



Cell-based screening of mitochondrial dysfunction and mitochondrial toxicity

Introduction

Mitochondrial dysfunction is implicated in numerous disease states and is also a major mechanism of drug-induced toxicity. Oxygen consumption is one of the most informative and direct measures of mitochondrial function. Traditional methods of measuring oxygen consumption are hampered by the limitations of low throughput and high complexity. The MitoXpress™-Xtra assay from Luxcel, solves these limitations by allowing convenient, plate-based analysis of mitochondrial function. The assay employs MitoXpress, one of a family of phosphorescent oxygen sensitive probes developed by Luxcel. The assay is based on the ability of O₂ to quench the excited state of the MitoXpress probe. As the test material respirates (e.g., isolated mitochondria, cell populations, small organisms, tissues and enzymes), O₂ is depleted in the surrounding solution/environment, which is seen as an increase in probe phosphorescence signal. Changes in oxygen consumption reflecting changes in mitochondrial activity are seen as changes in MitoXpress™ probe signal over time.

The assay is non-chemical and reversible, a decrease in oxygen consumption (an increase in O₂ levels) is seen as a decrease in probe signal. MitoXpress™ is analysed on standard fluorescence plate readers using standard 96- and 384-well microtitre plates. The MitoXpress™-Xtra assay combines the high data quality and information content of the oxygen electrode approach, with the throughput and convenience of microtitre plate based assays. These capabilities make MitoXpress™-Xtra the ideal tool for rapid compound screening, IC₅₀ generation and the application of structure-activity relationship approaches.

Protocol

Plate Preparation

- Prepare plates on a plate heater equilibrated to 30°C.
- Add 150µl of cells to test wells (suspension/tyrpsinised adherent cells). Alternatively, adherent cells may be pre-plated and 150µl of fresh media added prior to measurement
- Reconstitute MitoXpress™ probe in 1 ml H₂O to give a 1 µM stock solution and dispense 10 µl of this stock solution into to each well.
- For compound testing, add 1 µl of compound in an appropriate solvent to test wells. If required, incubate cells with compounds.

- Add a layer of mineral oil (preheated to 30°C) 100µl volume to each well. This increases assay sensitivity by minimising interference from ambient oxygen.

Measurement

- Insert the prepared plate into a fluorescence plate reader pre-set to 30° C.
- Measure MitoXpress™ oxygen probe signal at 1.5 min intervals for 30-200 min using excitation and emission wavelengths of 380 nm and 650 nm respectively. (Readers equipped with a time-resolved mode, may achieve improved performance using delay and gate time of 30 and 100 µsec).
- Cell respiration causes a reduction in the concentration of dissolved oxygen in the sample, resulting in an increase in MitoXpress™ probe signal.

Note: Sufficient cell numbers are required to produce measurable signal changes. The cell numbers are cell-type dependent. Highly glycolytic adherent cells must be trypsinised and concentrated prior to measurement.

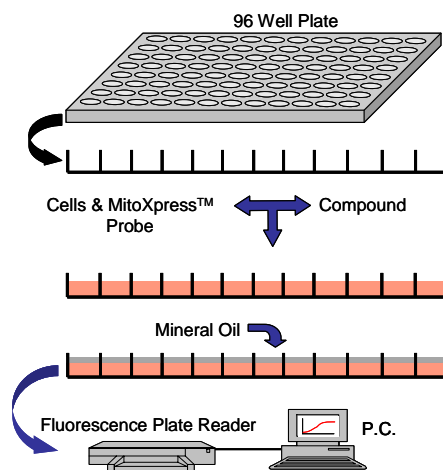


Fig. 1: Schematic diagram of MitoXpress™ cell-based screening assay.

Data Analysis

- For semi-quantitative analysis, the rate of signal increase can be used to compare treated and untreated samples or to compile dose response data.

Results and discussion

Monitoring cell respiration

The ability of the MitoXpress™ -Xtra assay to assess cell



Cell-based screening of mitochondrial dysfunction and mitochondrial toxicity

respiration is illustrated in Fig. 2. Dilutions curves for HepG2 cells (Fig. 2A) and primary rat hepatocytes (Fig. 2B) are presented.

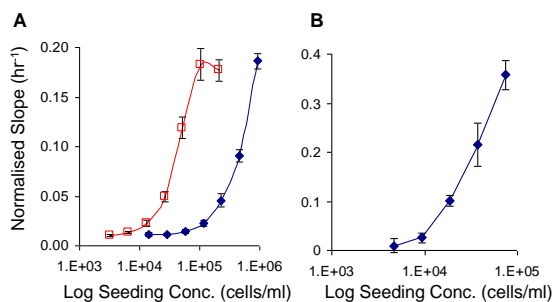


Fig. 2: Cell dilutions measured on 96-well plates using MitoXpress™ oxygen Probe. **A)** HepG2 cell dilution after an overnight (□) or 2 day culture period (●), **B)** primary rat hepatocyte cell dilution after an overnight culture period. Rates of probe signal change (slope of fluorescence signal) were normalised against initial intensity [1].

MitoXpress™ -Xtra and ATP analysis of cells

To contrast the sensitivities of oxygen consumption and cellular ATP concentrations as indicators of mitochondrial dysfunction; HepG2 cells were treated with a panel of classical electron-transport chain (ETC) modulators and both cellular oxygen consumption and cellular ATP concentrations were measured.

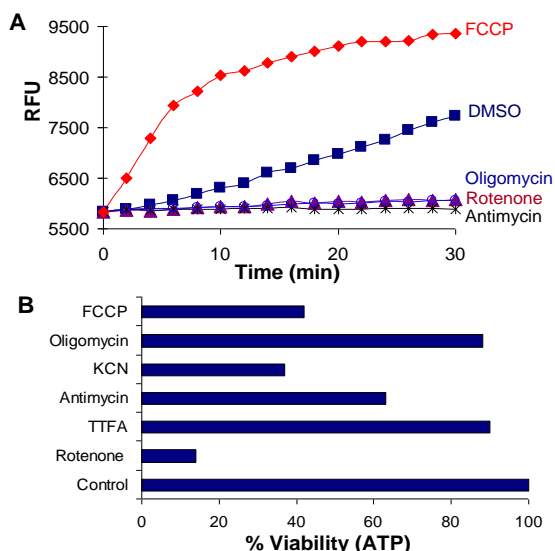


Fig 3: Parallel analysis of classical inhibitors on HepG2 cells. **A)** MitoXpress™ assessment of mitochondrial function immediately post-treatment, **B)** analysis of ATP concentrations 24 h post-treatment.

For MitoXpress™ probe measurements, cells were plated at 80,000 cells/well, allowed to adhere overnight and then assayed. MitoXpress™ data presented in Fig. 3A illustrates that drug-induced mitochondrial dysfunction is evident immediately post-treatment with both inhibition (Oligomycin, Rotenone, Antimycin) and uncoupling (FCCP) being detected. Despite this dysfunction and an additional 24 hour exposure, analysis of cellular ATP concentrations indicated high levels of 'viability' (Fig. 3B). This pattern is also reflected using other assays and cell lines.

MitoXpress™ -Xtra: comparison with other methods of cell screening

The dose-response relationship for the ETC inhibitor rotenone using four different viability assays: MitoXpress™, MTT, LDH and CyQuant is outlined in Fig. 4. These data indicate that treatment suppresses mitochondrial respiration, leading to loss of function and reduced oxygen consumption prior to cell death.

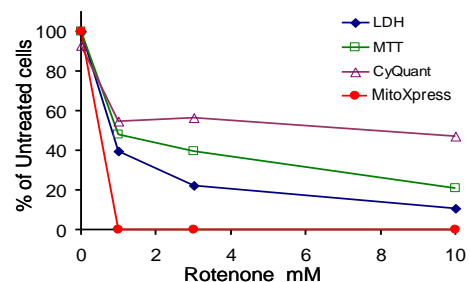


Fig. 4: Effects of Rotenone treatment on H-4-II-E cells analysed using MitoXpress™, CyQuant, MTT and LDH assays (24h exposure) [see ref 2 below].

These results demonstrate the effectiveness of MitoXpress™ in assessing mitochondrial function in whole cells as well as the importance of evaluating mitochondrial function in the context of drug toxicity. The specificity and sensitivity of the MitoXpress™ -Xtra assay is demonstrated by its ability to detect mitochondrial toxicity more rapidly and at lower drug doses than other assays. When combined with other assays, MitoXpress™ allows detailed evaluation of mechanisms of drug toxicity, adding significantly to the portfolio of information available for compound evaluation.

References

1. Marroquim *et al.*, *Toxicol. Sci.* 2007;97(2):539-47.
2. Hynes J. *et al.*, *Tox in Vitro.* 2006;20(5):785-92.
3. Hynes J. *et al.*, *Toxicol. Sci.* 2006;92(1):186-200.
4. Hynes J. *et al.*, *J. Immunol. Meth.* 2005;306:193-201.

CONTACT

Luxcel Biosciences Ltd, BioInnovation Centre, UCC, Cork, Ireland

CALL US ON: +353 (0) 21 4901447 FAX US ON: +353 (0)1 4811801 EMAIL US AT: info@luxcel.com

www.luxcel.com