Technical Manual

# Oxygen Consumption Rate (OCR) Fluorometric Assay Kit

- Catalogue Code: MAES0290
- Size: 96T/48T
- Research Use Only

## Table of contents

# 1. Key Features

### Detection method:

Fluorometric method (Ex/Em =405nm/675nm)

### Specification:

96T/48T

### Storage:

-20°C for 12 months

### Expiry:

See Kit Label

### Experiment Notes:

This kit is for **research use only.**

Instructions should be strictly followed. Changes of operation may result in unreliable results.

Do not use components from different batches of kit.

## 2. Background

Mitochondrial oxidative phosphorylation consumes oxygen to produce ATP, which provides energy for cell growth. Therefore, detection of cellular oxygen consumption is a key indicator of mitochondrial function.

## 3. Intended Use

This kit can measure oxygen consumption rate (OCR) of cell samples.

## 4. Detection Principle

The kit provides a fluorescent probe, which is sensitive to oxygen. The fluorescence of the probe increases with the decrease of oxygen in a closed environment, and the oxygen consumption rate of cells is judged by detecting the change of the fluorescence value.

## 5. Kit Components & Storage

| Item | Component | 48T | 96T | Storage |
|---|---|---|---|---|
| **Reagent 1** | **Probe** | 1.5 mL x 1 vial | 1.5 mL x 2 vials | -20°C, 12 months (shading light) |
| **Reagent 2** | **Sealing Solution** | 4 mL x 1 vial | 8 mL x 1 vial | -20°C, 12 months (shading light) |
| | **Black Clear-bottom Culture Plate** | 96 wells | | No requirement |

*Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other. For a small volume of reagents, please centrifuge before use.*

### Materials required but not supplied

**Instruments:**

- Fluorescence microplate reader (with temperature control function, Ex/Em=405nm/675nm)
- 37°C Incubator with $CO_2$ control.
- Centrifuge

**Reagents**

- Culture medium

## 6. Assay Notes:

1. Pre-warm the reagents to 37℃ in incubator, and set the fluorescence microplate reader temperature at 37℃ before detection.
2. Follow the operation steps to detect in time to avoid missing the best detection time.
3. During the testing process, it is recommended to maintain a stable testing environment and avoid shaking the culture plate.
4. The OCR value is related to the number of cells per well. When the fluorescence value per unit time does not change significantly, the number of cells can be adjusted.
5. Dilute drugs with culture medium.

## 7. Reagent Preparation:

1. Equilibrate all the reagents to 25°C before use. The probe can be aliquoted storage at-20°C, and avoid repeated freeze/thaw cycles is advised.
2. Preparation of working solution: Before testing, please prepare sufficient working solution according to the test wells. For example, prepare 1000μL of working solution (mix well 800μL of culture medium which is used to culture samples and 200μL of probe).

## 8. Assay Protocol

### Adherent cells

| | Blank well | Control well | Sample well |
|---|---|---|---|
| **Cell** | √ | √ | √ |
| **Working solution (μL)** | -- | 100 | 100 |
| **Culture medium (μL)** | 100 | -- | -- |
| **Drug (μL)** | -- | -- | 10 |
| **Drugs solvent (μL)** | 10 | 10 | -- |
| **Sealing solution** | 2 drops | 2 drops | 2 drops |

1. Set up blank well, control well and sample well in black clear-bottom culture plate, the cell density is 5×10^5 /mL. Add 100 μL cell suspension to each well (the cell density is 5×10^4 /well).
2. Culture overnight in a 5% CO2incubator at 37°C.
3. After culture, remove culture medium carefully and avoid cell falls off.
4. Add 100 μL of culture medium into blank wells. Add 100 μL of working solution into control wells and sample wells.
5. Incubate the culture plate for 30 min in fluorescence microplate reader (37℃).
6. Add 10 μL of drugs solvent into blank wells and control wells. Add 10 μL of drug into sample wells. Immediately add 2 drops (about 50 μL) of sealing solution to each well.

7. Stand the culture plate for 5 min in fluorescence microplate reader (37℃), and after this time consider the test started (t0). Measure the kinetics using the microplate reader (recommended filter settings: Ex/Em = 405/675 nm, 5 min interval for 90 min, Bottom reading).

### Suspension cells

| | Blank well | Control well | Sample well |
|---|---|---|---|
| **Cell culture medium suspension (μL)** | 100 | -- | -- |
| **Cell working solution suspension (μL)** | -- | 100 | 100 |
| **Drug (μL)** | -- | -- | 10 |
| **Drugs solvent (μL)** | 10 | 10 | -- |
| **Sealing solution** | 2 drops | 2 drops | 2 drops |

1. Use culture medium and working solution for suspension cells respectively, the recommended cell density is 5×10^ 6 /mL. Set up blank well, control well and sample well in black clear-bottom culture plate. Add 100 μL cell culture medium suspension to blank well (the cell density is 5×10^ 5 /well). Add 100 μL cell working solution suspension to control well and sample well (the cell density is 5×10^ 5 /well).
2. Incubate the culture plate for 30 min in fluorescence microplate reader (37℃).
3. Add 10 μL of drugs solvent into blank wells and control wells. Add 10 μL of drug into sample wells. Immediately add 2 drops (about 50 μL) of sealing solution to each well.
4. Stand the culture plate for 5 min in fluorescence microplate reader (37℃), and after this time consider the test started (t0). Measure the kinetics using the microplate reader (recommended filter settings: Ex/Em = 405/675 nm, 5min interval for 90 min, Bottom reading).

## 9. Calculations

The curve was drawn according to the fluorescence value (F) and time (min), the linear part was selected, the OCR of the cells was compared according to the slope of the linear part.

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