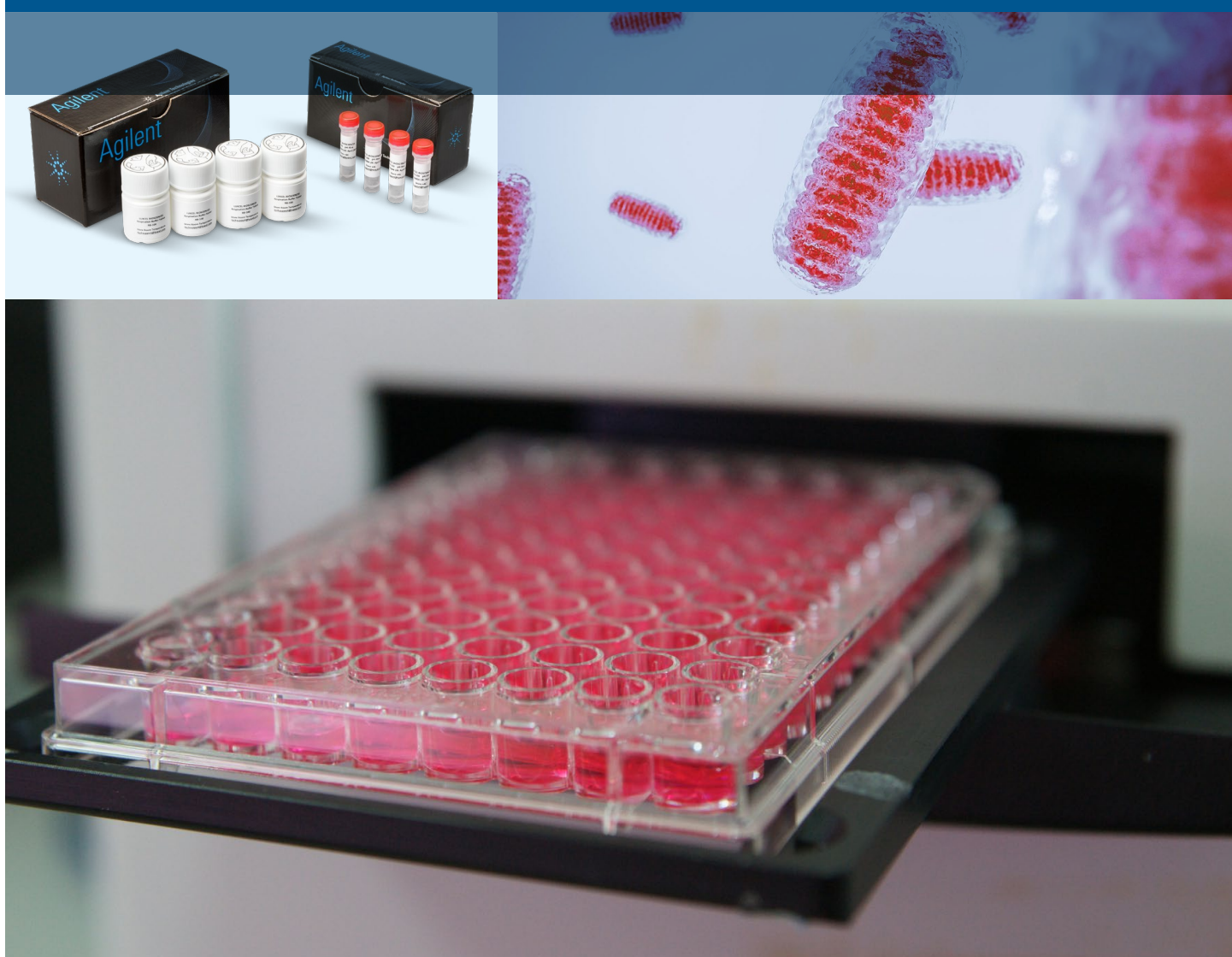


# Agilent Solutions for Measuring Cell Metabolism on your Plate Reader



# Unlock the full potential of your plate reader



Discover how Agilent's MitoXpress Xtra oxygen consumption assay, pH Xtra glycolysis assay, and 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

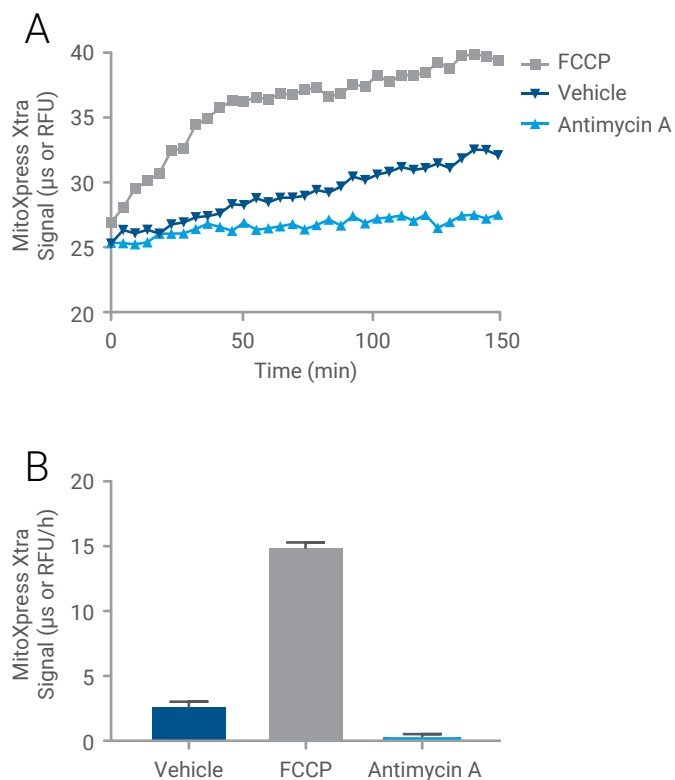
## Measuring cellular oxygen consumption



Oxygen consumption measurements are a key functional readout of cell metabolism and more specifically mitochondrial function. Using these types of measure 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.



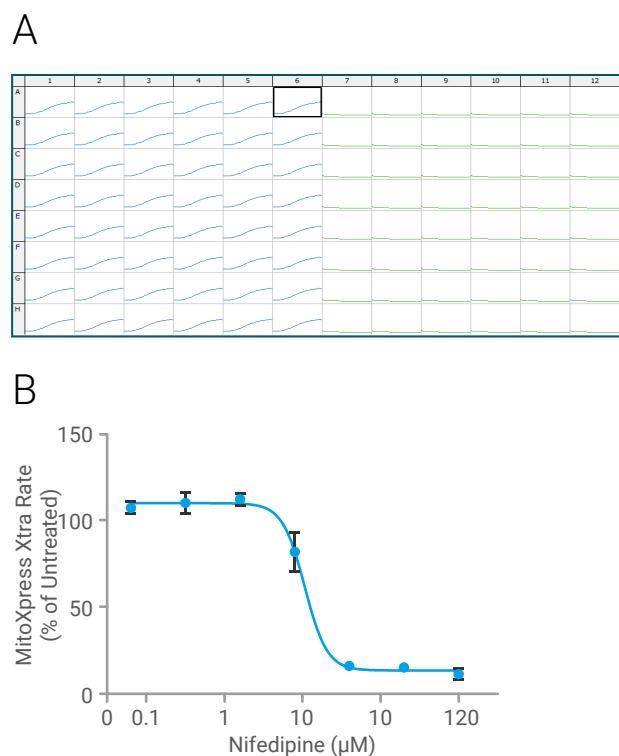
**Figure 1.** (A) Interrogation of Cor.4U (NCardia) oxygen consumption measured using MitoXpress Xtra. As cells respire, they deplete dissolved oxygen causing an increase in MitoXpress Xtra signal. Treatment with Antimycin A (inhibitor of mitochondrial respiration) inhibits oxygen consumption, therefore, 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.

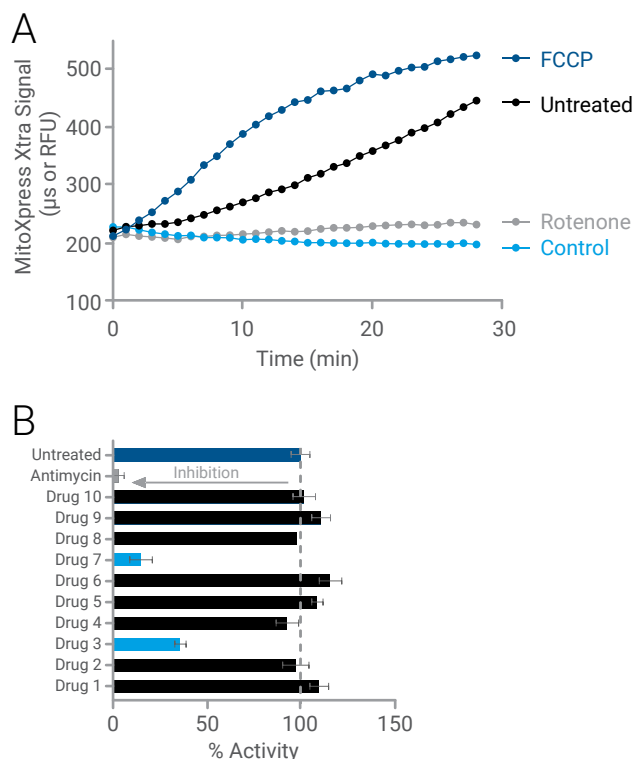
Because of the microplate format and assay performance, the MitoXpress Xtra assay offers a convenient solution for early screening of drug-induced mitochondrial liabilities and the generation of dose-response curves (Figure 2). These studies can be performed with a range of relevant *in vitro* models,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



**Figure 2.** (A) Oxygen consumption measurement in hiPS-HEP cells (Clontech) using MitoXpress Xtra 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.



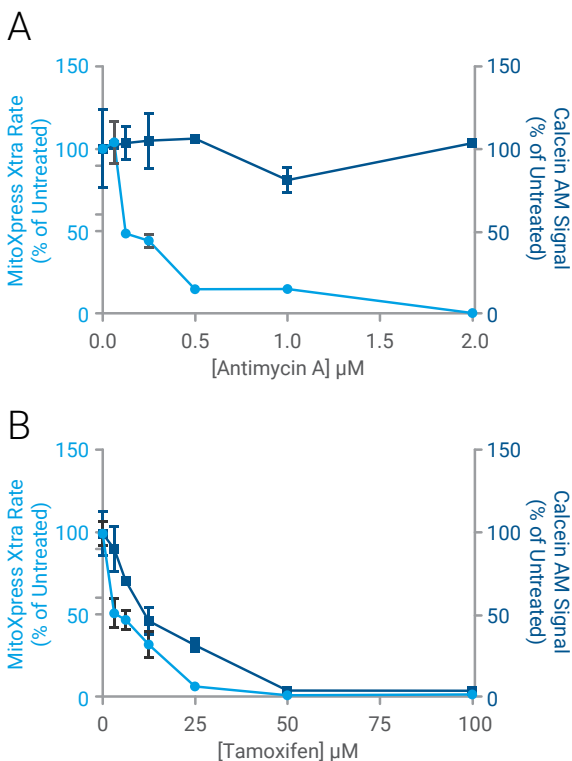
**Figure 3.** Respiration of isolated mitochondria (rat liver) (A) showing inhibition and uncoupling following treatment with classical mitochondrial modulators. (B) Screening with a panel of unknown drugs using rat liver mitochondria to identify drug-induced mitochondrial dysfunction (drug 3 and 7).

# 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.

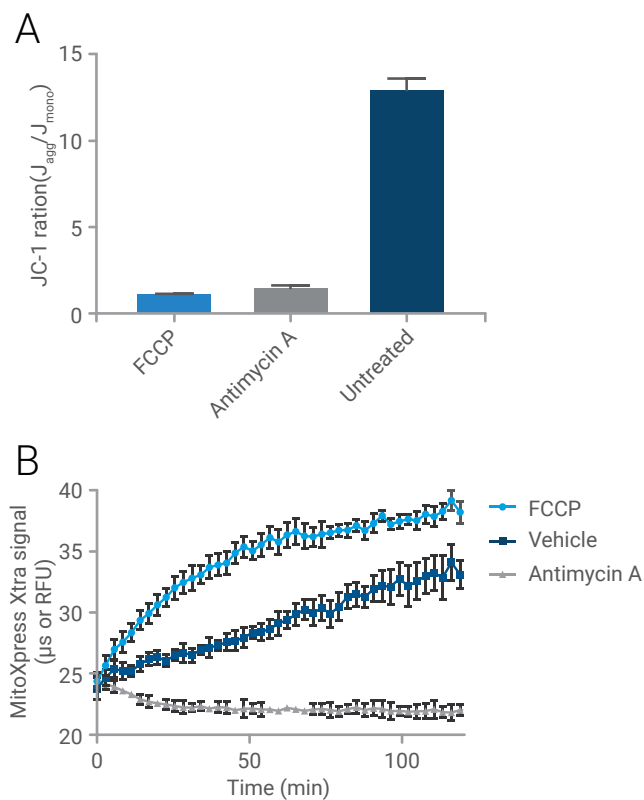
One example is to use a multiplexed measurement of cell viability (Calcein AM) and mitochondrial respiration (MitoXpress Xtra) to better understand the off-target effect of drug treatment on cell function (Figure 4).



**Figure 4.** Multiplexed measurement of MitoXpress Xtra 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.



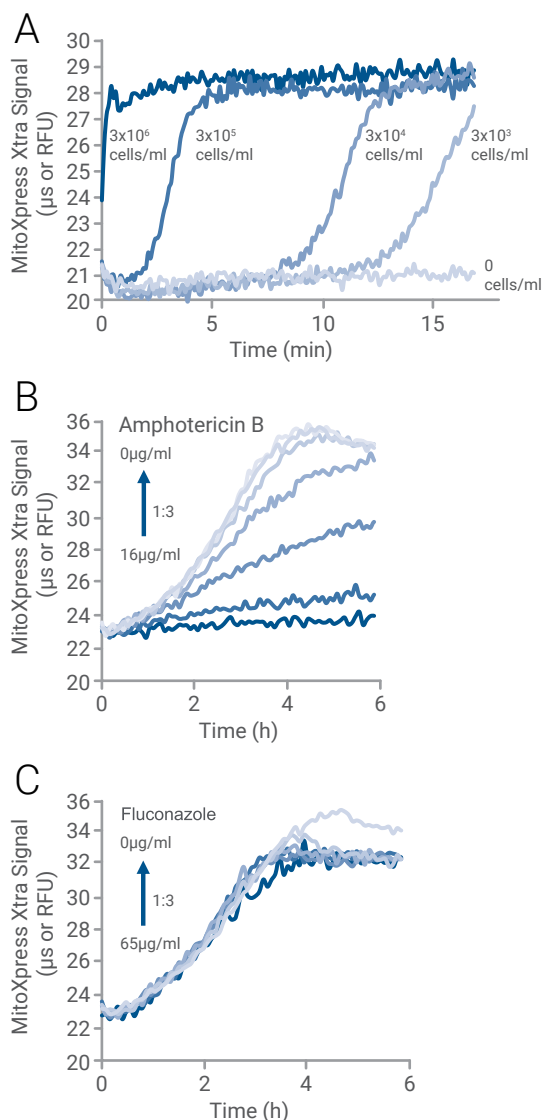
**Figure 5.** Measurement of MitoXpress Xtra and JC-1 in the same well. HepG2 cells were treated with Antimycin A or FCCP. Both compounds caused a decrease in MMP (A) FCCP caused a characteristic increase in oxygen consumption, whereas Antimycin A inhibited respiration (B).

# 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.



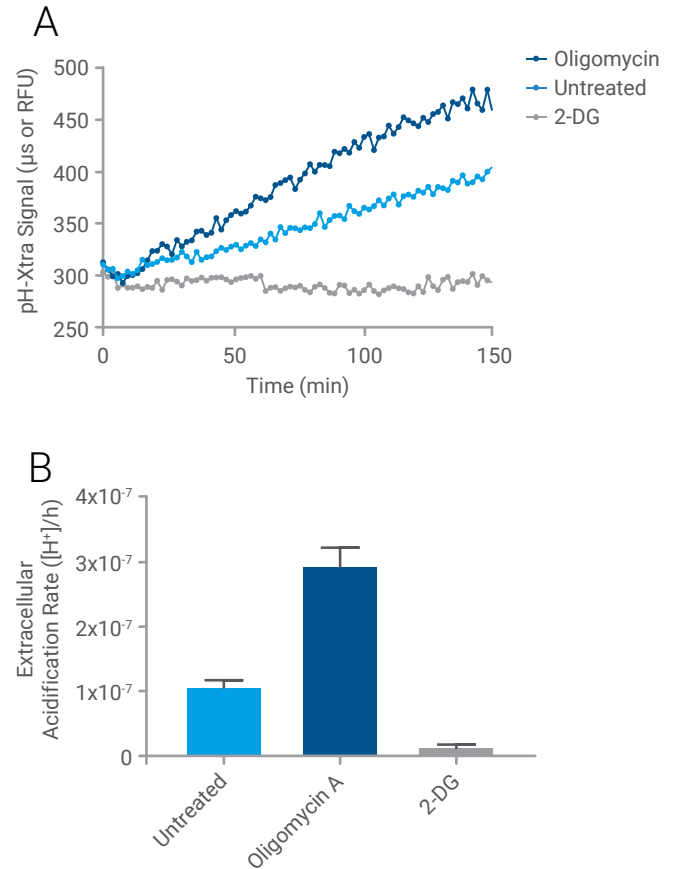
# pH Xtra glycolysis assay

## 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.

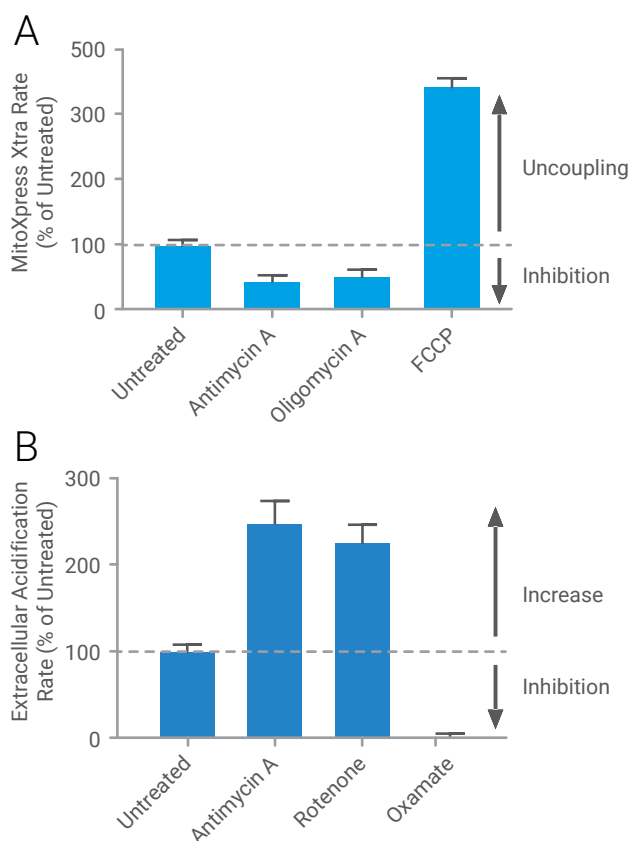


**Figure 7.** (A) Interrogation of A549 glycolytic activity measured using the pH Xtra glycolysis assay. Treatment with the hexokinase inhibitor 2-Deoxyglucose (2-DG) inhibits extracellular acidification, observed as a decrease in sensor signal change. Treatment with Oligomycin A, an inhibitor of mitochondrial 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  $[\text{H}^+]$  ion scales over time.

# MitoXpress Xtra and pH Xtra Solutions

## Measuring cell metabolism in 3D cultures

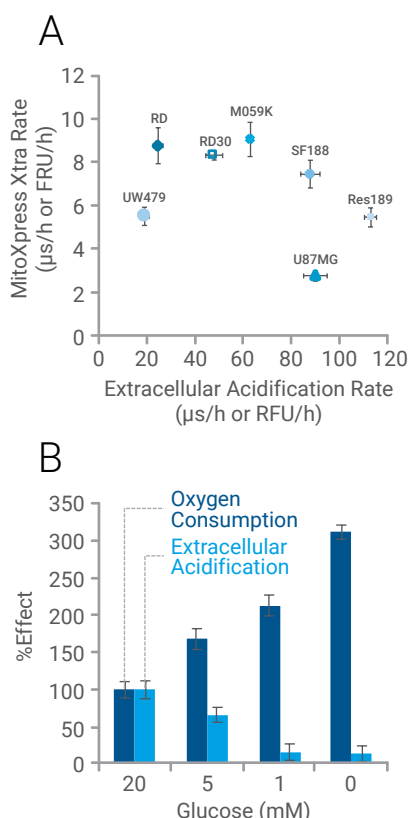
Plate reader based measurement of cellular metabolism can also be performed in suspension cultures and specific 3D culture systems (for example RAFT™, Mimetix®, and Alvetex®). Culturing cells in 3D facilitates the development of complex intracellular interactions, helping to narrow the gap between *in 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 MitoXpress Xtra assay. (B) The relative effect of drug treatment on extracellular acidification rates of HepG2 RAFT cultures measured using the 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 MitoXpress Xtra and pH Xtra. (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

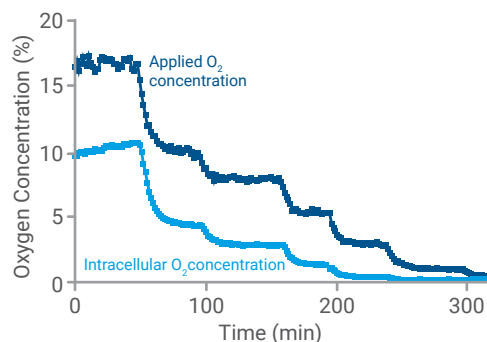
## Intracellular oxygenation and hypoxia



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.

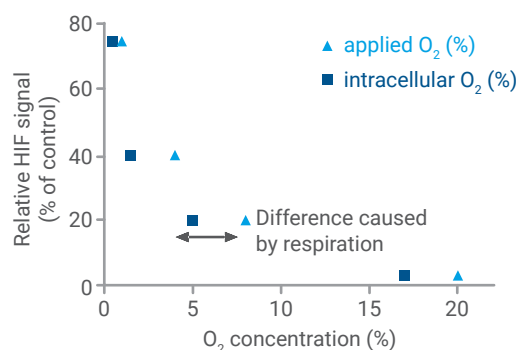
The MitoXpress Intra assay delivers a solution by measuring intracellular  $O_2$  concentrations in live cells. The reagent is taken up by the cell and can be monitored using fluorescence plate readers with the capacity to perform dual-read time-resolved fluorescence (TRF) measurements. The sensor then 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%  $O_2$ .



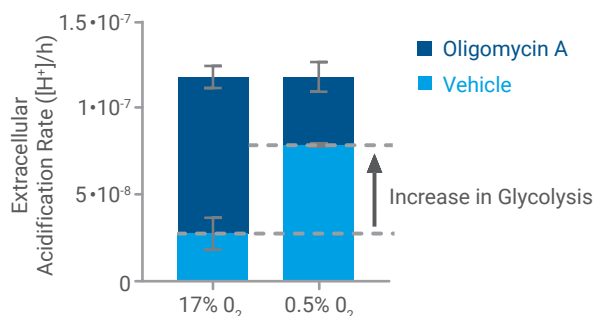
**Figure 11.** HIF-stabilization measured using a luminescent HIF-reporter in HT-1080 cells monitored at decreasing oxygen concentrations. HIF-stabilization is related to both the applied oxygen concentration (light blue) and the actual oxygenation levels experienced by the cells, as measured using MitoXpress Intra (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.

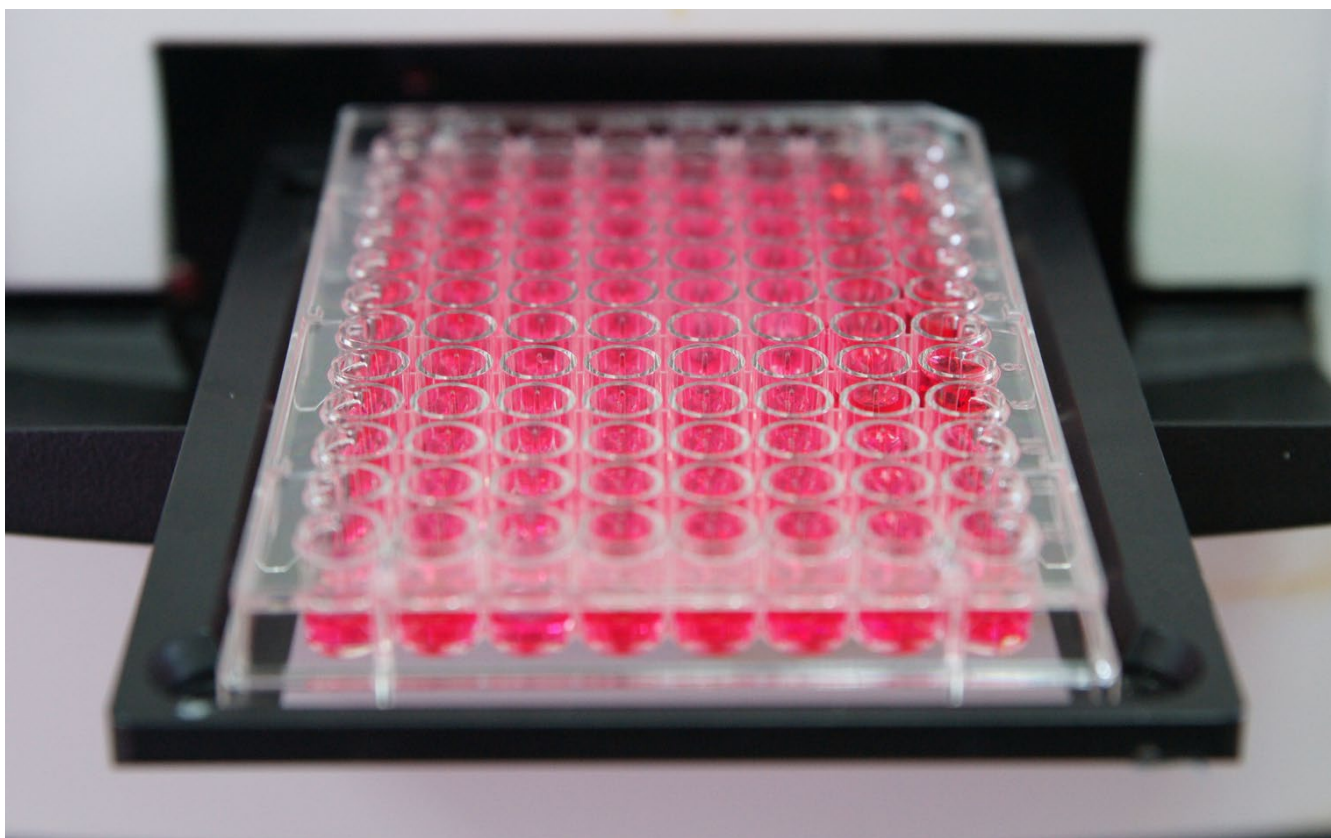
An example of this is shown in Figure 12 where multiplexed measurements of cellular oxygenation and glycolytic activity are achieved using MitoXpress Intra and the pH Xtra Glycolysis Assay. Measurements are performed under both hypoxic and atmospheric oxygen conditions with data underlining the impact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



**Figure 12.** Extracellular acidification of A549 cells measured using the pH Xtra Glycolysis Assay multiplexed with MitoXpress Intra 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.

# The Agilent Advantage

Metabolism underpins all cellular responses and is therefore, a core functional measurement in cell biology. With the range of fluorescence plate reader compatible metabolic sensors, Agilent now provides a comprehensive suite of solutions for *in vitro* interrogation of cell metabolism. The applications shown here are examples of how these assays can be used to analyze cellular metabolism and function. For additional resources, more detailed protocols, and to find out how our soluble metabolic sensors can help you to answer your research questions visit [www.agilent.com](http://www.agilent.com) or contact us directly at [cellanalysis.support@agilent.com](mailto:cellanalysis.support@agilent.com).



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