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

Catalog No：E-BC-F068

Specification：48T/96T

Measuring instrument：Fluorescence Microplate reader

(Ex/Em = 405nm/675nm)

Elabscience® 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
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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	1.5 mL × 1 vial	1.5 mL × 2 vials	-20°C, 12 months shading light
Reagent 2	Sealing Solution	8 mL × 1 vial	8 mL × 2 vial	-20°C, 12 months shading light
	Black Clear-bottom Culture Plate	96 wells × 2		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, 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/675 nm), Incubator(37℃， without CO_2)

Reagents:

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

Reagent preparation

① Equilibrate all the reagents to 25℃ before use. The probe can be aliquoted storage at -20°C, and avoid repeated freeze/thaw cycles is advised.

② The preparation of working solution:

Dilute the probe 5-15 times with culture medium to prepare working solution. It is recommended that the dilution of the initial probe be 5 times, which can be adjusted according to the pre-experiment.

Note: The diluent is the complete medium corresponding to the cell sample.

The key points of the assay

① Pre-warm the reagents to 37℃ in incubator, and set the fluorecence 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 When the fluorescence value per unit time did not change significantly, try to adjust the number of cells or increase the volume of working solution (not more than 200 μL).

⑤ Dilute the drug with complete medium or PBS(0.01M, pH7.4)

Operating steps

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

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% CO_2 incubator 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℃) or incubator (37°C, without CO_2).

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 Measure the kinetics using the fluorescence microplate reader at 37℃ bottom reading (recommended filter settings: Ex/Em:405 nm/675 nm, 2 min interval for more than 90 min).

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 of culture medium and working solution 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℃) or incubator (37°C, without CO_2).

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 Measure the kinetics using the fluorescence microplate reader at 37℃ bottom reading (recommended filter settings: Ex/Em:405 nm/675 nm, 2 min interval for more than 90 min).

Calculation

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.

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.