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Real-time measurement of intracellular O_2 in mammalian cells

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- **NEW O_2-sensitive cell-penetrating nanoparticle probe MitoXpress® Intra – Intracellular Oxygen Assay**
- **Cytosolic O_2 tension significantly influences signal transduction and cellular metabolism**
- **Dedicated measurement protocols, $[O_2]$ scale conversion and data analysis with one mouse click**

Introduction & Assay Principle

As illustrated in recent publications, the level of available oxygen in the cell significantly influences cell physiology, signal transduction and cellular response to drug treatment.[1,2,3] In spite of this knowledge, the majority of *in vitro* studies culture and study cells at ambient oxygen – ignoring the oxygen gradient between the atmosphere and the medium and between the medium and the intracellular cell environment. To facilitate the quantification of cellular oxygenation Luxcel Biosciences has developed the MitoXpress Intra – Intracellular Oxygen Assay kit, based on a proprietary O_2-sensitive cell-penetrating nanoparticle probe.[4]

In this application note we show how a MitoXpress Intra assay is performed on the CLARIOstar microplate reader equipped with an atmospheric control unit (ACU). 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. The MitoXpress Intra nanoparticle probe is taken up by cells during an overnight loading period and responds in real time to any changes in intracellular oxygen concentration in both 2D culture as well as a wide range of 3D systems, including Matrigel, RAFT™, microtissues, Alvetex®, Mimetix® and other scaffolds. Oxygen quenches the phosphorescent emission from the probe, such that phosphorescence is proportional to $[O_2]$.

Cellular respiration can reduce the levels of intracellular oxygen concentration, creating a local oxygen gradient. In the specific example shown here (Fig. 1) for cells cultured under ambient oxygen, the intracellular $[O_2]$ measured using MitoXpress Intra was found to be ~14% for metabolically inactive cells and ~7% for metabolically active cell types. However, when the environmental O_2 concentration is reduced to ~6% using an ACU, the intracellular $[O_2]$ falls to ~4.5% for metabolically less active cells, and is close to anoxia for metabolically active cells.

As the real oxygen concentration experienced by cells in culture is a function of environmental O_2 concentration, cell metabolism and seeding density, MitoXpress Intra provides the ideal tool to intelligently modulate these parameters to achieve a desired and specific intracellular oxygen concentration. Similarly, MitoXpress Intra is an ideal tool to monitor real-time changes in intracellular $[O_2]$ in response to treatments that perturb mitochondrial function and cell metabolism.

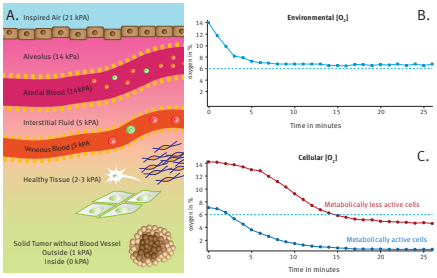

Fig. 1: (A) Schematic illustration showing changing O_2 tension in tissues. (B) *In vitro* cell culture environmental $[O_2]$ set to ~6% using an ACU and (C) corresponding cellular $[O_2]$ measured by MitoXpress Intra for metabolically active (blue) and metabolically less active cells (red).

Material & Methods

- MitoXpress Intra (MX-300, www.luxcel.com)
- CLARIOstar microplate reader equipped with ACU

Instrument settings

Detection Mode:	Dual-read, TR-F, bottom reading
Method:	Plate mode kinetic
No. of cycles:	300
Cycle time:	60 seconds
No. of flashes per well:	100
Well multichromatics:	Yes
Injection volume:	Various using onboard injectors
Target temperature:	37°C
Target O_2 concentration:	Various
Target CO_2 concentration:	Set off

Optic Settings

	Excitation	Dichroic	Emission	Int. start	Int. time	Gain
1	F: Ex TR	F: LP TR	F: 645-20	30 µs	30 µs	1900
2	F: Ex TR	F: LP TR	F: 645-20	70 µs	30 µs	1900

Data Calculation

A data transform by the MARS software is required to convert signal to lifetime and from lifetime to $\%O_2$. The CLARIOstar and FLUOstar Omega readers come with pre-installed measurement protocols and $[O_2]$ scale that allow the user single mouse click data conversion.

Results & Discussion

Cellular response to decreased ambient oxygen

Sample data are presented in Fig. 2 illustrating the effect of cell respiration on the O_2 concentration experienced

by liver cells grown within a 3D collagen culture (RAFT™). The ACU was used to create a stepwise series of atmospheric environments between 19 % and close to 0 % oxygen.

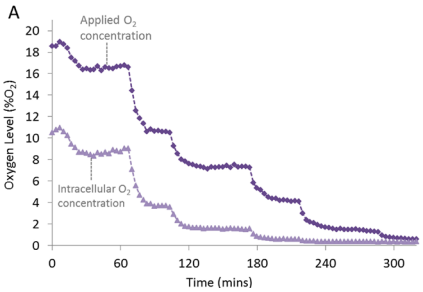

Fig. 2: Monitoring O_2 concentrations in samples containing 3D Hep2 cells in response to decreasing atmospheric O_2 conditions realized by the CLARIOstar ACU.

The initial oxygen concentration set by the microplate reader ACU is ~19 %, however the measured oxygen concentration of cells in the 3D culture is around 10 %. This reduced [O_2] is due to rapid local depletion of oxygen, consumed through cellular respiration by this metabolically active cell type. At each environmental oxygen concentration set, the actual [O_2] experienced by these liver cells is between 2 to 10 % lower. At the levels of environmental oxygen typically used by researchers studying hypoxia (~5%) it can be seen that the local [O_2] of the cells is close to zero.

Effect of compound addition

Sample data are presented in Fig. 3 and Fig. 4 illustrating the immediate and longer term effect from the addition of drugs that affect mitochondrial function and cell metabolism, in 2D and 3D culture, respectively.

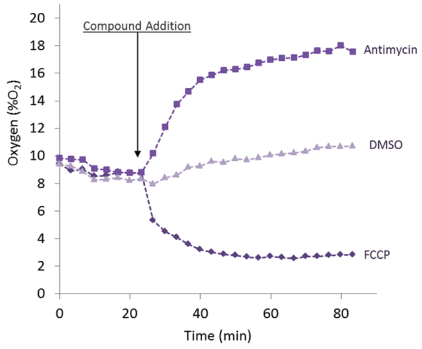

Fig. 3: Monitoring intracellular O_2 concentrations in a fully confluent monolayer of HepG2 cells.

Sequential compound additions are a feature of the CLARIOstar's onboard injectors, which offer the possibility to study opposing effects of different compounds on cellular [O_2] in the same well.

Cell respiration reduces the O_2 concentration from ambient to a baseline of ~10 % cells grown as a monolayer and to ~9% for cells grown in 3D culture. Increasing oxygen consumption rate by treatment with the mitochondrial uncoupler FCCP causes an acute and dramatic decrease in [O_2] to ~2-3 %, while complete inhibition of respiration by the mitochondrial inhibitor antimycin returns O_2 to ambient concentrations. Addition of DMSO serves as control and has no significant influence on oxygen concentration.

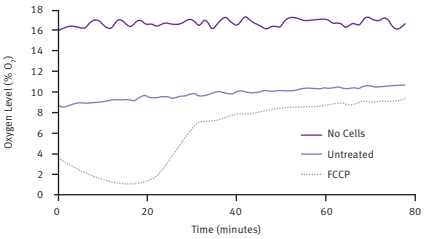

Fig. 4: Measuring the effect of drug treatment on cellular oxygenation, with HepG2 3D RAFT™ structures using MitoXpress Intra at 21% applied oxygen.

Conclusion

The physiological O_2 and substrate environment in which cells are cultured significantly affect signal transduction and cellular response to drug treatment. For the first time Luxcel's MitoXpress Intra – Intracellular Oxygen Assay kit provides researchers with an easy to use tool, with which they can measure the actual [O_2] of their cell culture system achieving greater understanding of the impact on cellular physiology.

The CLARIOstar equipped with an ACU is an excellent choice to measure intracellular [O_2]. The instrument is equipped with temperature and atmospheric control as well as shaking options for all requirements. These features are necessary for doing long term cell culturing while measuring alterations in the cells metabolisms caused e.g. by a decreased ambient oxygen content. Onboard injectors can be used to add compounds.

References

1. Chapple SJ. *et al.* (2015) *Free Radic Biol Med.* [Epub].
2. Zhdanov AV. *et al.* (2015) *Exp Cell Res.* **(330)** 13–28.
3. Potter M. *et al.* (2016) *Adv Exp Med Bio.* **(876)** 257-263.
4. Hynes and Carey (2015) *Mitochondrial Medicine 1*, **(1264)** Probing Mitochondrial Function, Methods in Molecular Biology.

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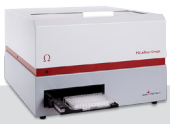

Omega Series