## Overview

<table>
<thead>
<tr>
<th>Product name</th>
<th>Intracellular Oxygen Concentration Assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Detection method</td>
<td>Fluorescent</td>
</tr>
<tr>
<td>Sample type</td>
<td>Adherent cells, Suspension cells</td>
</tr>
<tr>
<td>Assay type</td>
<td>Quantitative</td>
</tr>
<tr>
<td>Assay time</td>
<td>1h 30m</td>
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</tbody>
</table>
| Species reactivity            | **Reacts with:** Human  
**Predicted to work with:** Mammals |

### Product overview

Intracellular Oxygen Concentration Assay (ab197245) is an easy mix and measure 96 well fluorescence plate reader based approach for the analysis of intracellular oxygen concentration at the cell monolayer. 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. The probe signal increases with a reduction in intracellular oxygen and decreases with an increase in intracellular oxygen. The probe is excitable at 360-400 or 535 nm and emits at 630-680 nm. Optimal filter combinations are Ex/Em = 340/642 nm.

The flexible plate reader format, allows multiparametric or multiplex combination with a range of other reagents and it is suitable for HTP automation.

### Notes

Learn more about the full range of **assays to measure glycolysis, oxygen consumption, fatty acid oxidation and metabolic flux in live cells**.

Or review the full **metabolism assay guide** for other assays for metabolites, metabolic enzymes, mitochondrial function, and oxidative stress.

### Platform

Microplate reader
Storage instructions
Store at +4°C. Please refer to protocols.

<table>
<thead>
<tr>
<th>Components</th>
<th>Identifier</th>
<th>96 tests</th>
<th>4 x 96 tests</th>
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</thead>
<tbody>
<tr>
<td>Intracellular O₂ probe</td>
<td>1 vial</td>
<td></td>
<td></td>
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</tbody>
</table>

Excitation and emission spectra of Intracellular O₂ probe, showing normalized excitation (Ex 360-400nm; Peak 380nm) and emission (Em 630-670nm; Peak 650nm).

Excitation and emission spectra of O₂ probe

Illustrating dual read TR-F measurement.

HEK293T cell oxygenation. HEK293T cells were cultured in 2D and measured at ambient oxygen. Intracellular O₂ levels were ~ 14%.
Reducing instrument O₂ to 6% caused cellular oxygenation to drop to ~2%. Assay performed using a CLARIOstart equipped with an ACU module (BMG Labtech).
Sample Calibration Data. Intra O₂ probe Lifetime profiles measured at decreasing [O₂] with parallel glucose oxidase treatment to achieve 0% O₂.

Relationship between probe lifetime (τ) and applied [O₂]. Applying a first order exponential fit generates a calibration function of \( O₂\% = A_1 \times \exp(-\frac{\tau}{t_1}) \). Example: \( O₂\% = 659.3 \times \exp(-\frac{\tau}{8.475}) \).

Measuring the impact of cell metabolism on iPS-derived cardiomyocyte oxygenation. During measurement, cells are treated with antimycin (ETC inhibitor) and isoproterenol (β-adrenoreceptor agonist).

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