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

Catalog No：E-BC-F070

Specification：48T/96T

Measuring instrument：Fluorescence Microplate Reader

(Ex/Em = 405nm/650nm)

Elabscience® Enhanced Oxygen Consumption Rate (OCR)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
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

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 oxygen consumption rate (OCR) of cell samples.

Detection principle

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. 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.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Probe	0.55 mL × 1 vial	1.1 mL × 1 vial	2-8°C, 12 months shading light
Reagent 2	Sealing Solution	8 mL × 1 vial	8 mL × 2 vials	2-8°C, 12 months shading light
	Black Clear-bottom Culture Plate	96 wells × 2		No requirement
	Plate Sealer	4 pieces		
	Sample Layout Sheet	2 pieces		

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, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

Fluorescence microplate reader (With temperature control function, Ex/Em=405 nm/650 nm)

Reagents:

Complete medium, PBS (0.01M, pH7.4)

Reagent preparation

① Equilibrate all the reagents to 25°C before use. The probe is a suspension. It can be shaken well and aliquoted storage at 2-8°C protected from light.

② The preparation of working solution:
The probe must be shaken well before use. Dilute the probe 10 times with complete medium to prepare working solution. The working solution should be used up within 8 h.

The key points of the assay

① Pre-warm the reagents to 37°C in incubator, and set the fluorecence microplate reader temperature at 37°C 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 Dilute the drug with complete medium or PBS(0.01M, pH7.4).

⑤ The probe and the working solution are prone to sedimentation. They must be thoroughly mixed before use.

Operating steps

Pre-warm the reagents to 37°C in incubator, and set the fluorecence microplate reader temperature at 37°C before detection.

Adherent cells:

	Blank well	Control well	Sample well
Cell	--	√	√
Working solution (μL)	100	100	100
Drug (μL)	--	--	10
Drugs solvent (μL)	10	10	--
Sealing solution	2 drops	2 drops	2 drops
Measure the kinetics using the fluorescence microplate reader at 37°C bottom reading (recommended filter settings: Ex/Em: 405 nm/650 nm, 2 min interval for more than 90 min).			

① Set up blank well, control well and sample well in black clear-bottom culture plate, the cell density is 5×10^5 /mL (**The optimal cell density required for the experiment needs to be determined through pre-experiment exploration**). Add 100 μL cell suspension to control well and sample well (the cell density is 5×10^4 /well).

② Culture overnight in a 5% CO_2 incubator at 37°C.

③ After culture, remove culture medium carefully and avoid cell falls off.

④ Add 100 μL of working solution (**mix well before use**) into each well.

⑤ Incubate the culture plate for 30 min in fluorescence microplate reader (37°C) or incubator (37°C, without CO_2).

⑥ Add 10 μL of drug into sample wells. Add 10 μL of drugs solvent into blank wells and control wells. Immediately add 2 drops (about 80 μL) of sealing solution to each well (If the drug action time is longer than 1 h, it is recommended to perform drug treatment on the cells before inoculation).

⑦ Measure the kinetics using the fluorescence microplate reader at 37°C bottom reading (recommended filter settings: Ex/Em: 405 nm/650 nm,

2 min interval for more than 90 min).

Suspension cells:

	Blank well	Control well	Sample well
Working solution (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
Measure the kinetics using the fluorescence microplate reader at 37°C bottom reading (recommended filter settings: Ex/Em: 405 nm/650 nm, 2 min interval for more than 90 min).			

① Resuspend the cells using the working solution (mix well before use), the recommended cell density is 5×10^5 /mL (**The optimal cell density required for the experiment needs to be determined through pre-experiment exploration**). Set up blank well, control well and sample well in black clear-bottom culture plate. Add 100 μL of working solution to blank well. Add 100 μL cell working solution suspension to control well and sample well (the cell density is 5×10^4 /well).

② Incubate the culture plate for 30 min in fluorescence microplate reader (37°C) or incubator (37°C, without CO_2).

③ Add 10 μL of drug into sample wells. Add 10 μL of drugs solvent into blank wells and control wells. Immediately add 2 drops (about 80 μL) of sealing solution to each well (If the drug pretreatment time is longer than 1 h, it is recommended to perform drug treatment on the cells before inoculation).

④ Measure the kinetics using the fluorescence microplate reader at 37°C bottom reading (recommended filter settings: Ex/Em:405 nm/650 nm, 2 min interval for more than 90 min).

Calculation

Cell sample:

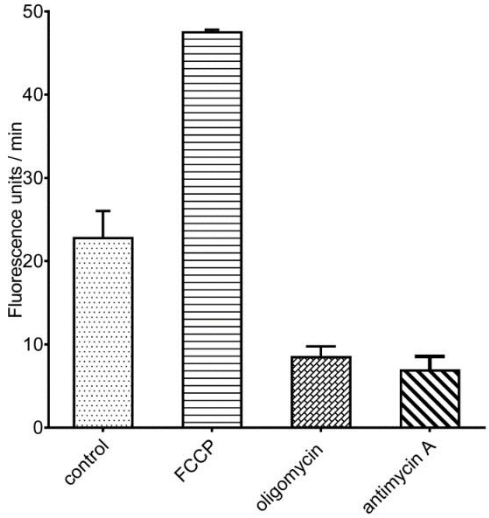
$$\text{OCR (Fluorescence units/min)} = \frac{F_2 - F_1}{\Delta T}$$

Note:

The curve was drawn according to the fluorescence value (F) and time (min). Select the time period $T_1 \sim T_2$ when the fluorescence value is linear with time to calculate the OCR. The fluorescence value detected at T_1 is F_1, the fluorescence value detected at T_2 is F_2. ΔT is the time of fluorescence value change from $T_2 - T_1$, min.

Appendix I Performance Characteristics

Oxygen consumption rates of A549 cells treated with FCCP, oligomycin, and antimycin A:



A549 cells were seeded at a density of 3×10^5/mL and cultured overnight. The concentration of FCCP is 2 μmol/L, the concentration of oligomycin is 1 μmol/L, and the concentration of antimycin A is 1 μmol/L.

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.