ab197245 – Intracellular Oxygen Concentration Assay

Instructions for Use

For the measurement of intracellular oxygen in populations of live mammalian cells

This product is for research use only and is not intended for diagnostic use.
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1. Introduction

Molecular oxygen is a key substrate of all aerobic organisms and the terminal acceptor of the electron transport chain. This highly informative marker of cellular metabolism and mitochondrial function is closely regulated by the ATP/ADP ratio, the levels of available oxygen and the action of signalling molecules such as NO and Ca$^{2+}$.

Analysis of molecular oxygen facilitates the elucidation of critical biochemical pathways; including cell survival and death, mitochondrial function, toxicological impact of compounds and metabolic alterations caused by various stimuli or disease states. To date, routine analysis of intracellular molecular oxygen has been limited by the lack of suitable means of sensing oxygen within the cell monolayer.

**Intracellular Oxygen Concentration Assay (ab197245)** is a flexible fluorescence approach for analysis of intracellular oxygen concentration at the cell monolayer using plate-based time-resolved fluorometry. The assay is based on the ability of oxygen to quench the excited state of the oxygen-sensitive probe. The probe is taken up via nonspecific energy dependent endocytosis and, after washing, the cells are monitored on a fluorescence plate reader (dual-read TR-F required for full oxygen quantitation). The probe phosphorescence is quenched by intracellular oxygen in a non-chemical, reversible manner allowing the measurement of average intracellular O$_2$ levels and facilitating real-time monitoring of relative
changes in cellular oxygen consumption without requiring specialised imaging equipment.

The ability to monitor such fluctuations is particularly informative as it allows the assessment of transient changes in metabolic activity and provides access to a critical parameter in the study of hypoxia which is beyond the capacity of extracellular sensing methodologies.

2. Product Overview

Intracellular O$_2$ Probe is optimised for use on standard 96-well plates and high-sensitivity time-resolved fluorescent plate readers, using ratiometric measurement of time-resolved phosphorescence intensity for calculation of probe lifetime. One vial of Intracellular O$_2$ Probe is sufficient for ~100 data points.

For the extracellular analysis of molecular oxygen, use Extracellular Oxygen Consumption Agent (ab197243) or Oxygen Consumption Reagent (ab197242).
3. Assay Summary

Add cells and Intracellular O₂ Probe to 96-well plate

Return cells to culture overnight

Wash cells with pre-heated respiration medium

Read plate using fluorescence plate reader at 640 nm and 660 nm

4. Materials Supplied

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
<th>Storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intracellular O₂ Probe</td>
<td>1 vial</td>
<td>+2-8ºC (in the dark)</td>
</tr>
</tbody>
</table>
5. Protocol for Assessment of Intracellular Oxygen by Time Resolved Fluorometry

5.1 Probe preparation and cell loading

5.1.1 Reconstitute the probe in 1 mL of ultra-pure water prior to use.

5.1.2 Dilute the reconstituted probe 1:10 in standard culture media (equilibrated to 37°C) for incubation with cells. Prepare immediately prior to use.

5.1.3 Cells are grown to full confluence in a suitable medium supplemented with Fetal Bovine Serum, L-glutamine and Penicillin/Streptomycin on cell culture treated 96 well plates.

5.1.4 Replace seeding medium with 100 μL of normal growth media (10%FBS) containing Intracellular O2 Probe at 10 μg/ml. Return cells to culture overnight for 16-20 hours.

5.1.5 Prior to measurement wash cells twice with pre-heated media.

5.1.6 For measurement add 150 μL/well of same medium and set plate reader temperature control to 37°C.
5.2 Measurement

Samples are assessed using a suitable time resolved plate reader. The Intracellular O2 Probe is excitable at 340-400 nm and emission is collected between 640 nm and 660 nm. Phosphorescent intensities are measured at delay times of 30 μs and 70 μs (with 30 μs window time) with the ratio of these intensities subsequently converted to phosphorescent lifetimes as shown in Figure 1.

![Figure 1](image)

**Figure 1:** Measurement principle for calculation of phosphorescence lifetime from ratiometric time-resolved phosphorescence intensity.

5.3 Typical Data Output

When analysing a fully confluent monolayer, ETC activity results in measurement usually beginning at oxygen concentrations below air saturated levels. Reduced ETC activity results in an increase in intracellular oxygen concentration resulting in a decrease in probe signal while increased ETC activity results in a decrease in intracellular oxygen concentration resulting in an
increase in probe signal (Fig. 2). Increases may be prolonged or more transient, depending on the processes involved and the measurement conditions.

**Figure 2:** Diagram representing Intracellular O2 Probe profiles in response to drug treatment.

6. Data Output

6.1 Loading & Measurement
Cells are incubated with probe overnight. Figure 3 presents a pseudo-colour microsecond FLIM image of one well of a loaded 96-well plate showing significant cell loading. Probe emission from each well is assessed kinetically in response to altered oxygen concentration on recommended time-resolved fluorescence readers as outlined (Fig. 1).
Figure 3: FLIM image of a 96 Well Plate well seeded with HepG2 loaded with Intracellular $O_2$ Probe

6.2 Probe Response
Measurement of phosphorescent lifetime of the Intracellular $O_2$ Probe facilitates measurement of the basal level of intracellular oxygen within the cell monolayer. The response of the probe is demonstrated in antimycin-treated HepG2 cells measured on a fluorescent plate reader equipped with an atmospheric control unit. This instrument allows the ambient $O_2$ concentration to be reduced stepwise. The time taken for the non-respiring monolayer to equilibrate to this new $O_2$ concentration can be observed (Fig. 4) with the new steady state probe signal reflecting this new concentration. Such calibrations also allow measured lifetime values to be automatically transposed into $O_2$ concentrations, thereby allowing probe response to be viewed in this scale.
Figure 4: Kinetic probe trace showing the response of the cell monolayer to decreasing ambient oxygen.

6.3 Assay Performance & Cell Responses

The magnitude, consistency of monolayer (de)oxygenation is monitored. Profiles are seen to be highly consistent with uncoupling causing enhanced oxygen consumption seen by an immediate decrease in intracellular oxygen resulting in an increase in probe lifetime while inhibition causes the reverse. Increasing the FCCP concentration increases the speed of the deoxygenation, while increasing the cell number increases the depth of the deoxygenation (data not shown). Treatment of HCT116 cells with the RyR1 agonist ryanodine also causes a transient deoxygenation of the monolayer via a calcium mediated increase in metabolic rate (Fig. 5B), a response not readily apparent using extracellular analysis.
**Figure 5:** The effect of HepG2 cells treated with an electron transport chain inhibitor (Antimycin) and uncoupler (FCCP). Consistency of response & inhibition and uncoupling of ETC activity (A) and kinetic analysis of metabolic responses agonist treatment (B).
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