Measuring changes in cellular metabolism by monitoring extracellular acidification and oxygen consumption in real-time

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Introduction

Dysregulation of cellular energy metabolism is a common factor in a variety of disorders including diabetes and obesity as well as cancer to name a few. The current technologies used to assess mitochondrial function via extracellular acidification (ECA) and oxygen consumption rate (OCR) are often designed in such a way that they ignore the impact of O2 concentration on cellular bioenergetics. Here we describe the implementation of the CLARIOstar with ACU in conjunction with advanced phosphorescent dyes to measure O2 consumption and glycolytic flux in an open-flow respirometry system. This system enables measurement of respiration rate at steady state, where O2 supplied equals O2 consumed. The phosphorescent oxygen and pH probe (Cayman Chemical) combined with the CLARIOstar and ACU provides a ‘push button’ multiplexed system with the potential for monitoring additional cell health outcomes. Experiments can be performed under user defined [O2], using either adherent cells or cell suspensions where relevant, with throughput comparable to existing technologies.

Assay Principle

Open-flow respirometry is a useful, physiologically relevant approach that allows for the measurement of mitochondrial respiration rates at dynamic steady-state oxygen concentrations. To improve throughput, this system was translated to a 96-well plate format. This requires the use of at least 2 negative control wells that exhibit zero respiration [Figure 1]. Results from experimental samples are subtracted from controls to calculate respiration rate.

Materials & Methods

- Phosphorescent oxygen and pH probe (Cayman Chemical)
- CLARIOstar with ACU (BMG LABTECH)
- Black, clear bottom, 96-well plates (Greiner)
- H9C2 cells and HepG2 cells
- Other reagents from commercially available sources

The indicated cell types were plated at a density of 7 X 104 cells/well. Reagent preparation, cell seeding, instrument set up, and assay protocols were all performed as indicated in kit literature. For dose-response experiments, serial dilutions of antimycin A were prepared in DMSO to create a 10 point half-log dilution series with a maximum concentration of 10 μM (final DMSO concentration = 0.01%).

The measurement settings are also provided in the CLARIOstar test protocol, available at:
www.bmglabtech.com/cayman/

The test protocol includes use of onboard reagent injectors to add control compounds. Oligomycin is used to decrease OCR and increase ECA. FCCP is used to increase OCR. These control compounds are included in the test kit.

Instrument settings

<table>
<thead>
<tr>
<th></th>
<th>MitoXpress® Xtra</th>
<th>pH-Xtra®</th>
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<tr>
<td>Dichroic</td>
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<td>Start: 100 µs, Time: 100 µs</td>
<td>Integration window 2</td>
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<td>Settling time</td>
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<tr>
<td>Number of flashes per well: 100</td>
<td>Oxygen: 10%</td>
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<tr>
<td>Volume: 5 µl, pump speed: 430 µl/s</td>
<td>Injections</td>
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<tr>
<td>1 (Oligomycin): injection cycle 20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 (FCCP): injection cycle 40</td>
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Fig. 1: Open-flow in a 96-well plate. Respiration rate is calculated from the indicated formula where Q = respiration (nmols O2/min/well), m = linear diffusion rate of O2 from gas phase to liquid phase, C* = zero respiration wells at steady state, C0 = experimental sample wells at the same time point.

1 CLARIOstar® with atmospheric control unit (ACU) provides a key element in an open-flow respirometry system

2 OCR and ECA measured simultaneously in real-time under physiologically relevant atmospheric conditions.

References:


Calculation of ECA and OCR were performed in MARS, BMG LABTECH’s data analysis software. A data analysis template is available at the web address indicated above. Subsequent analysis of dose-response and Z’ data were also performed in MARS.

Results & Discussion

When the phosphorescent oxygen and pH probes are used with the CLARIOstar with ACU, real-time changes in ECA and OCR can be observed (Figure 2). After an initial 20 min equilibration, a steady-state signal for OCR can be observed. The expected changes in OCR and ECA in response to control compounds are also observed following their injection for the triplicate samples displayed.

As an example of the capabilities of this test for compound screening a dose response to the known mitochondrial inhibitor antimycin A was prepared. Figure 3 shows the anticipated concentration dependent effect of antimycin A on OCR. From this plot an IC₅₀ = 120.8 nM is determined. Similar results can be obtained for ECA analyses [data not shown].

Finally, we calculated the Z’ statistic for this assay (Figure 4). The value of above 0.59 is indicative of an excellent assay that is well suited to screening purposes.

Conclusion

The phosphorescent oxygen and pH probe (Cayman Chemical) and the CLARIOstar with ACU provide a robust and efficient means of measuring cellular bioenergetics at [O₂] that are physiologically relevant. Additional utility can be derived from the ability to easily use non-adherent cells and to perform additional multiplexing to examine additional cellular responses.

References