Cellular metabolism made easy:

Microplate assays for mitochondrial function & glycolytic flux

LUXCEL BIOSCIENCES

ILLUMINATING DISCOVERY

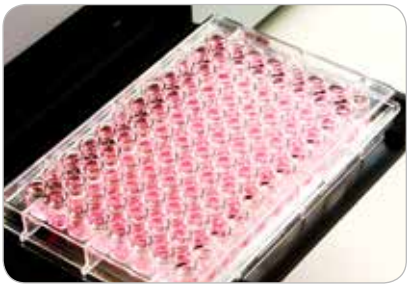
BMG LABTECH

The Microplate Reader Company

Resuspend

Add Reagent

Measure



A simple push-button solution to measuring cell metabolism on your plate reader

Luxcel Biosciences provide simple mix-and-measure assays for the assessment of cell metabolism and mitochondrial function:

- **MitoXpress® Xtra HS** measures oxygen (O_2) consumption
- **pH-Xtra™** measures glycolytic flux
- **MitoXpress® Intra** measures intracellular O_2 concentration

These assays are compatible with all commonly used *in vitro* models from isolated mitochondria to 3D cell cultures.

Combining these products with optimised BMG LABTECH hardware and configured MARS data analysis software provides a simple **'push button'** measurement solution and user-friendly data analysis, allowing the scientist to focus on the science.

Measuring Cellular O_2 Consumption

The **MitoXpress® Xtra Oxygen Consumption Assay [HS Method]** (Cat No: MX-200) offers a simple, direct, real-time measure of cellular respiration and mitochondrial function. As cell respiration reduces O_2 concentration, the **MitoXpress® Xtra** reagent signal increases, with the rate of increase reflecting the level of O_2 consumption.

These signals are processed automatically using MARS software allowing user friendly measurement of isolated mitochondria, whole cells and a wide range of 3D cultures. The assay is also suitable for measurement of isolated enzymes, bacteria, yeasts and small aquatic organisms.

Measuring Glycolytic Flux

The **pH-Xtra™ Glycolysis Assay** (Cat No: PH-200) offers a simple, direct, real-time measure of glycolytic flux. Measurement is performed in a customised measurement buffer, prepared by dissolving a buffer tablet provided with the **pH-Xtra™** kit.

As cell respiration acidifies the measurement buffer, **pH-Xtra™** reagent signal increases, with the rate of increase reflecting the extracellular acidification rate (ECAR). As lactate production is the main contributor to this acidification, ECA measurements are a convenient and informative measure of glycolytic flux.

Intracellular O_2 Concentration and Hypoxia

The **MitoXpress Intra® Intacellular Oxygen Assay** (Cat No: MX-300) measures intracellular O_2 concentrations in both 2D and 3D culture systems.

The reagent is taken up by the cell during an overnight loading period, and responds in real time to any changes in intracellular O_2 concentration.

Cell respiration can significantly impact the O_2 concentration being experienced by the cell model, allowing researchers to relate metabolic responses to available O_2. These parameters are particular important in areas such as ischemia, cancer metabolism and hypoxia.

The most direct and sensitive measure of drug-induced mitochondrial dysfunction

Drug Toxicity

Drug-induced mitochondrial toxicity (Mitotox) has been implicated with a variety of drug classes and has been shown to contribute to toxicity in the liver, heart, kidney, muscle, and the central nervous system.

MitoXpress® Xtra provides a direct high-throughput measure of mitochondria function through assessing the activity of the electron transport chain (ETC) and is therefore a powerful tool in investigating mitochondrial toxicity.

Isolated Mitochondria

Isolated mitochondria offer a very useful model for the investigation of mitochondrial toxicity. Rat liver mitochondria are typically used with mitochondria in state 2 (without ADP) used for uncoupler screening and mitochondria in state 3 (with ADP) used for inhibitor screening. Sample data are presented in Fig. 1.

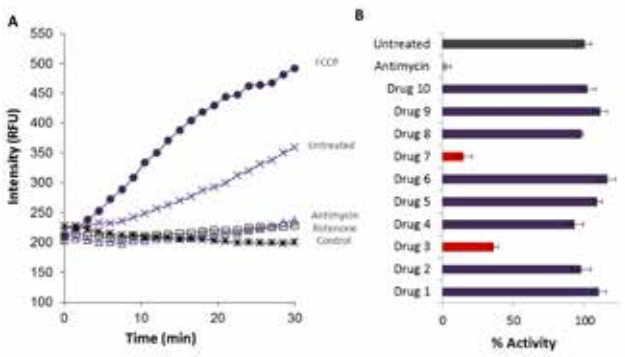


Fig. 1: A) *Glutamate/malate driven respiration of rat liver mitochondria measured in state 2 (without ADP) showing uncoupling (FCCP) and inhibition (antimycin & rotenone).* ***B)*** *Screening of unknown drug compounds using rat liver mitochondria identifying compound 3 and 7 as ETC inhibitors.*

Cell-based Mitotox Assays

Mitotox can also be assessed using cell-based assays, with hepatotoxicity and cardiotoxicity being significant focus areas. Data from the analysis of stem cell-derived hepatocytes (hiPS-HEP™, Cellartis) are presented in Fig. 2 demonstrating good screening performance.

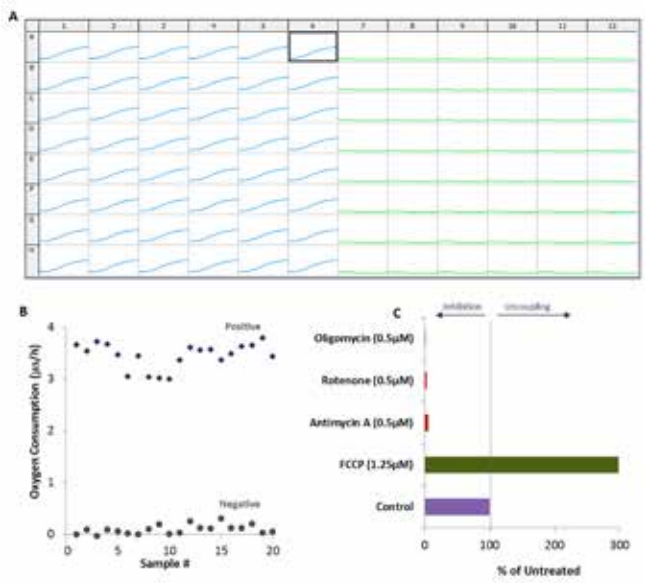


Fig. 2: *hiPS-HEP™ cells measured using MitoXpress® Xtra showing a Z' Factor of ~0.7* ***(A&B)****. Perturbed ETC function is measured using a panel of ETC modulators* ***(C).***

An additional level of mechanistic insight can be achieved by combining both **MitoXpress® Xtra** O_2 consumption and **pH-Xtra™** glycolytic flux measurements. This simultaneous assessment of the main ATP generating pathways allows true mitochondrial toxicity to be delineated from a non-specific mitochondrial insult. Sample data are presented in Fig. 3 with specific mitochondrial impairment indicated by decreased O_2 consumption and increased glycolytic flux as the cell attempts to maintain ATP supply (HepG2, Pfizer).

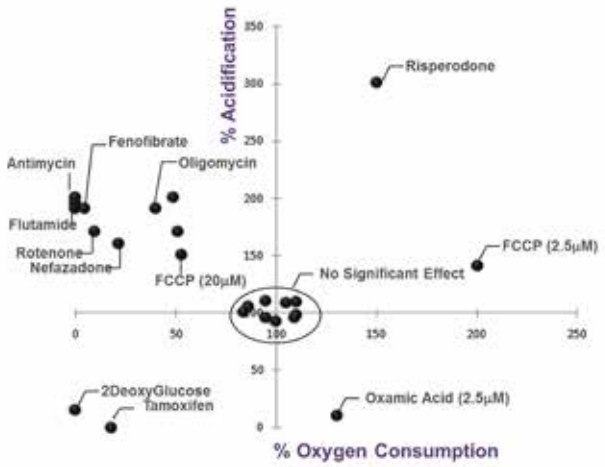


Fig. 3: *MitoXpress® Xtra and pH-Xtra™ based drug screening.*

Physiologically Relevant O_2 Concentrations

A growing appreciation that typical cell culture conditions reflect a hyperoxic state for most cell types has led to a trend towards the use of lower and more physiologically relevant O_2 concentrations in *in vitro* testing.

MitoXpress® Intra allows real-time measurement of intracellular O_2 concentrations in microplate format, a critical parameter in understanding the relationship between available O_2 and cell metabolism.
Using MARS data analysis software, data can be visualised in O_2 scale. These measurements are particularly powerful when combined with an Atmospheric Control Unit (ACU) whereby the concentration of O_2 and CO_2 on the reader can be modulated.

Measuring Cellular Oxygenation

MitoXpress® Intra allows real-time measurement of transient changes in metabolic activity through assessing cellular oxygenation.
Sample data are presented in Fig. 4, illustrating the steady-state O_2 concentration in a confluent HepG2 monolayer, and the impact of perturbed metabolism on that concentration. Cell respiration reduces O_2 concentration from ambient to 10% O_2. Increasing O_2 consumption rate by treating with FCCP causes a further decrease to approx. 3% while inhibition of respiration returns O_2 to close to ambient concentrations.

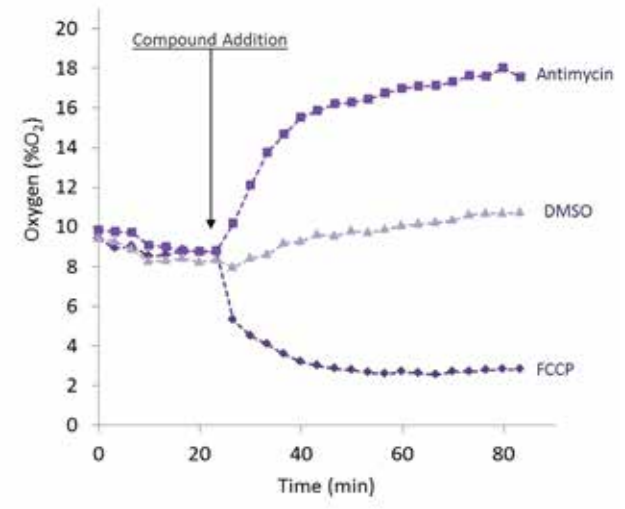


Fig 4: Monitoring intracellular O_2 concentrations in a fully confluent monolayer of HepG2 cells (compounds added using on-board injectors).

3D Tissue Oxygenation and Glycolytic Flux

MitoXpress® Intra can also be used to monitor O_2 concentrations within 3D cultures and, through multiplexing with **pH-Xtra™**, the relationship between O_2 availability and glycolytic flux can be examined in detail. These tools, combined with O_2 depletion modelling using BMG LABTECH ACU systems, facilitate measurement at 'normoxic' O_2 levels. Such analysis is beyond the capability of extracellular measurements.

Sample data are presented in Fig. 5 illustrating the effect of cell respiration on the O_2 concentrations experienced by cells within a 3D collagen culture. The concentration experienced by these cells is between 2 and 10% lower than the applied concentration.
Reduced O_2 availability can have a significant impact on the balance between aerobic and glycolytic metabolism, as measured using **pH-Xtra™**.

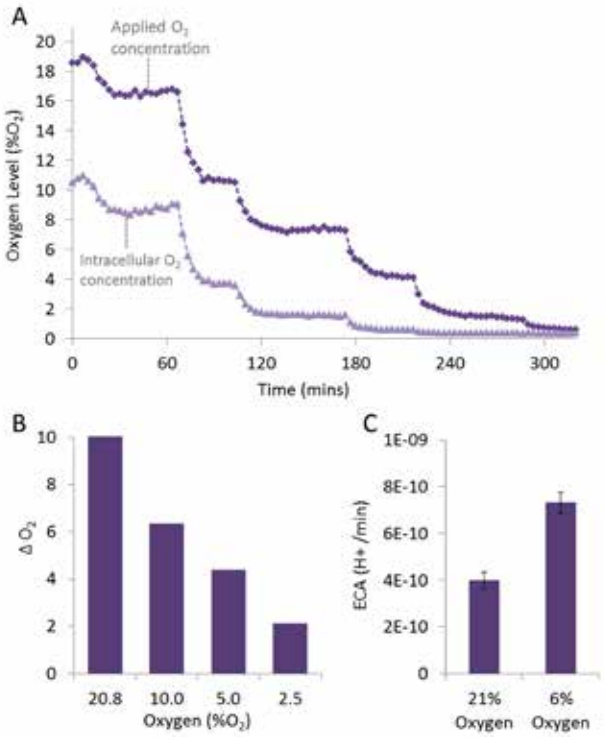


*Fig. 5: **A)** Monitoring Analysis of applied O_2 concentration and resultant intracellular O_2 concentration in 3D HepG2 culture (RAFT™, Lonza). **B)** Difference between applied and intracellular O_2 concentration across a range of applied O_2 concentrations. **C)** The impact of applied O_2 concentration on glycolytic with reduced O_2 availability causing concomitant increases in ECA. Data generated on a BMG LABTECH microplate reader with integrated ACU.*

Cancer Metabolism and Warburg

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 O_2 is termed the Warburg effect or aerobic glycolysis and can be investigated using a combination of **MitoXpress® Intra, MitoXpress® Xtra** and **pH-Xtra™**. In combination, these tools allow the balance between Oxidative Phosphorylation (OxPhos) and glycolytic ATP production to be assessed and the impact of tumour oxygenation to be determined.

The combined use of **MitoXpress® Xtra** and **pH-Xtra™** allows the assessment of cellular metabolic balance as a baseline for subsequent metabolic investigation. Data presented in Fig. 6 illustrate the balance between OxPhos and glycolysis across a range of cell types.

This balance can be modulated by substrate availability and environmental condition, with the impact of increasing glucose concentration presented Fig. 6.

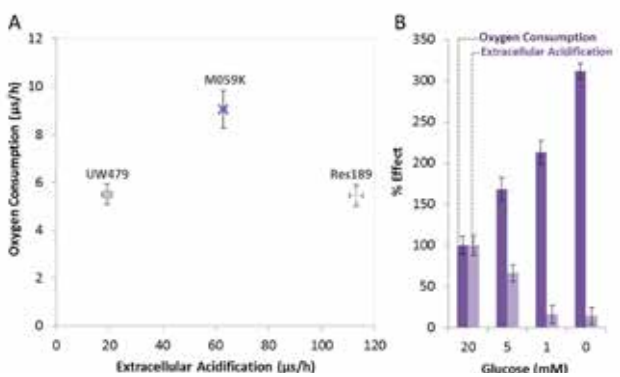


*Fig. 6: **A)** 2D analysis of metabolic balance with increasing dependence on OxPhos represented on the x-axis and increased dependence on glycolytic metabolism represented on the y-axis. **B)** Effect of glucose on metabolic balance of U87MG cells with increasing glucose concentration causing a decrease in ETC activity and an increase in glycolytic flux. (Data courtesy of Dr. Karl Morten & Dr. Michelle Potter , University of Oxford UK).*

Metabolic Multiplexing

Additional information on cellular bioenergetics can be generated through multiplexing with other metabolically relevant endpoints including mitochondrial membrane potential (MMP), ATP and ROS generation.

This facilitates a more holistic assessment of the relationships between metabolic balance, ROS production, perturbed MMP, ATP generation, O_2 availability and substrate utilisation; all of which are relevant to the metabolic alterations associated with malignant transformation.

In addition, the use of an ACU allows the impact of reduced O_2 availability on these parameters to be determined. An example is presented in Fig. 7 where **MitoXpress® Xtra** and JC-1 are measured in the same test well allowing simultaneous analysis of ETC activity and MMP (Cayman Chemical Cat#600880). This approach can also provide deeper data density and more mechanistic information through kinetic measurement.

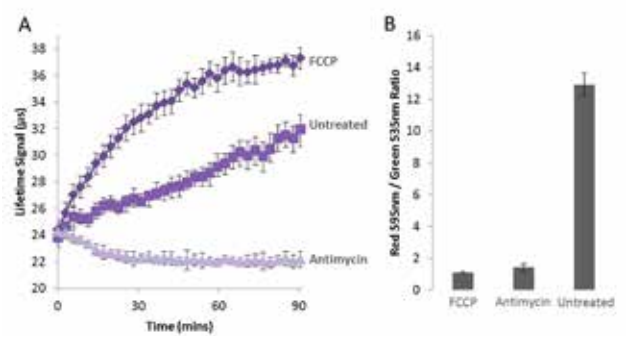


*Fig. 7: MitoXpress® Xtra **(A)** and JC-1 **(B)** measurement of HepG2 cells treated with Antimcyin and FCCP. The uncoupler FCCP causes a characteristic collapse in MMP and a resultant increase in O_2 consumption. The inhibitor Antimycin blocks the ETC thereby inhibition O_2 consumption and as a result MMP is run down by MMP consuming cellular activities.*

Some other application areas

- Obesity, diabetes & fatty acid oxidation
- Neurological diseases, ischemia & stroke
- Stem cell biology
- Immune response
- Microbes, yeasts and enzymes

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

BMG LABTECH microplate readers are an ideal choice for the detection of **Luxcel's MitoXpress® Xtra, pH-Xtra™** and **MitoXpress® Intra** assays, no matter whether in fluorescence intensity, standard time-resolved fluorescence (TRF) or lifetime mode.

CLARIOstar®

The **CLARIOstar®** with BMG LABTECH's proprietary LVF Monochromators™ is the most sensitive monochromator-based reader on the market. The **CLARIOstar®** is the ideal microplate reader and provides the highest levels of flexibility and performance for all Luxcel assays.

The following dedicated features guarantee the best level of performance:

- Exceptional performance in TRF
- Dual read TRF lifetime mode
- Convenient TRF, FI and OD_{600} multiplexing
- Top and bottom reading with automated focal height adjustment
- Dedicated high-performance filters

Perfect Environment

The CLARIOstar plate reader offers the best environmental control for all live cell-based assays. The reader comes with a uniform temperature regulation up to 45°C and with the Atmospheric Control Unit (ACU) module.

The ACU is a microprocessor-controlled unit that can regulate CO_2 and O_2 within the reader to reproduce the optimal physiological as well as hypoxic conditions needed for live cell-based assays.

FLUOstar® Omega

The **FLUOstar® Omega** is a budget-friendly filter-based microplate reader that provides the perfect combination of flexibility and performance for any Luxcel assay.

Excellent sensitivity in fluorescence intensity, an advanced TRF optic head for outstanding performance in TRF, dedicated high-performance filters, accurate temperature control and top and bottom reading are features that make the **FLUOstar® Omega** an excellent detection system for all Luxcel kits.

You are only two mouse clicks away from your cell metabolism results

BMG LABTECH's intuitive Control Software allows users to easily define measurement settings and protocols, whereas the MARS Data Analysis Software effortlessly performs a variety of mathematical calculations and data reductions. Both softwares are included with every reader and can be installed on multiple PC systems at no extra cost.

The longstanding collaboration between BMG LABTECH and Luxcel has streamlined a perfectly optimized flow that allows users to read and analyze data in a very intuitive and rapid way.

With BMG LABTECH microplate readers and software, scientists avoid time-consuming measurement optimization and data reduction steps and can concentrate on what they value most, their research!

Dedicated Measurement Protocols

Control and MARS softwares already come with Luxcel-optimized measurement protocols and data analysis templates. Dedicated measurement protocols for **MitoXpress® Intra**, **MitoXpress® Xtra** and **pH-Xtra™**, provide the user with assay-optimized settings. Users can simply start the data acquisition with one mouse click, and do not have to worry about adjusting and optimizing measurement settings.

Fig. 8: Dedicated measurement protocol buttons for MitoXpress®-Intra, -Xtra and pH-Xtra™ kits.

Assay-specific Templates

BMG LABTECH's templates are a unique feature of the MARS software and facilitate simple data processing. With a single mouse click, Luxcel-dedicated templates automatically perform all required calculations and display all processed results.

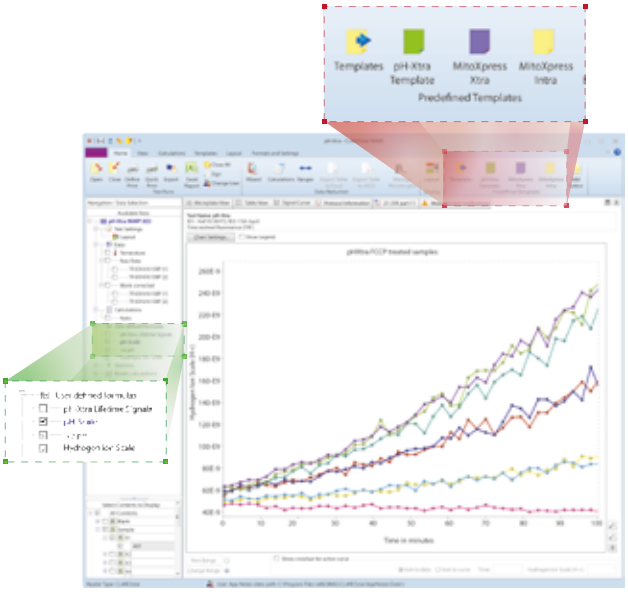


Fig. 9: pH-Xtra assay data converted to H⁺ scale by applying a MARS template.

With the ACU module, O_2 and CO_2 gas percentages are recorded and displayed together with the measured data.

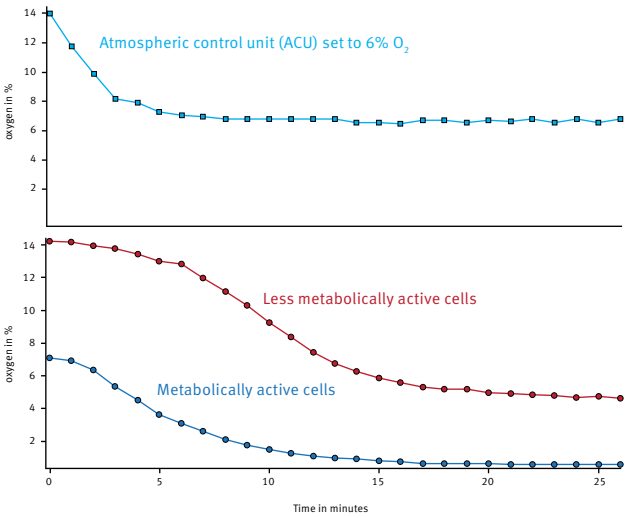
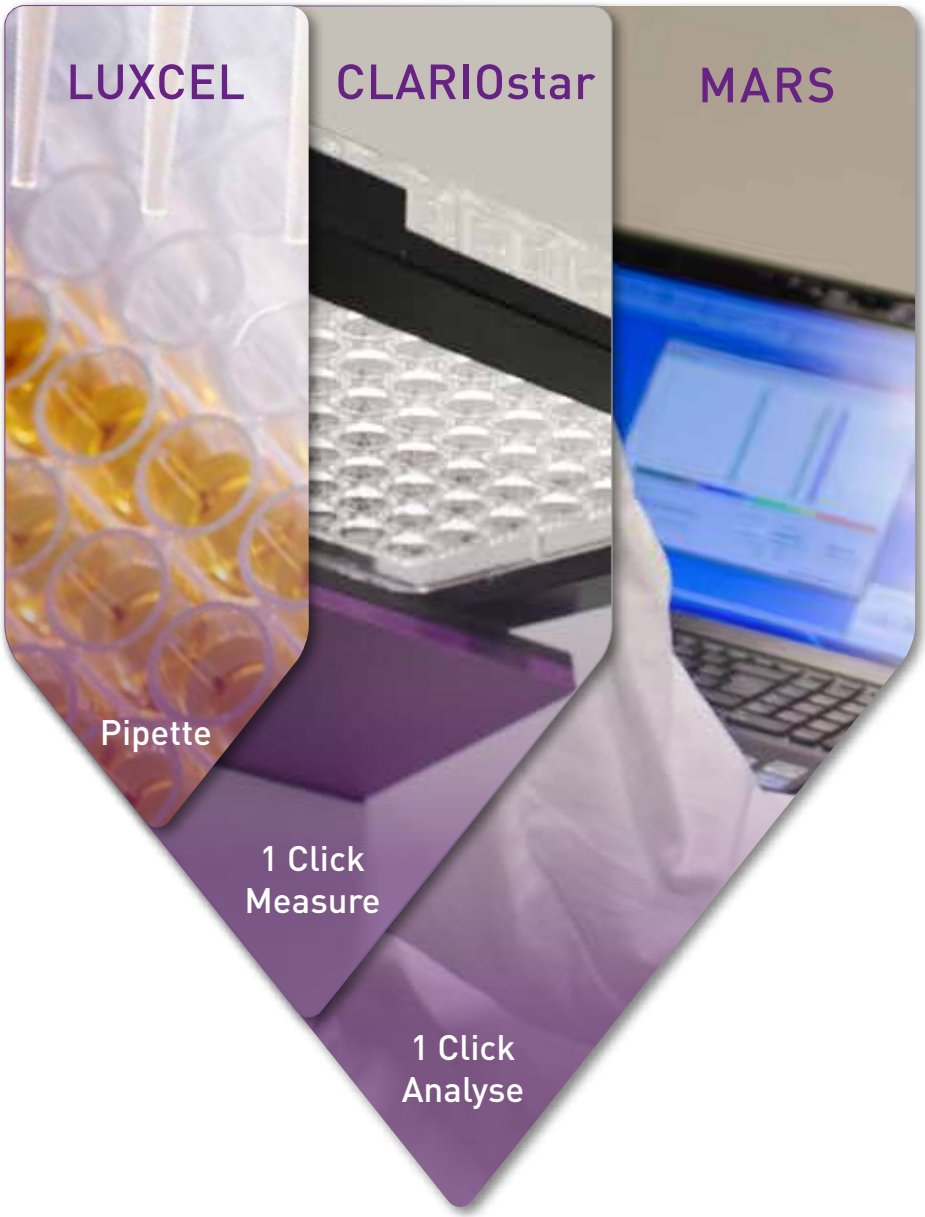


Fig. 10: O_2 sensing: effect of ACU-induced O_2 decrease on two cell lines.

The combination of kit-optimized settings, measurement protocols, and data analysis templates allows Luxcel assays to be measured and analyzed on every BMG LABTECH microplate reader with only two mouse clicks.

Just 3 simple steps to your results



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