

(FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !)

Catalog No: E-BC-F063

Specification: 48T/96T

**Measuring instrument: Fluorescence microplate reader
(Ex/Em=380 nm/650 nm)**

Elabscience® Cellular Intracellular Oxygen Fluorometric Assay Kit

This manual must be read attentively and completely before using this product.

If you have any problem, please contact our Technical Service Center for help:

Toll-free: 1-888-852-8623

Tell: 1-832-243-6086

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Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

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Intended use

This kit can be used to measure cellular intracellular oxygen.

Detection principle

This kit utilizes O₂-sensitive probes whose phosphorescence is quenched by oxygen. The probe signal increases with decreasing intracellular O₂ concentration and decreases with rising O₂ levels, allowing real-time monitoring of intracellular oxygen dynamics via fluorescence intensity changes.

Kit components & storage

| Item | Component | Size 1(48 T) | Size 2(96 T) | Storage |
|-----------|----------------------------------|------------------|------------------|--------------------------------|
| Reagent 1 | Probe | 0.06 mL × 1 vial | 0.12 mL × 1 vial | -20°C, 12months, shading light |
| | Black Clear-bottom Culture Plate | 96wells | | No requirement |
| | Plate Sealer | 2 pieces | | |
| | Sample Layout Sheet | 1 piece | | |

Note: All the reagents should be stored according to the table. The reagents from different kits can not be mixed or used interchangeably. For liquid reagents with small volumes or powders, centrifuge them before use to prevent loss.

Instruments

Fluorescence microplate reader (Ex/Em=380 nm/650 nm)

Materials required but not provided

Complete Medium

Reagent preparation

① Equilibrate all the reagents to 25°C before use.

② The Preparation of Probe Working Solution:

Before testing, prepare a sufficient amount of Probe Working Solution according to the test wells. For example, prepare 1000 μL of a Probe Working Solution (mix 10 μL of Probe and 990 μL of Complete Medium thoroughly). Probe Working Solution should be freshly prepared before use.

The key point of assay

① Light-sensitive: Keep probe protected from light at all times.

② Store probe at -20°C in aliquots to avoid repeated freeze-thaw cycles.

Operating steps

Flow Cytometry Analysis

- ① Using a 96-well black clear-bottom plate, set up blank, control, and experimental groups (drug treatment groups as needed). Use 3–5 replicate wells per sample. Seed 100 μL of cell suspension per well at a density of $4\text{--}8 \times 10^5$ cells/mL ($4\text{--}8 \times 10^4$ cells/well).
 - ② Incubate the plate overnight in a 37°C, 5% CO₂ incubator.
 - ③ After overnight incubation, carefully aspirate the culture medium to avoid detaching the cells.
 - ④ Add 100 μL of complete medium to the blank group, and 100 μL of probe working solution to the other groups.
 - ⑤ Incubate the plate overnight in a 37°C, 5% CO₂ incubator (this step allows equilibration of the probe).
 - ⑥ After overnight incubation, carefully aspirate the supernatant from each well without disturbing the cells. Wash each well once with complete medium, then add 100 μL of fresh complete medium to each well.
 - ⑦ Place the plate in a controlled gas environment (set conditions according to the specific experimental requirements) and incubate for 1 hour (incubation time may be adjusted depending on cell status and probe concentration; can be shortened or extended as needed). Using a microplate reader, perform a 1-hour kinetic measurement at 37°C with 2-minute intervals. Set the excitation wavelength to 380 nm and emission wavelength to 650 nm. Measure the fluorescence intensity in each well to monitor changes in intracellular oxygen level.
- If a gas control module connected to the microplate reader is available, perform a 1-hour kinetic measurement of fluorescence intensity.

If real-time detection is not possible, use an alternative method to isolate the plate from air, and measure the fluorescence intensity at the initial and end time points, recorded as F_1 and F_2 .

Calculate $\Delta F = F_2 - F_1$. Compare the ΔF values between the experimental and control groups to assess changes in oxygen concentration. A larger ΔF indicates a more pronounced change in oxygen concentration.

Appendix I Example Analysis

Using H9c2 cells as an example, cells were seeded according to the experimental protocol. After probe equilibration, cells were treated with Antimycin A or FCCP and incubated under hypoxic conditions for 1 hour. Treatment with 1 $\mu\text{mol/L}$ Antimycin A inhibited mitochondrial respiration, reducing intracellular oxygen consumption. As a result, intracellular oxygen levels remained relatively high, leading to a lower ΔF compared to the control group.

Treatment with 1 $\mu\text{mol/L}$ FCCP increased the cellular respiration rate within a certain time frame (the duration should be determined based on specific experimental conditions and is not fixed), accelerating intracellular oxygen consumption. This resulted in a significant decrease in intracellular oxygen concentration, reflected by a higher ΔF compared to the control group.

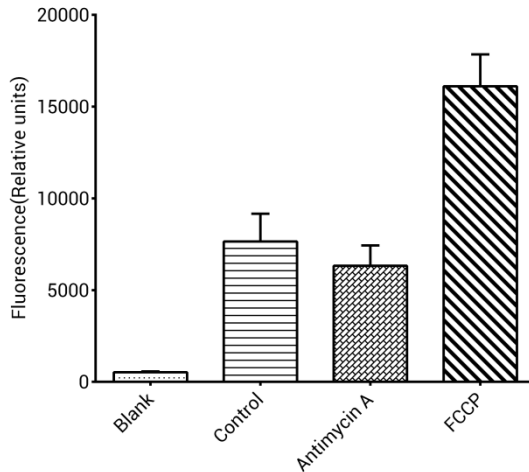


Figure 1. Detection of intracellular oxygen levels in H9c2 cells.

Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.