium.



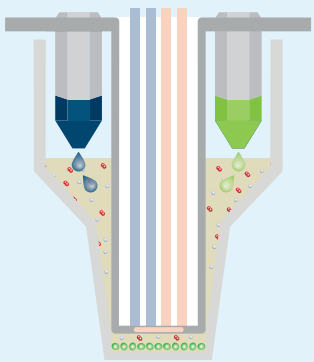
### Measure

The probes lower to form the extremely small volume of the transient microchamber.



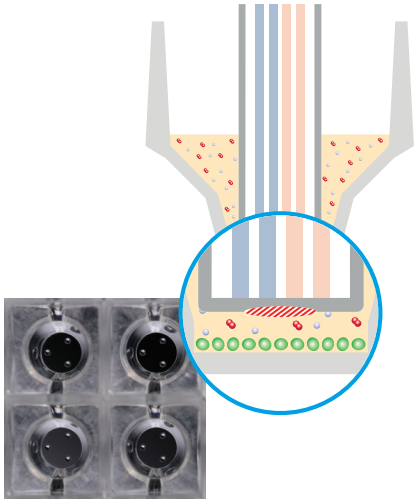

### Inject

After a series of basal measurements, constituents are injected from the drug ports into the culture medium at user-programmable intervals.

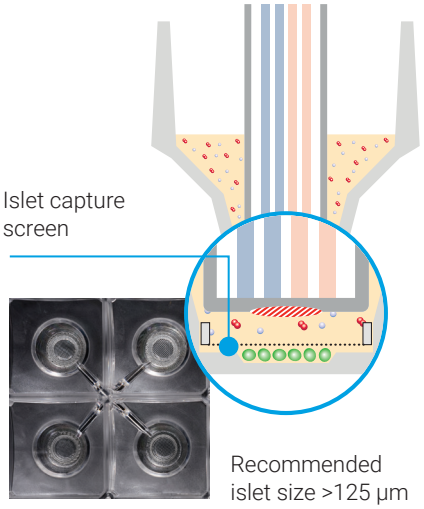


# Cell Culture Microplate Types and Specifications

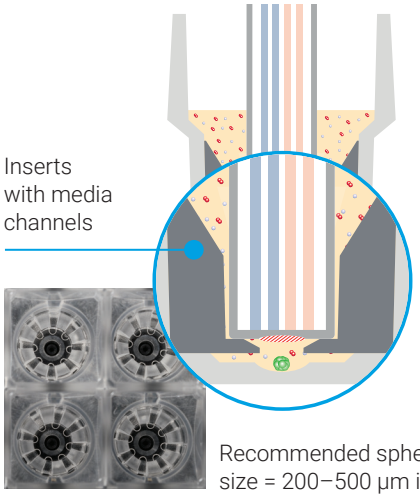
## Standard Microplate



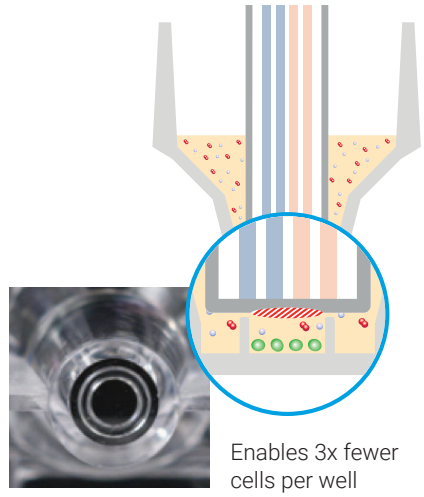
## Islet Capture Microplate




## Spheroid Microplate




## XF HS Miniplate




Seahorse XF Cell Culture Microplates and Miniplates can be modified with substrates to improve cellular adherence. You can purchase precoated Poly-D-Lysine plates or modify manually (such as collagen or Cell-Tak).

| Analyzer | Plate Type | Number of Wells | Total Volume (µL) | Microchamber Volume (µL) | Height of Microchamber (µm) | Material | Cell Types | Isolated Mitochondria | Part Number |
|---|---|---|---|---|---|---|---|---|---|
| XFe96 or XF96 | V3 PS | 96 | 250 | 2.28 | 200 | PS | 2-D, monolayer | Yes | 101085-004 |
| XFe96 | Spheroid | 96 | 250 | 1.88 | 250 | PS | 3-D, spheroids | No | 102978-100 |
| XFe96 or XF96 | V3 PS (PDL) | 96 | 250 | 2.28 | 200 | Poly-D-Lysine on PS | 2-D, suspension and monolayer | Yes | 103729-100 |
| XFe24 or XF24 | V7 PS | 24 | 1000 | 5.65 | 200 | PS | 2-D, monolayer | Yes | 100777-004 |
| XFe24 or XF24 | V28 PS | 24 | 1000 | 22 | 812 | PS | 2-D, monolayer | No | 100882-004 |
| XFe24 or XF24 | Islet | 24 | 1000 | 16.6 | 1,422 | PS | 3-D, islets | No | 101122-100 |
| XF HS Mini or XFp | V3 PS | 8 | 250 | 2.28 | 200 | PS | 2-D, monolayer | Yes | 103022-100 |
| XF HS Mini or XFp | V3 PS (PDL) | 8 | 250 | 2.28 | 200 | Poly-D-Lysine on PS | 2-D, suspension and monolayer | Yes | 103722-100 |
| XF HS Mini | PS | 8 | 250 | 0.71 | 200 | PS | High sensitivity* 2-D; monolayer | Yes | 103725-100 |
| XF HS Mini | PS (PDL) | 8 | 250 | 0.71 | 200 | Poly-D-Lysine on PS | High sensitivity* 2-D; suspension and monolayer | Yes | 103727-100 |

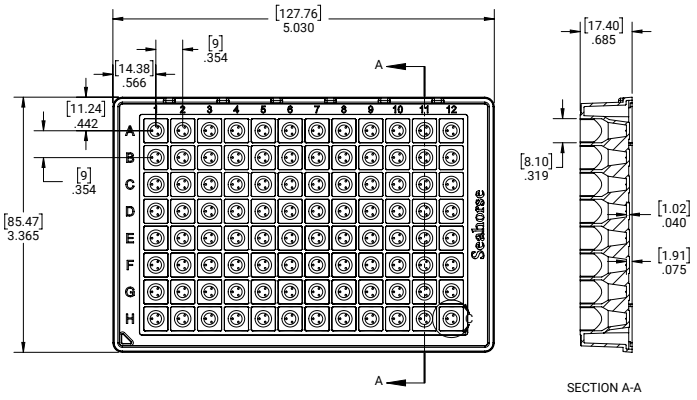
*PS = Polystyrene*

** = XF HS Miniplate enables fewer cells per well.*

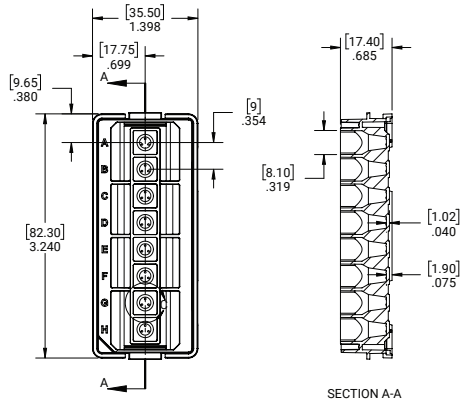
# Specifications for Cell Culture Microplates

## XFe96 Cell Culture Microplate

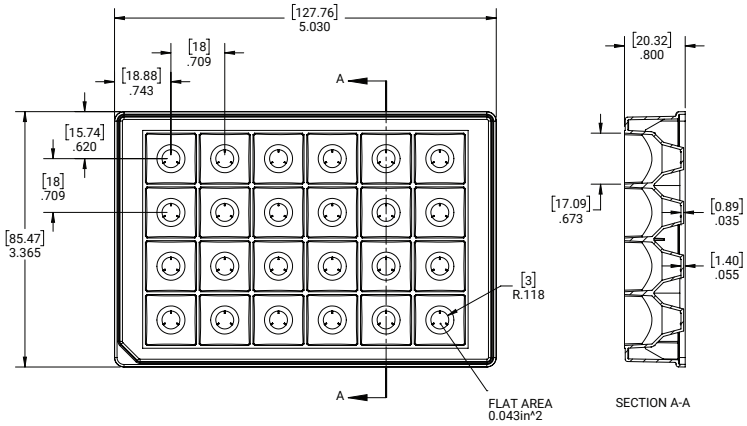
**Dimensions are [mm] in.**



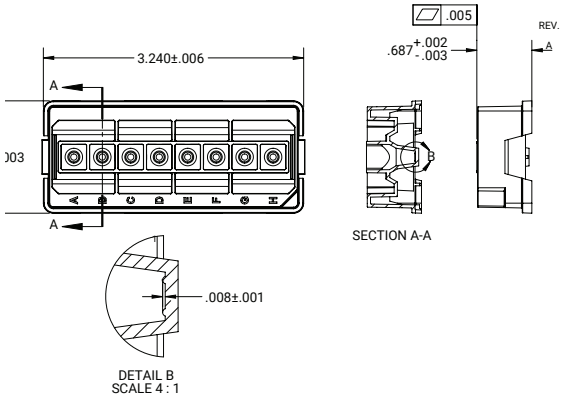

## XFp Cell Culture Miniplate




## XFe24 Cell Culture Microplate




## XF HS Miniplate




### Cell culture microplate dimensions

| Plate Type | Max Volume (µL) | Well Depth (mm) | Well Diameter Top/Bottom (mm) | Plate Length (mm) | Plate Width (mm) | Plate Height (mm) | A1 Row Offset (mm) | A1 Column Offset (mm) | Well-to-Well Row Center Spacing (mm) | Well-to-Well Column Center Spacing (mm) | Well Bottom Elevation (mm) | Well Bottom Thickness (mm) | Well Bottom Area (mm2) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| XFe96 | 250 | 15.49 | 8.1/3.81 | 127.76 | 85.47 | 17.4 | 11.24 | 14.38 | 9 | 9 | 0.89 | 1.02 | 11.40 |
| XFe24 | 1000 | 18.92 | 17.09/6 | 127.76 | 85.47 | 20.32 | 15.74 | 18.88 | 18 | 18 | 0.51 | 0.89 | 28.26 |
| XF HS Mini / XFp | 250 | 15.49 | 8.1/3.81 | 35.5 | 82.3 | 17.4 | 9.65 | 17.75 | 9 | N/A | 0.89 | 1.02 | 11.40 |
| XFe24 Islet | 1000 | 19.18 | 17.09/3.15 | 127.76 | 85.47 | 20.32 | 15.74 | 18.88 | 18 | 18 | 0.5 | 0.65 | 7.79 |
| XFe96 Spheroid | 250 | 15.75 | 8.1/3.18 | 127.76 | 85.47 | 17.4 | 11.24 | 14.38 | 9 | 9 | 0.89 | 0.76 | 7.94 |

## Ordering information for Agilent Seahorse XF plastic ware

### XFe96/XF96 Analyzers

| Product Description | Product Content | Part Number |
|---|---|---|
| Seahorse XFe96 FluxPak | 18 XFe96 Sensor Cartridges, 20 XF96 V3 PS Tissue Culture Microplates | 102416-100 |
| Seahorse XFe96 FluxPak Mini | 6 XFe96 Sensor Cartridges, 10 XF96 V3 PS Tissue Culture Microplates | 102601-100 |
| Seahorse XFe96 Spheroid FluxPak | 6 XFe96 Sensor Cartridges, 6 XFe96 Spheroid Culture Micoplates | 102905-100 |
| Seahorse XF96 V3 PS Culture Microplates | 10 polystyrene plates | 101085-004 |
| Seahorse XFe96 Spheroid Plate | 1 polystyrene plate, 1 mm (divot diameter) | 102959-100 |
| Seahorse XFe96 Spheroid Microplates | 6 polystyrene plates, 1 mm (divot diameter) | 102978-100 |
| Seahorse XFe96 FluxPak Mini (PDL Microplates) | 6 XFe96 Sensor Cartridges, 6 XFe96 PDL Microplates | 103729-100 |
| Seahorse XFe96 PDL Microplates | 6 XFe96 PDL Microplates | 103730-100 |

### XFe24 Analyzers

| Product Description | Product Content | Part Number |
|---|---|---|
| Seahorse XFe24 FluxPak | 18 XFe24 Sensor Cartridges, 20 XF24 V7 PS Tissue Culture Microplates | 102340-100 |
| Seahorse XFe24 FluxPak Mini | 6 XFe24 Sensor Cartridges, 10 XF24 V7 PS Tissue Culture Microplates | 102342-100 |
| Seahorse XFe24 Islet Capture FluxPak | 6 XFe24 Sensor Cartridges, 6 XF24 Islet Capture Culture Microplates | 103518-100 |
| Seahorse XF24 V7 PS Culture Microplates | 10 polystyrene plates | 100777-004 |
| Seahorse XF24 V28 PS Culture Microplates | 10 polystyrene plates | 100882-004 |
| Seahorse XF24 Islet Capture Microplates | 6 polystyrene plates | 101122-100 |

### XFp Analyzers

| Product Description | Product Content | Part Number |
|---|---|---|
| Seahorse XFp FluxPak | 12 XFp Sensor Cartridges, 12 XFp PS Tissue Culture Microplates | 103022-100 |
| Seahorse XFp Cell Culture Miniplates | 12 polystyrene plates | 103025-100 |
| Seahorse XFp FluxPaks (PDL Miniplates) | 12 8-well sensor cartridges, 12 XFp Miniplates | 103721-100 |
| Seahorse XFp PDL Miniplates | 12 XFp PDL Miniplates | 103722-100 |

### XF HS Mini Analyzers

| Product Description | Product Content | Part Number |
|---|---|---|
| Seahorse XFp FluxPak | 12 XFp PS Tissue Culture Microplates | 103022-100 |
| Seahorse XFp Cell Culture Miniplates | 12 polystyrene plates | 103025-100 |
| Seahorse XFp FluxPak (PDL Miniplates) | 12 8-well sensor cartridges, 12 XFp PDL Miniplates | 103721-100 |
| Seahorse XFp PDL Miniplates | 12 XFp PDL Miniplates | 103722-100 |
| Seahorse XF HS Mini FluxPak | 12 8-well sensor cartridges, 12 XF HS Miniplates | 103723-100 |
| Seahorse XF HS Mini FluxPak (PDL Miniplates) | 12 8-well sensor cartridges, 12 XF HS PDL Miniplates | 103724-100 |
| Seahorse XF HS Miniplates | 12 XF HS Miniplates | 103725-100 |
| Seahorse XF HS PDL Miniplates | 12 8-well sensor cartridges, 12 XF HS PDL Miniplates | 103727-100 |

*PS = Polystyrene*

*PDL = Poly-D-Lysine*

# Agilent Seahorse XF Assay Kits and Reagents

## Standardized, optimized, live-cell metabolic assays for use with Agilent Seahorse Analyzers

Agilent provides a wide range of Seahorse XF assay kits, reagents, and media for studying cellular function, including metabolism and energetics and assessing mitochondrial and glycolytic function in real time. These kits are designed to simplify workflows and improve research productivity, enabling generation of consistent data on your Seahorse XF Analyzer.

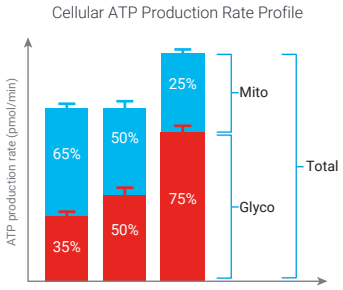
Together with Agilent's excellent technical support, these kits will greatly enhance your overall experience of performing XF assays.

### Key features and benefits include:

- An optimized open-and-go format for simplified protocol and reduced assay complexity
- Direct reconstitution of compounds in assay medium—eliminating the need for DMSO or other solvents
- A single-use concept to reduce waste and eliminate the need for storage of used compounds
- Rigorous testing and validation for every lot with cells to deliver consistent and robust results—eliminating the cost associated with failed experiments

Agilent

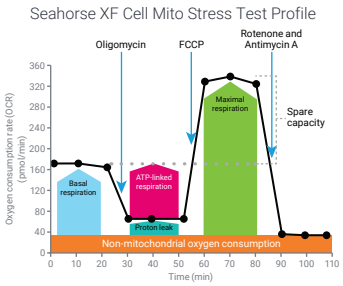
Trusted Answers




## Agilent Seahorse XF Real-Time ATP Rate Assay Kits

(p/n **103592-100** and **103591-100**)

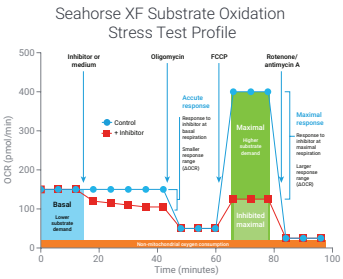
This is the only assay that quantifies the production rates of adenosine triphosphate (ATP) from mitochondrial respiration and glycolysis simultaneously, in real time with live cells. It offers a more informative and quantitative approach to assess the interplay between cellular functions and energy metabolism in response to gene modification, compound exposure, and/or other types of interventions.




## Agilent Seahorse XF Cell Mito Stress Test Kits

(p/n **103015-100** and **103010-100**)

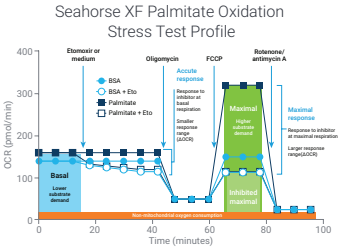
This widely-used assay kit is an optimized solution for assessing mitochondrial function. In this assay, key parameters obtained include ATP-linked respiration, maximal respiration, and spare respiratory capacity. The assay provides insights into the mechanism of mitochondrial dysfunction and enables investigation of functional differences among cell types, drug candidates, as well as genetic or biochemical interventions.




## Agilent Seahorse XF Substrate Oxidation Stress Test Kits

(p/n **103672-100**, **103673-100**, and **103674-100**)

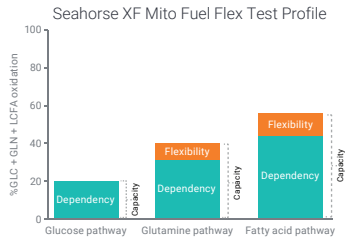
A suite of powerful assays enabling interrogation of cellular substrate oxidation under high energy demand conditions in live cells. These kits facilitate the investigation of how cells alter or shift oxidation of mitochondrial substrates (e.g., long-chain fatty acids, glucose/ pyruvate, and/or glutamine) to perform key cellular functions of activation, proliferation and differentiation, survival in microenvironments, as well as responses to genetic or pharmaceutical interventions.




## Agilent Seahorse XF Palmitate Oxidation Stress Test Kit

(p/n **103693-100**)

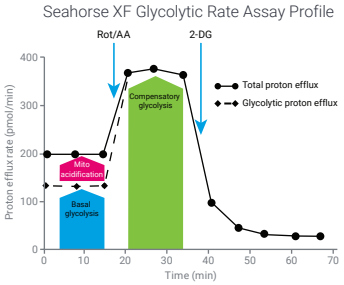
This conventional fatty acid oxidation (FAO) assay kit provides ready-to-use reagents, including the XF palmitate-BSA FAO substrate, etomoxir, oligomycin, FCCP, rotenone/ antimycin A, and L-carnitine. It enables determination of the intrinsic rate and capacity of a cell to oxidize long-chain fatty acids (LCFAs) in the absence or limitation of other exogenous substrates, facilitating investigation of how interventions (e.g., genetic manipulations, drug exposures) specifically affect the LCFAs oxidation process.




## Agilent Seahorse XF Mito Fuel Flex Test Kits

(p/n **103260-100** and **103270-100**)

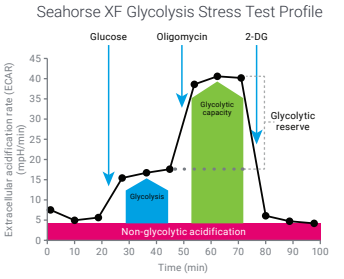
A method for measuring basal-state mitochondrial substrate oxidation in live cells. This assay utilizes a set of inhibitors to reveal the cells' ability to switch oxidative pathways in meeting basal energetic demands and provides information regarding the relative contributions of glucose, glutamine, and LCFA oxidation to basal respiration.




## Agilent Seahorse XF Glycolytic Rate Assay Kit

(p/n **103344-100** and **103346-100**)

This assay is an optimized complete solution (kit, media, software) that quantifies the rate of glycolytic activity in live cells by accounting for both glycolytic and mitochondrial ($CO_2$) acidification. This kit delivers robust, informative, and precise glycolysis data that enable the study of transient responses and metabolic switches in cellular disease models. This low-cost, real-time, functional assay is a superior alternative to the end-point lactate assay.




## Agilent Seahorse XF Glycolysis Stress Test Kit

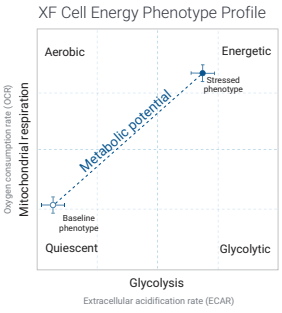
(p/n **103020-100** and **103017-100**)

A relative test of glycolytic function in live cells, this assay measures the capacity of the glycolytic pathway by driving cells toward glycolysis and assesses the ability of study models to increase glycolytic activity to meet metabolic and bioenergetic demands.

## Agilent Seahorse XF Plasma Membrane Permeabilizer

(p/n **102504-100**)

Perform isolated mitochondria type assays without isolating mitochondria! This exclusive reagent permeabilizes the plasma membrane of intact cells in culture without damage to mitochondrial membranes, enabling experimental control of substrate provision to the mitochondria and detailed characterization of key components in mitochondrial function, such as transporters, enzymes, and electron transport chain complexes.




## Agilent Seahorse XF Cell Energy Phenotype Test Kit

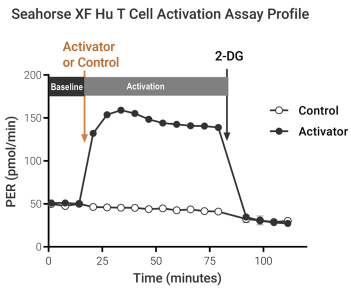
(p/n **103325-100** and **103275-100**)

This assay rapidly determines the energy phenotype of cells and reveals their metabolic potential, providing a qualitative view of cellular energy metabolism. It may be used for screening compounds that modulate mitochondrial function and/or glycolytic activity.

## Agilent Seahorse XF Palmitate-BSA FAO Substrate

(p/n **102720-100**)

A bio-available, preconjugated substrate with a quantified free palmitate concentration, used for *in vitro* studies of LCFA oxidation by intact cells. It is supplied as a part of the Seahorse XF Palmitate Oxidation Stress Test kit and is also available alone for other types of applications.




## Agilent Seahorse XF Hu T Cell Activation Assay Kit

**(p/n 103759-100 and p/n 103766-100)**

The assay measures human T cell activation response in minutes, via monitoring proton efflux rates (PER) associated with glycolysis-dependent energy production, thus providing an early window into the dynamics of immunometabolism that drive many key aspects of immune responses. This metabolism-based T cell activation assay kit, together with standardized protocols and cloud-based Agilent Seahorse Analytics software, offers an optimized turnkey solution and delivers unique insights into the mechanisms underlying T cell activation.

## Product ordering information

| P/n | Product Description | Unit | Product Content | Compatible Analyzer |
|---|---|---|---|---|
| 103592-100 | Seahorse XF Real-Time ATP Rate Assay Kit[3] | EA | Six (6) pouches[1] | XFe96, XF96, XFe24 |
| 103015-100 | Seahorse XF Cell Mito Stress Test Kit[3] | EA | Six (6) pouches[1] | XFe96, XF96, XFe24, XF24 |
| 103672-100 | Seahorse XF Long Chain Fatty Acid Oxidation Stress Test Kit | EA | Three (3) pouches[1] | XFe96, XF96, XFe24, XF24 |
| 103673-100 | Seahorse XF Glucose/Pyruvate Oxidation Stress Test Kit | EA | Three (3) pouches[1] | XFe96, XF96, XFe24, XF24 |
| 103674-100 | Seahorse XF Glutamine Oxidation Stress Test Kit | EA | Three (3) pouches[1] | XFe96, XF96, XFe24, XF24 |
| 103693-100 | Seahorse XF Palmitate Oxidation Stress Test Kit | EA | Contains one (1) each of these products: 103672-100, 102720-100, 103689-100 | XFe96, XF96, XFe24, XF24 |
| 103260-100 | Seahorse XF Mito Fuel Flex Test Kit | EA | Six (6) pouches[1] | XFe96, XF96, XFe24, XF24 |
| 103325-100 | Seahorse XF Cell Energy Phenotype Test Kit | EA | Twelve (12) pouches[1] | XFe96, XF96, XFe24, XF24 |
| 103344-100 | Seahorse XF Glycolytic Rate Assay Kit[3] | EA | Six (6) pouches[1] | XFe96, XF96, XFe24 |
| 103020-100 | Seahorse XF Glycolysis Stress Test Kit | EA | Six (6) pouches[1] | XFe96, XF96, XFe24, XF24 |
| 103591-100 | Seahorse XFp Real-Time ATP Rate Assay Kit[3] | EA | Six (6) pouches[2] | XFp, XF HS Mini |
| 103010-100 | Seahorse XFp Cell Mito Stress Test Kit[3] | EA | Six (6) pouches[2] | XFp, XF HS Mini |
| 103270-100 | Seahorse XFp Mito Fuel Flex Test Kit | EA | Six (6) pouches[2] | XFp, XF HS Mini |
| 103275-100 | Seahorse XFp Cell Energy Phenotype Test Kit | EA | Twelve (12) pouches[2] | XFp, XF HS Mini |
| 103346-100 | Seahorse XFp Glycolytic Rate Assay Kit[3] | EA | Six (6) pouches[2] | XFp, XF HS Mini |
| 103017-100 | Seahorse XFp Glycolysis Stress Test Kit | EA | Six (6) pouches[2] | XFp, XF HS Mini |
| 102720-100 | Seahorse XF Palmitate-BSA FAO Substrate | EA | 3 × 2 mL palmitate-BSA<br>3 × 2 mL BSA controls | All XF Analyzers |
| 102504-100 | Seahorse XF Plasma Membrane Permeabilizer | EA | One (1) vial of 25 µL PMP in 10 µM concentration | All XF Analyzers |
| 103759-100 | Seahorse XF Hu T Cell Activation Assay Kit, 200 tests | EA | 1 × 2 mL ImmunoCult human CD3/CD28 T cell activator,<br>2 × 2-deoxy-D-glucose | XFe/XF96, XFp, XF HS Mini Analyzers |
| 103766-100 | Seahorse XF Hu T Cell Activation Assay 96-well Pack, 200 tests | Pk | 1 × p/n 103759-100<br>2 × XFe96 PDL FluxPak | XFe/XF96 Analyzers |
| 102353-100 | Seahorse XF Base Medium (2 × 1 L) | 2/pk | | All XF Analyzers |
| 103334-100 | Seahorse XF Base Medium, 500 mL | EA | | All XF Analyzers |
| 103335-100 | Seahorse XF Base Medium, without phenol red, 500 mL | EA | | All XF Analyzers |
| 103575-100 | Seahorse XF DMEM Medium, pH 7.4, 500 mL | EA | | All except for XF24 |
| 103680-100 | Seahorse XF DMEM Assay Medium Pack | PK | Contains one (1) each of 103575-100, 103577-100, 103578-100, 103579-100 | All except for XF24 |
| 103576-100 | Seahorse XF RPMI Medium, pH 7.4, 500 mL | EA | | All except for XF24 |
| 103681-100 | Seahorse XF RPMI Assay Medium Pack | PK | Contains one (1) each of 103576-100, 103577-100, 103578-100, 103579-100 | All except for XF24 |
| 103577-100 | Seahorse XF 1.0 M Glucose Solution, 50 mL | EA | | All XF Analyzers |
| 103578-100 | Seahorse XF 100 mM Pyruvate Solution, 50 mL | EA | | All XF Analyzers |
| 103579-100 | Seahorse XF 200 mM Glutamine Solution, 50 mL | EA | | All XF Analyzers |

[1] One XF pouch contains sufficient reagents to run one 24-well or 96-well plate

[2] One XFp pouch contains sufficient reagents to run one 8-well mini-plate

[3] These kits can also be purchased together with the Seahorse XF DMEM Medium, pH 7.4 and XF supplements/substrates as starter packs:
Catalog numbers for Seahorse XF or XFp Real-Time ATP Rate Assay Starter Packs are 103677-100 and 103676-100, respectively.
Catalog numbers for Seahorse XF or XFp Cell Mito Stress Test Starter Packs are 103708-100 and 103707-100, respectively.
Catalog numbers for Seahorse XF or XFp Glycolytic Rate Assay Starter Packs are 103710-100 and 103709-100, respectively.

Agilent

Trusted Answers

# Unlock the Full Potential of Your Plate Reader


Discover how the Agilent MitoXpress Xtra oxygen consumption assay, Agilent pH Xtra glycolysis assay, and Agilent MitoXpress Intra intracellular oxygen assay can help you to:

- Measure mitochondrial activity and glycolysis in live cells.
- Move beyond indirect end-point cell-based assays to direct informative mix-and-measure assessments of mitochondrial function, glycolytic activity, and cellular oxygenation.
- Multiplex with other relevant assays.
- Expand beyond the monolayer to measure suspension cells, microbial, and specific 3D cultures.
- Elevate your throughput with simple mix-and-measure metabolism assays in standard microplates.
- Conveniently measure isolated mitochondria in high throughput.
- Probe the connection between oxygen availability and altered cell metabolism.

# MitoXpress Xtra Oxygen Consumption Assay

## Measuring cellular oxygen consumption


Oxygen consumption measurements are a key functional readout of cell metabolism and more specifically mitochondrial function. Using these types of measures to examine the metabolism of live cells provides important mechanistic insights into cellular function and the role of perturbed metabolism in disease progression.

The MitoXpress Xtra oxygen consumption assay is a valuable tool to investigate metabolism. It offers a simple kinetic measurement of aerobic metabolism that can be performed on standard microplates using fluorescence plate readers. As respiration occurs, the concentration of oxygen in the sample decreases. This causes an increase in MitoXpress Xtra signal providing a measure of oxygen consumption.

Figure 1 shows an investigation of the oxygen consumption of human iPSC-derived cardiomyocytes (Cor.4U, NCardia) using the MitoXpress Xtra assay. In cells treated with the uncoupler FCCP, the rate of signal change increased due to an increase in respiration. In contrast, treatment with an inhibitor of mitochondrial respiration, Antimycin A, had the opposite effect. The rate of signal change decreased due to reduced oxygen consumption. These measurements can be carried out in a wide range of media formulations, facilitating flexible assay design.

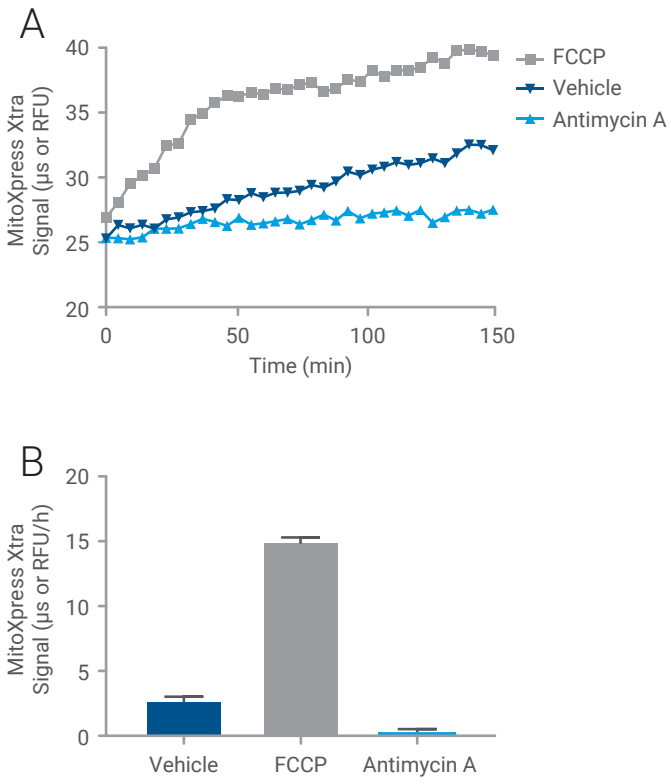



**Figure 1.** (A) Interrogation of Cor.4U (NCardia) oxygen consumption measured using the Agilent MitoXpress Xtra assay. As cells respire, they deplete dissolved oxygen causing an increase in MitoXpress Xtra signal. Treatment with Antimycin A (inhibitor of mitochondrial respiration) inhibits oxygen consumption, therefore, reducing the rate of sensor signal change. Treatment with FCCP (uncoupler), increases oxygen consumption, which increases the rate of sensor signal change. (B) These metabolic effects can be assessed by analyzing of the rate of change of MitoXpress Xtra signal, where lower rates of change indicate reduced aerobic metabolic activity.

# MitoXpress Xtra Solutions for Toxicology

## Screening for drug-induced toxicity

Drug-induced mitochondrial dysfunction has been implicated with various drug classes and has been shown to significantly contribute to toxicity in the liver, heart, kidney, muscle, and central nervous system.

Because of the microplate format and assay performance, the MitoXpress Xtra assay offers a convenient solution for early screening of drug-induced mitochondrial liabilities and the generation of dose-response curves (Figure 2). These studies can be performed with a range of relevant *in vitro* models, including primary hepatocytes and hiPSC derived cardiomyocytes and hepatocytes.

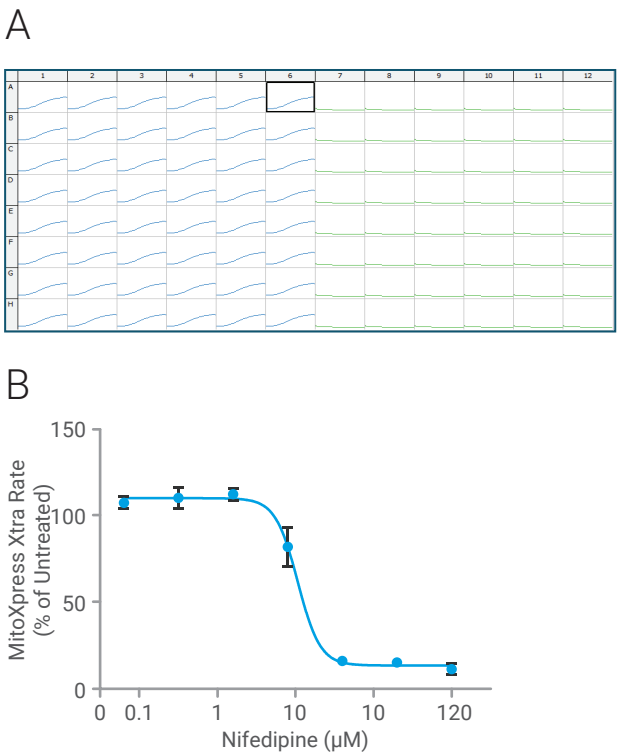



**Figure 2.** (A) Oxygen consumption measurement in hiPS-HEP cells (Clontech) using the Agilent MitoXpress Xtra assay showing a Z' factor of ~0.7. (B) Dose-response curve for Nifedipine (Calcium channel blocker) on mitochondrial respiration in Cor.4U cardiomyocytes. Values were obtained from slopes normalized to vehicle control.

## Measuring respiration in isolated mitochondria

Measuring the respiration of isolated mitochondria has never been more accessible, due to MitoXpress Xtra. This assay enables the direct, convenient, high-throughput, microplate-based assessments of electron transport chain (ETC) activity on conventional fluorescence plate readers (Figure 3A). This facilitates convenient compound screening and dose-response analysis (Figure 3B). In addition, using specific substrate and inhibitor combinations enables more detailed mechanistic assessments for individual ETC complexes and associated mitochondrial proteins. In comparison to traditional polarographic approaches, the comparatively low sample volume required by the MitoXpress Xtra assay allows for more replicates per isolation. These low sample volume requirements significantly increase the amount of data that can be generated from a single mitochondrial preparation, particularly when 384 well plates are used.

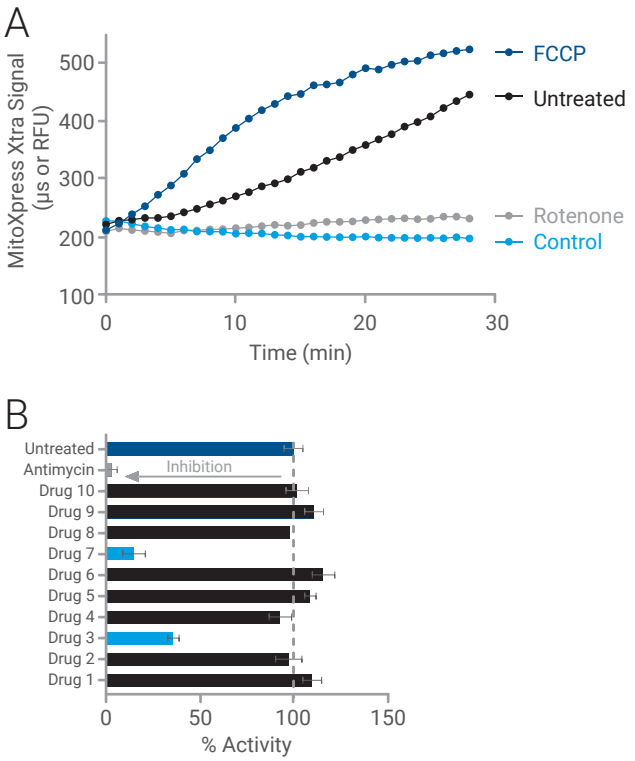



**Figure 3.** Analysis of respiration of isolated mitochondria (rat liver) using the Agilent MitoXpress Xtra assay (A) showing inhibition and uncoupling following treatment with classical mitochondrial modulators. (B) Screening with a panel of unknown drugs using rat liver mitochondria to identify drug-induced mitochondrial dysfunction (drug 3 and 7).

# MitoXpress Xtra Solutions for Multiplexing

## Multiparametric and multiplexed assessment of mitochondrial function

Further insight into cellular response can be achieved by combining MitoXpress Xtra or pH Xtra assays with measurement of other relevant plate reader compatible parameters, such as mitochondrial membrane potential (MMP), reactive oxygen species (ROS), or cellular ATP content. This enables researchers to better characterize the impact of treatments or changes in conditions on cellular function. It also helps to contextualize observed metabolic perturbations without having to carry out parallel treatments or use disparate technology platforms.

One example is to use a multiplexed measurement of cell viability (Calcein AM) and mitochondrial respiration (MitoXpress Xtra) to better understand the off-target effect of drug treatment on cell function (Figure 4).

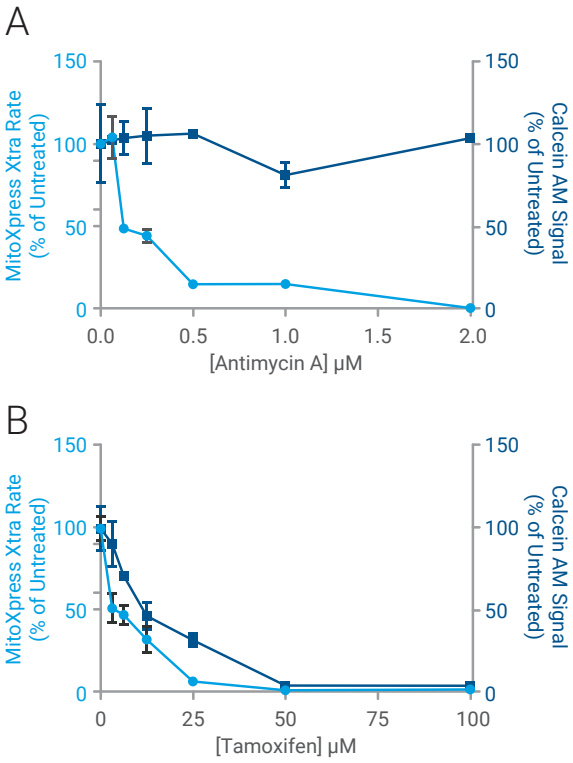



**Figure 4.** Multiplexed measurement of Agilent MitoXpress Xtra signal and Calcein AM in HepG2 cells treated with (A) Antimycin A and (B) Tamoxifen for 24 h. Both drugs reduce mitochondrial respiration (light blue). Tamoxifen showed a significant impact on viability, while Antimycin A treatment shows no significant reduction in cell viability (dark blue). This suggests that the impact of Antimycin A on respiration results from a more direct mitochondrial mechanism.

Indicators of mitochondrial function such as MMP and the generation of ROS can also be assessed in parallel with oxygen consumption. These parameters are of particular interest when investigating the role of the mitochondria in cell physiology.

Figure 5 shows the multiplexed measurement of MitoXpress Xtra and MMP (using JC-1) in HepG2 cells treated with the metabolic modulators; FCCP and Antimycin A. Both compounds decrease membrane potential, however, distinct effects on oxygen consumption can be seen, demonstrating how measuring multiple relevant parameters can add additional insight.

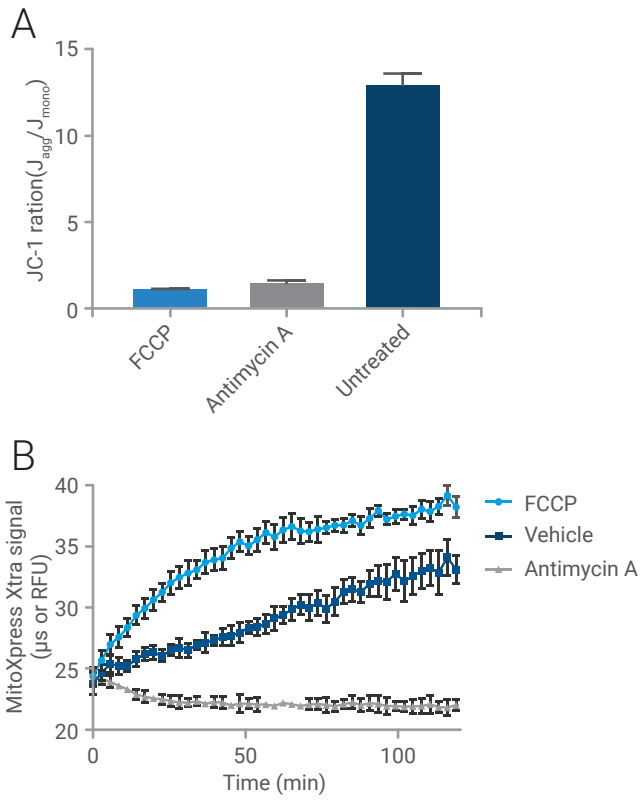



**Figure 5.** Measurement of Agilent MitoXpress Xtra signal and JC-1 in the same well. HepG2 cells were treated with Antimycin A or FCCP. Both compounds caused a decrease in MMP (A) FCCP caused a characteristic increase in oxygen consumption, whereas Antimycin A inhibited respiration (B).

# MitoXpress Xtra Solutions for Measuring Microbial Metabolism

## Measuring microbial proliferation, metabolism, and response to antibiotics

The MitoXpress Xtra assay offers a simple, sensitive plate-reader based method to measure prokaryotic cell growth or screen for antimicrobial compounds without the need to conduct multiple sample dilutions or lengthy agar-based investigations. It also offers a valuable means to study microbial metabolism.

The plate reader format provides the throughput and resolution necessary for screening and convenient generation of $IC_{50}$ or MIC data. Figure 6 shows how this method was applied to assess microbial proliferation, generate dose-response data, and assess the specific metabolic effects of compound treatment for two compounds in yeast.

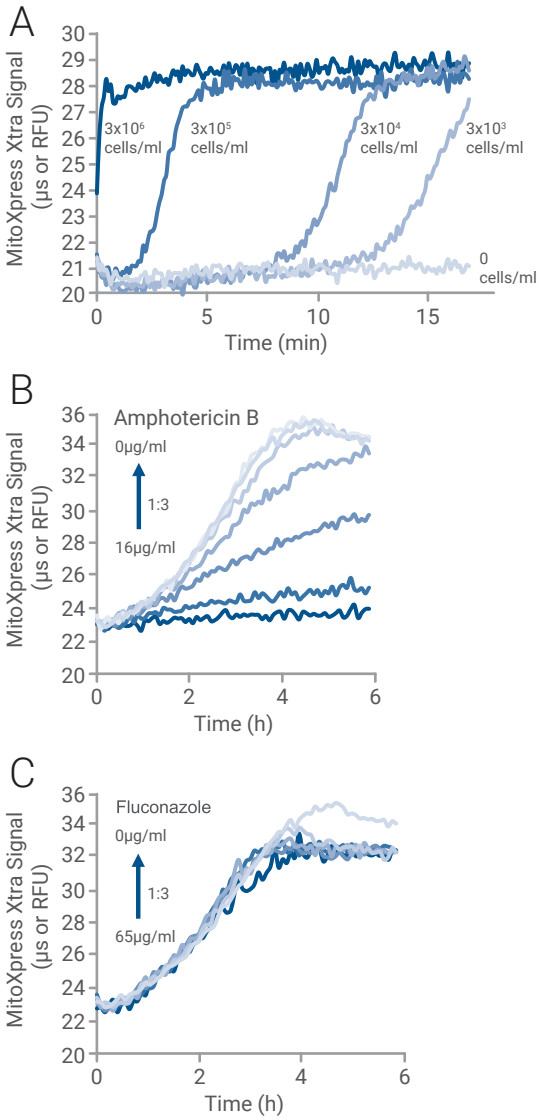



**Figure 6.** *C. albicans* oxygen depletion profiles measured at decreasing seeding concentrations. (A) *C. albicans* were treated with increasing concentrations of Amphotericin B (B) and Fluconazole. (C) Amphotericin B caused dose-dependent decreases in oxygen consumption while Fluconazole caused no decrease in oxygen consumption. These observations correlate with the mode of drug action.

# pH Xtra Glycolysis Assay

## Measuring glycolytic activity


Extracellular acidification measurements are a highly informative means of investigating glycolytic activity and are conveniently performed on time-resolved fluorescence (TRF) enabled plate readers using the pH Xtra glycolysis assay. Extracellular acidification is caused in large part by the conversion of pyruvate to lactate, which results in a reduction in assay buffer pH. The pH Xtra sensor sensitively detects this reduction in pH as an increase in sensor signal.

These pH measurements provide important insights into the central role played by altered glycolytic activity in a wide array of physiological and pathophysiological processes, including cancer and cellular adaptation to hypoxia.

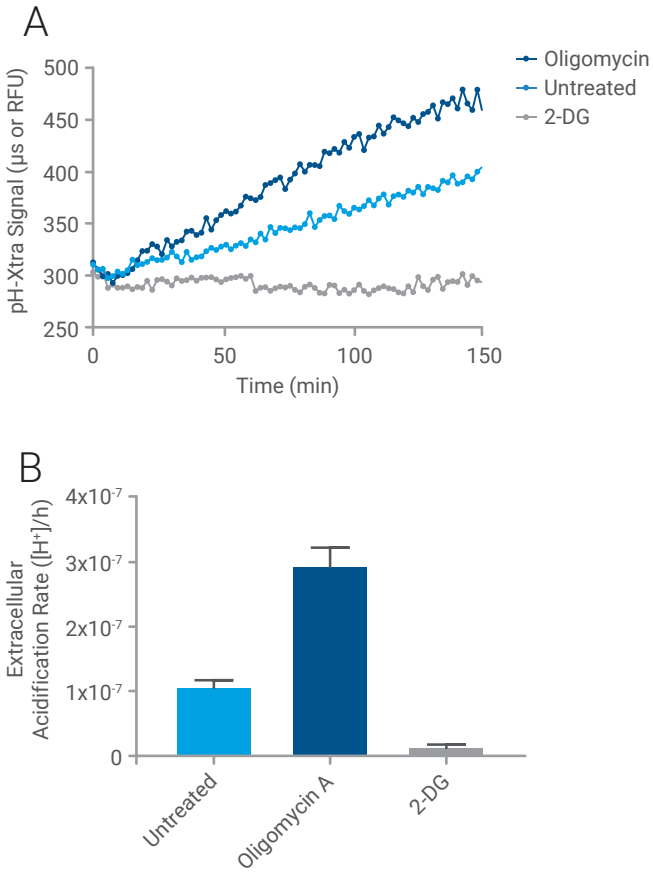



**Figure 7.** (A) Interrogation of A549 glycolytic activity measured using the Agilent pH Xtra glycolysis assay. Treatment with the hexokinase inhibitor 2-Deoxyglucose (2-DG) inhibits extracellular acidification, observed as a decrease in sensor signal change. Treatment with Oligomycin A, an inhibitor of mitochondrial ATP generation, leads to increased glycolytic ATP production to maintain cellular energy homeostasis. (B) Changes in extracellular acidification can be conveniently assessed in either pH or [$H^+$] ion scales over time.

# MitoXpress Xtra and pH Xtra Solutions

## Measuring cell metabolism in 3D cultures

Plate reader based measurement of cellular metabolism can also be performed in suspension cultures and specific 3D culture systems (for example RAFT, Mimetix, and Alvetex). Culturing cells in 3D facilitates the development of complex intracellular interactions, helping to narrow the gap between *in vitro* and *in vivo* biological systems. Figure 8 shows how the MitoXpress Xtra and pH Xtra assays were used to measure cell metabolism in 3D matrices (RAFT, Lonza) without disrupting the integrity of the 3D structure.

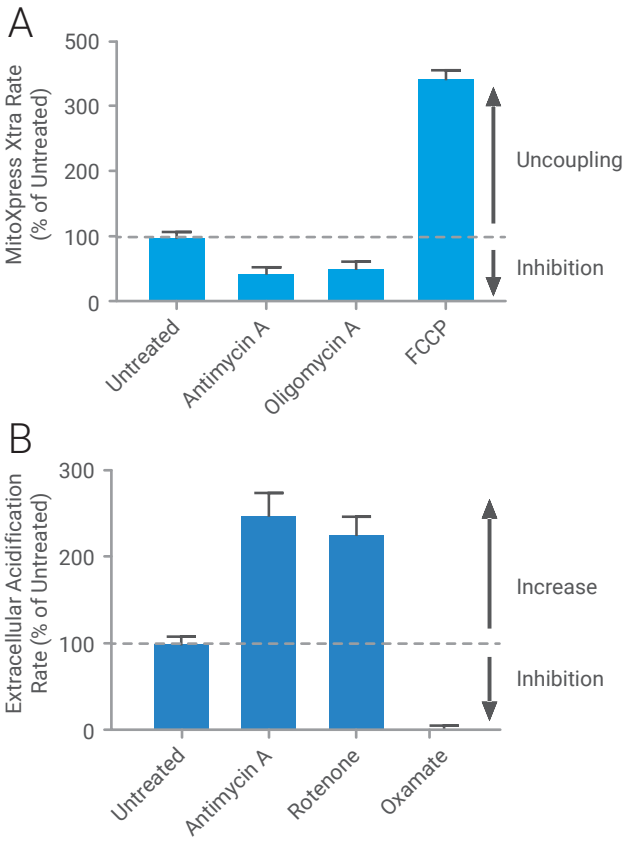



**Figure 8.** (A) Relative effect of drug treatment on oxygen consumption in A549 3D RAFT cultures measured using the Agilent MitoXpress Xtra assay. (B) The relative effect of drug treatment on extracellular acidification rates of HepG2 RAFT cultures measured using the Agilent pH Xtra glycolysis assay.

## Combined measurement of glycolysis and oxygen consumption

The combined use of MitoXpress Xtra and pH Xtra allows for the assessment of cellular metabolic poise as a baseline for subsequent metabolic investigation. Figure 9 illustrates how these combined assays are used to study the balance between oxygen consumption and glycolysis across a range of cell types. The figure also shows how varying substrate availability can modulate this metabolic balance.

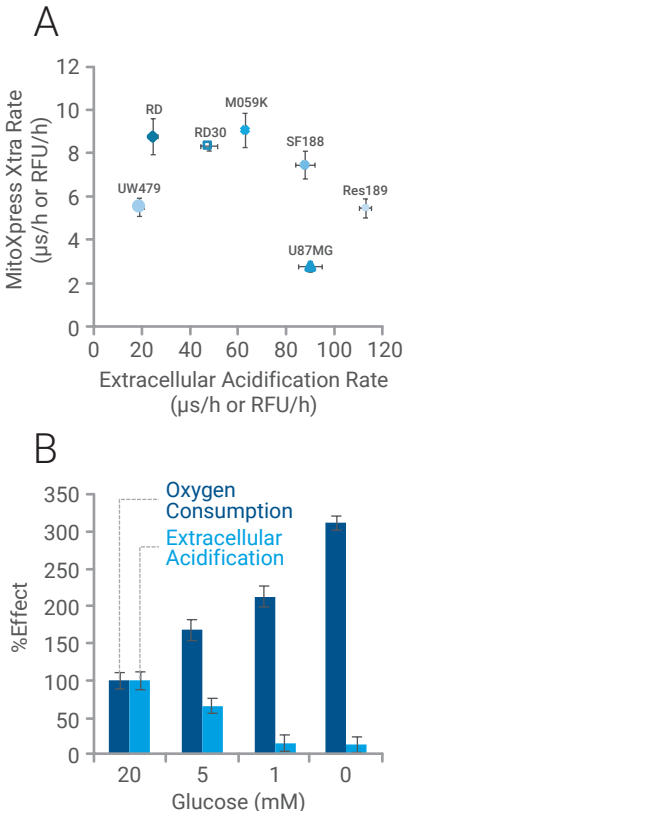



**Figure 9.** (A) Combined analysis of glycolysis and mitochondrial respiration in cancer cell lines using AgilentMitoXpress Xtra and Agilent pH Xtra assays. (B) Effect of glucose availability on the metabolism of U87MG cells. Increasing glucose availability led to a decrease in respiration and an increase in glycolysis (Data courtesy of Dr. Karl Morten, University of Oxford, UK).

# MitoXpress Intra Intracellular Oxygen Assay

## Intracellular oxygenation and hypoxia


Oxygen availability at the cellular level has a significant influence on cell physiology, signal transduction, and metabolism. As a result, lower more physiologically relevant oxygen concentrations are being used in *in vitro* studies, especially in cancer metabolism, drug discovery, neuronal, and cardiovascular research. A key element of such *in vitro* models, however, is the ability to monitor cellular oxygenation, as fluctuations in this dynamic parameter can confound data interpretation. Understanding the depth and duration of the oxygenation condition experienced by the cell model is therefore of significant importance.

The MitoXpress Intra assay delivers a solution by measuring intracellular $O_2$ concentrations in live cells. The reagent is taken up by the cell and can be monitored using fluorescence plate readers with the capacity to perform dual-read time-resolved fluorescence (TRF) measurements. The sensor then responds in real time to any changes in intracellular oxygen concentration caused by changes in atmospheric conditions or cellular respiration.

In addition to being an informative monitoring tool, the assay also enables researchers to relate metabolic responses to available oxygen levels. These parameters are extremely important in areas such as ischemia, cancer metabolism, and hypoxia. Figure 10 shows how the MitoXpress Intra assay can be used to reveal the significant differences between experienced and applied cellular oxygen levels. These differences depend on cell number, proliferation, type, and metabolic poise, therefore, the depth of hypoxia cells experience cannot be inferred from environmental oxygen concentration.

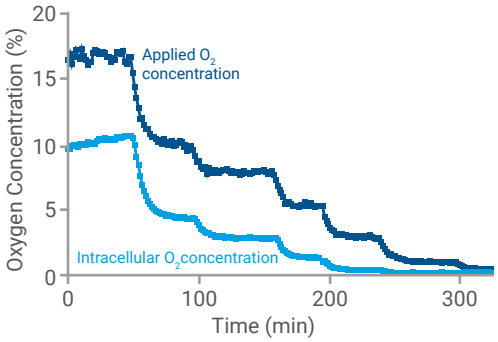



**Figure 10.** Monitoring $O_2$ concentrations in samples containing HepG2 cells grown in 3D culture (RAFT, Lonza) in response to decreasing atmospheric $O_2$ conditions. At atmospheric $O_2$, cells experience average oxygenation levels of ~10 %, while at 5% environmental $O_2$, oxygenation levels approach 0% $O_2$.

This reduction in oxygenation can have significant physiological consequences, and if not measured, can lead to data misinterpretation due to the lack of visibility of the oxygenation levels experienced by the cell model under study. This is exemplified in Figure 11 where 40% HIF stabilization is observed at a cellular oxygenation level of ~1.5% $O_2$. However, without measuring oxygenation it would have been assumed that this level of stabilization related to an oxygen level of ~5% $O_2$.

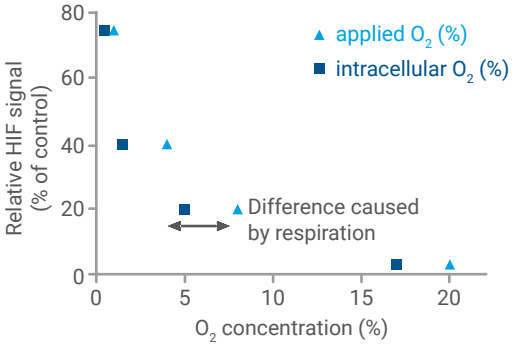



**Figure 11.** HIF-stabilization measured using a luminescent HIF-reporter in HT-1080 cells monitored at decreasing oxygen concentrations. HIF-stabilization is related to both the applied oxygen concentration (light blue) and the actual oxygenation levels experienced by the cells, as measured using the Agilent MitoXpress Intra assay (dark blue). These data illustrate that, unless cellular oxygenation is measured, erroneous conclusions will be made as to the relationship between oxygen availability and HIF stabilization. (Data courtesy of Dr. Karl Morten, University of Oxford, UK).

# MitoXpress Intra Solutions

## Studying cancer metabolism in hypoxic conditions

Monitoring cellular oxygenation in real time using MitoXpress Intra allows researchers to accurately determine the depth of hypoxia experienced by a cell model. Like the MitoXpress Xtra assay, MitoXpress Intra can be combined with other plate reader based assays, including measures of viability, MMP, and ROS. This is particularly relevant for cancer metabolism research, where the connection between hypoxic tumor environment, metabolic flexibility, and the cancer phenotype is of significant importance.

An example of this is shown in Figure 12 where multiplexed measurements of cellular oxygenation and glycolytic activity are achieved using MitoXpress Intra and the pH Xtra Glycolysis Assay. Measurements are performed under both hypoxic and atmospheric oxygen conditions with data underlining the impact of oxygenation on glycolytic activity.

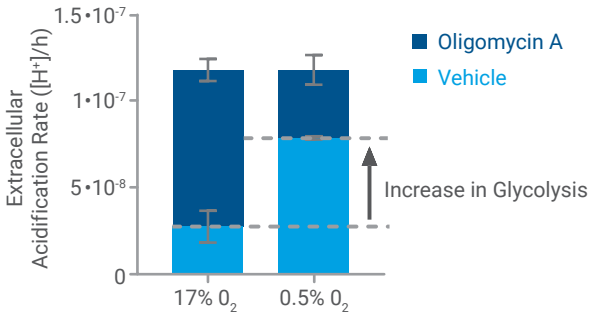



**Figure 12.** Extracellular acidification of A549 cells measured using the Agilent pH Xtra glycolysis assay multiplexed with the Agilent MitoXpress Intra assay to measure intracellular oxygen levels under hypoxic conditions imposed by the atmospheric control unit of the plate reader. The basal extracellular acidification rate of vehicle-treated cells was increased approximately threefold in hypoxia (light blue), whereas glycolytic capacity, as measured by treatment with Oligomycin (dark blue, an inhibitor of mitochondrial ATP generation) remained constant.

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