# Microplate reader solutions for Agilent's Cell Metabolism Assays

BMG LABTECH

*The Microplate Reader Company*

# Plate reader solutions for measuring cellular metabolism

## Measuring cell metabolism

Discover how Agilent's range of soluble metabolic sensors, the MitoXpress Xtra oxygen consumption assay, and the pH-Xtra glycolysis 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.
- Expand beyond the monolayer to measure in suspension, and specific 3D cell cultures.
- Elevate your throughput with simple mix-and-measure metabolism assays in standard microplates.

## 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. As respiration occurs, the concentration of oxygen in the sample decreases, this causes an increase MitoXpress Xtra signal providing a measure of the rate of oxygen consumption.

Figure 1 shows the investigation of the oxygen consumption of human iPSC-derived cardiomyocytes (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.

## Measuring glycolytic activity

Extracellular acidification measurements are a highly informative means of investigating glycolytic activity and are conveniently performed on time-resolved fluorescence (TRF) intensity 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 probe sensitively detects this reduction in pH as an increase in probe 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 2 shows the investigation of glycolytic activity of lung cancer cell line A549 measured using the pH-Xtra Glycolysis Assay. In cells treated with the hexokinase inhibitor 2-Deoxyglucose (2-DG), extracellular acidification is inhibited, observed as a decrease in probe signal change. In contrast treatment with Oligomycin A, an inhibitor of mitochondrial ATP generation, leads to increased glycolytic ATP production to maintain cellular energy homeostasis.

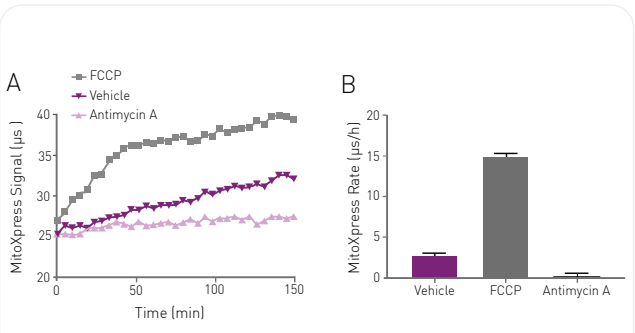


***Fig. 1:*** (A) Interrogation of iPSC-derived cardiomyocytes (NCardia) oxygen consumption measured using MitoXpress Xtra. (B) These metabolic effects can be assessed by analyzing of the rate of change of MitoXpress Xtra signal, where lower rates of change indicate reduced aerobic metabolic activity.

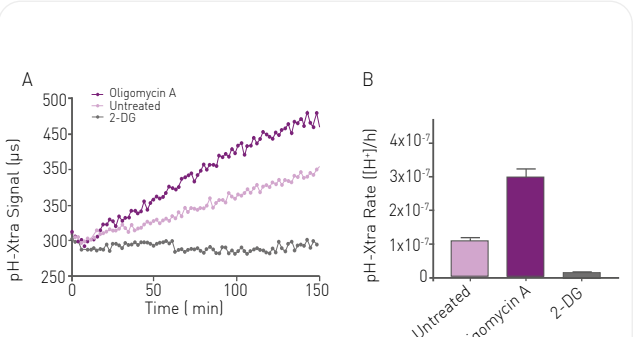


***Fig. 2:*** (A) Interrogation of A549 glycolytic activity measured using the pH-Xtra Glycolysis Assay. (B) Changes in extracellular acidification can be conveniently assessed in either pH or [H+] ion scales over time.

# The perfect platform for the analysis of cell metabolism and cellular respiration

## The best choice

The CLARIOstar® *Plus* microplate reader is the ideal choice for the detection of all cell-based assays. In particular, it provides the perfect platform for Agilent's MitoXpress® Xtra, pH-Xtra™ and MitoXpress® Intra assays, no matter whether measured in fluorescence intensity, standard time-resolved fluorescence (TRF) or lifetime mode. The following dedicated features guarantee the best possible results:

- Exceptional performance in TRF
- Dual read TRF lifetime mode
- Convenient TRF, FI and $OD_{600}$ multiplexing
- Top/bottom reading with auto-focus adjustment
- Dedicated high-performance filters

***Fig. 3:*** *CLARIOstar Plus microplate reader with ACU module.*

## Perfect environment

The Atmospheric Control Unit (ACU) module regulates $CO_2$ and $O_2$, reproducing physiological as well as hypoxic conditions needed by live cell-based assays in the reader.

In combination with temperature control and different shaking options, the ACU provides an ideal 'walk-away' solution for any long-term cell-based assay.

## Gas ramps

As a unique feature, the CLARIOstar *Plus* with ACU offers the capability to run $O_2$ gas ramps. For instance, the ACU can deprive $O_2$ and then rapidly re-oxygenate back to physiological conditions, keeping meanwhile steady $CO_2$ levels. This capability enhances live cell-based assays, as disease models such as ischemia/reperfusion can be reproduced *in vitro* in a microplate reader.

## Two mouse clicks to your results

Years of hands on assay experience and software enhancements has streamlined an ideally optimised flow that allows users to read and analyze data in a very intuitive and rapid way.

The BMG LABTECH software already comes with measurement protocols and data analysis templates optimized for MitoXpress® Intra, MitoXpress® Xtra and pH-Xtra™.

Dedicated measurement protocols provide the user with assay-optimised settings, no adjustment required. Users can simply start the data acquisition with one mouse click.


***Fig. 4:*** *Dedicated measurement protocol buttons.*

Templates are a unique feature and extremely facilitate data processing. With a single mouse click, Agilent-dedicated templates automatically perform all required calculations and display all processed results.

The combination of kit-optimized settings, measurement protocols, and data analysis templates allows Agilent assays to be measured and analysed with only two mouse clicks. This avoids time-consuming measurement optimization and data reduction steps.

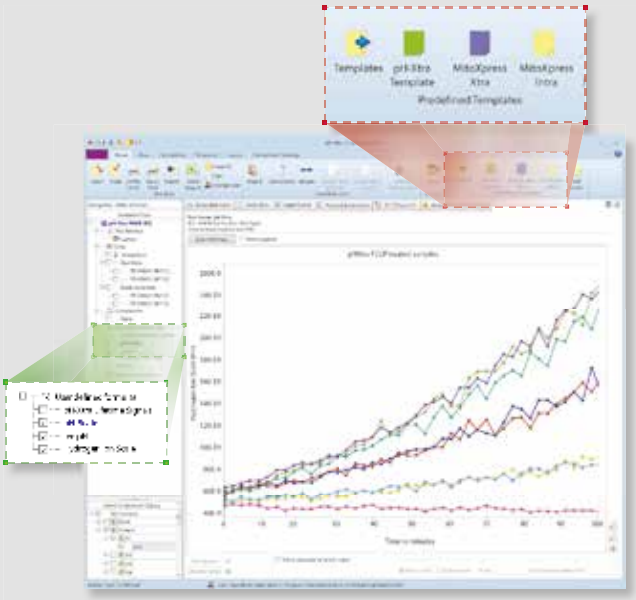

***Fig. 5:*** *pH-Xtra assay data converted to $H^+$ scale by applying a MARS template.*

# Combined measurement of glycolysis and respiration assessing cancer metabolism

*Dr. Karl Morten, University of Oxford, UK*

Malignant transformation is associated with particular metabolic alterations including increased glycolysis, increased lactic acid production and reduced pyruvate oxidation. This increased dependence on glycolysis in the presence of oxygen is termed the Warburg effect or aerobic glycolysis and can be investigated using a both MitoXpress Xtra and pH-Xtra. 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 6 illustrates how these combined assays are used to study the balance between oxidative phosphorylation and glycolysis across a range of cell types. The figure also shows how varying substrate availability can modulate this metabolic balance. U87MG metabolism is altered due to the availability of glucose, whereby increased glucose availability led to decreased mitochondrial respiration, and increase in glycolysis.

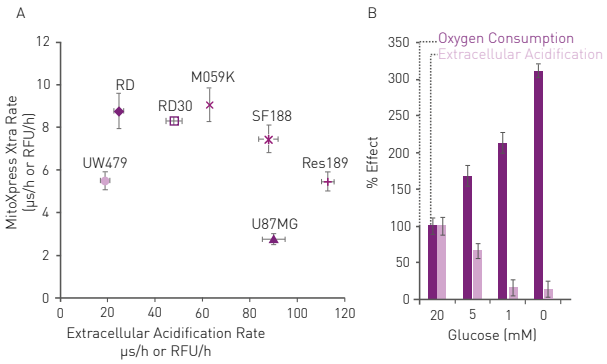


***Fig. 6:*** *(A) Combined analysis of glycolysis and mitochondrial respiration in cancer cell lines using MitoXpress Xtra (y-axis) and pH-Xtra (x-axis) respectively. (B) Effect of glucose availability on the metabolism of U87MG cells. Increasing glucose availability led to a decrease in respiration and an increase in glycolysis (Data courtesy of Dr. Karl Morten, University of Oxford, UK).*

# MitoXpress Xtra solutions for hepato/cardio toxicology

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 7). MitoXpress Xtra assay offers rapid screening of a larger number of compounds to detect acute impact of drug treatment on cells, as a primary screen. The simple workflow can identify both drugs with an inhibitory effect on mitochondrial respiration and with an uncoupling effect. These studies can be performed with a range of relevant *in vitro* models, including primary hepatocytes, hiPSC derived cardiomyocytes and hepatocytes or the HepG2 cell model.

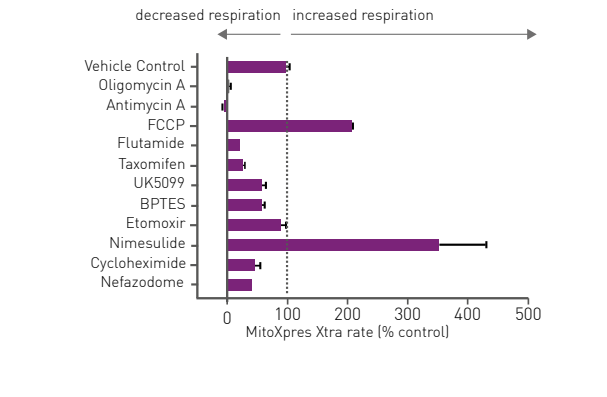


***Fig. 7:*** *Oxygen consumption rates in HepG2 cells treated acutely with single concentrations of drugs. Data were normalized to rates from vehicle treated cells. MitoXpress Xtra rates >100% indicate an increase in respiration; rates <100% inhibition.*

# Exposing cells to ischemia-reperfusion conditions and monitoring their oxygenation

*Andrea Krumm (1), C. Carey (2), M. Schwalfenberg (3), J. Hynes (2), R. Kettenhofen (3) (1) BMG LABTECH GmbH, Ortenberg, (2) Agilent Technologies, Cork, Ireland, (3) Ncardia, Cologne, Germany 07/2017*

The investigation of ischemia-reperfusion (I/R) effects requires an experimental set-up that imitates rapid changes in $O_2$ environment while recording its biological effects.
The I/R model presented here uses the CLARIOstar *Plus* plate reader with Atmospheric Control Unit (ACU) for software-controlled $O_2$ and $CO_2$ regulation. Changes in oxygenation of HepG2 cells and cardiomyocytes were reported by MitoXpress-Intra. The CLARIOstar *Plus* with ACU facilitates precise programmable control of both $O_2$ and $CO_2$, enabling the simulation of a hypoxic insult of defined depth and duration. Additionally, it enables rapid and controlled reperfusion. The use of MitoXpress Intra in real time allows ACU parameters to be modulated so that, at the cellular level, the desired depth and duration of hypoxic insult, and the required reperfusion rates are achieved.

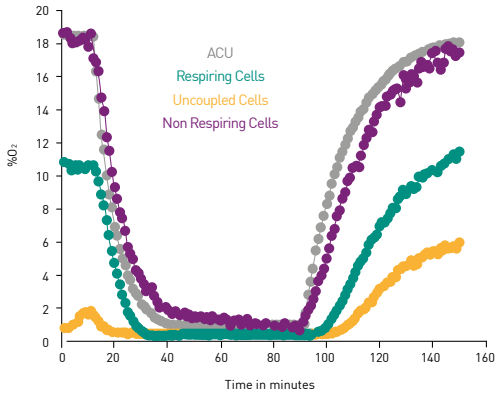


***Fig. 8:*** *Ischemia-reperfusion proof-of-concept.by modulating $O_2$ on HepG2 cells. Cellular oxygenation is monitored in respiring, non-respiring (Antimycin treated), and uncoupled (FCCP treated) cells.*

For more information please refer to BMG LABTECH application note 309.

# Measuring cell metabolism in 3D cultures

*C. Carey, J. Hynes, Agilent Technologies, Cork, Ireland, (3) Ncardia, Cologne, Germany 07/2017*

BMG LABTECH fluorescence 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 9 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 providing convenient, sensitive and high throughput measure of mitochondrial function, metabolism and cellular energy flux. This in combination with MARS data analysis and pH conversion, can facilitate a deep insight into the metabolic behaviour of the 3D culture and into how metabolism is perturbed by compound treatment or environmental conditions

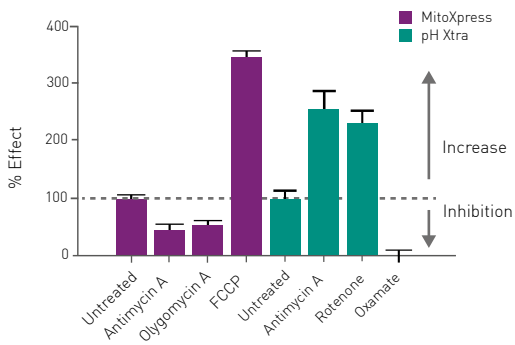


***Fig. 9:*** *Relative effect of drug treatment on oxygen consumption in A549 3D RAFT cultures measured using the MitoXpress Xtra assay. The relative effect of drug treatment on extracellular acidification rates of HepG2 RAFT cultures measured using the pH-Xtra Glycolysis Assay.*

For more information please refer to BMG LABTECH application note 248.

# Only three simple steps to your results!


Product Information: MitoXpress & pH Xtra Consumables
Brochure: Agilent Solutions for Measuring Cell Metabolism on your Plate Reader
Agilent Cell Analysis Portfolio
Our market leading technologies in real-time live cell analysis have helped researchers push new boundaries across a number of research areas. Learn about our complete portfolio of solutions by visiting our website at www.agilent.com/chem/discoverxf

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BMG LABTECH
The Microplate Reader Company

**Headquarters Germany**
BMG LABTECH GmbH
Allmendgrün 8
77799 Ortenberg
Tel. +49 781 96968 -0
germany@bmglabtech.com

**Australia**
BMG LABTECH Pty. Ltd.
2/24 Carbine Way
Mornington, Victoria, 3931
Tel. +61 3 5973 4744
australia@bmglabtech.com

**France**
BMG LABTECH SARL
7, Rue Roland Martin
94500 Champigny s/Marne
Tel. +33 1 48 86 20 20
france@bmglabtech.com

**Japan**
BMG LABTECH JAPAN Ltd.
1-6-2, Shimo-cho
Omiya-ku
330-0844 Saitama City
Tel. +81 48 647 7217
japan@bmglabtech.com

**UK**
BMG LABTECH Ltd.
8 Bell Business Park
Smeaton Close
Aylesbury
Bucks
HP19 8JR
Tel. +44 1296 336650
uksales@bmglabtech.com

**USA**
BMG LABTECH Inc.
13000 Weston Parkway
Suite 109
Cary, NC 27513
Tel. +1 877 264 5227
usa@bmglabtech.com

www.bmglabtech.com

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