

ILLUMINATING DISCOVERY[®]

Real-time fluorescence plate reader-based *in vitro* cell based assay kits

MitoXpress[®] Xtra Oxygen Consumption Assay

Real-time analysis of mitochondrial respiration and function.



- Determine metabolic
 phenotype
- Assess metabolic flexibility & substrate preference
- Study effects of:
 - various treatments
 - genetic modifications
 - respiration and mitochondrial function
- Directly evaluate mitochondrial toxicity of drug treatments

Mitochondrial function is crucial for cellular metabolism, survival and energy generation. Impaired mitochondrial respiration is associated with pathological conditions such as cancer, ischemic reperfusion damage and neurodegenerative diseases. Mitochondrial toxicity is also a common reason for safety related drug attrition and withdrawal from the market after significant time and expense at early development stage. Luxcel Biosciences' MitoXpress[®] Xtra Oxygen Consumption Assay allows realtime measurement of extracellular oxygen consumption rate (OCR) of whole cells or isolated mitochondria and is a reliable method to determine respiration rates for metabolic characterization and also to evaluate toxic effects of treatments on mitochondrial function in a high-throughput format.

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Luxcel Biosciences' MitoXpress[®] Xtra Oxygen Consumption Assay

- · Applicable to a wide range of in vitro models:
 - Adherent and Suspension cells
 - · Permeabilised cells
 - · Isolated mitochondria or enzymes
 - Tissues, 3D-cultures and spheroids
 - · Bacteria, yeasts and moulds
- Simple "mix-and-measure" protocol allows multiparametric analysis with a range of other assay kits, for example the pH-Xtra[™] Glycolysis Assay.
- Reversible, making transient and fast changes in oxygen consumption observable
- Companion kits are available for more detailed analysis of metabolic phenotype; for example the MitoXpress[®] (FAO) Fatty Acid Oxidation assay.

A major advantage of using Luxcel Biosciences' kits is that they are designed for use with most fluorescence plate readers and standard 96- and 384-well microtitre plates!

• NO in lab waiting time for specialised equipment to become available and NO capital expenditure required

MitoXpress[®] Xtra Oxygen Consumption Assay

MX-200 Kit Component Details¹

Component	ltem	Description
MitoXpress® Xtra reagent	1 vial	Oxygen sensing probe
HS mineral oil	1 dropper	Sample sealing to avoid oxygen
User Manual	x1	Detailed instructions on assay set-up and data analysis

¹For people conducting multiple ongoing research experiments these kits also come in a multipack of 4 the MX-200/4 with 4 vials and 4 dropper bottles of the reagent and mineral oil.

Real-time observation of oxygen consumption in live cells



Figure 1: Typical Signal profile of MitoXpress[®] Xtra for cells treated with compounds that modulate mitochondrial function. Cellular respiration reduces extracellular oxygen levels, causing the MitoXpress[®] Xtra reagent signal to increase, with the rate of increase reflecting the rate of oxygen consumption. The Inhibitor impacts the electron transport chain, reducing cellular respiration, while Uncoupler drives it to its maximal rate. Dose response curves show good inter assay reproducibility and allow easy calculation of IC₅₀

Measuring mitochondrial respiration (OCR), glycolysis & ATP



Figure 2: Cellular Energy Flux for HepG2 cells treated with compounds modulating the oxygen consumption rate (OCR) or extracellular acidification rate (ECAR), shown as a percentage relative to untreated control cells. Comparative measurements with MitoXpress[®] Xtra and pH-Xtra[™] Glycoloysis Assay show the shift between mitochondrial respiration and glycolysis and the cellular control of energy (ATP; measured 1h post-treatment).¹

REFERENCES

¹ Data modified from: Hynes, J., *et al.*, Anal. Biochem., (2009), 390(1), p. 21-8.

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