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

Catalog No：E-BC-F078

Specification：48T/96T

Measuring instrument：Fluorescence Microplate Reader

(Ex/Em=405 nm/675 nm)

Elabscience® Mitochondrial Stress 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
Tel: 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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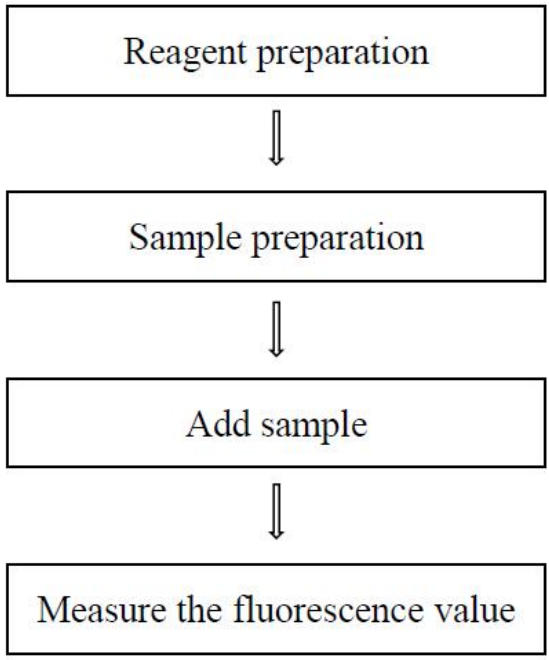
Assay summary



Intended use

This kit can be used to measure the oxygen consumption rate (OCR) of living cells to reflect mitochondrial stress.

Detection principle

Mitochondrial stress refers to the stress state that occurs when mitochondria are damaged or overloaded under the influence of external or internal factors (such as oxidative stress, nutritional deficiency, toxins, etc.). This kind of stress can affect the energy production of mitochondria, leading to insufficient cellular energy and possibly triggering cell damage, inflammatory responses or promoting cell apoptosis. Long-term mitochondrial stress is closely related to various diseases (such as neurodegenerative diseases, cardiovascular diseases, metabolic diseases, etc.) and the aging process.

This kit provides an oxygen-sensitive fluorescent probe, which increases the fluorescence of the probe by the cells consuming oxygen. After adding the regulators oligomycin, carboxycyanide-4-trifluoromethoxylhydrazone (FCCP) and antimycin A, the effect of the metabolic process on maximum respiration can be visually presented.

The addition of oligomycin blocked ATP synthesis, slowed the electron transfer rate, reduced the respiratory rate and decreased oxygen consumption. FCCP is used to study the respiratory activity of mitochondria in the uncoupled state. After the uncoupled oxidative phosphorylation of FCCP, the proton pump of the electron transport chain (ETC) is no longer limited by ATP synthesis, the electron transport rate increases, and the oxygen consumption significantly increases, which is used to determine the maximum respiratory capacity. Antimycin A inhibits the main respiratory chain, and the electron transfer dependent on complex III stops, resulting in a significant decrease in basal oxygen consumption, which is used to measure the reserve respiratory rate.

Kit components & storage

Item	Component	Size1 (48 T)	Size2 (96 T)	Storage
Reagent 1	Probe	1.5 mL × 2 vials	1.5 mL × 2 vials	-20℃, 12 months, shading light
Reagent 2	Sealing Solution	8 mL × 1 vial	8 mL × 2 vial	-20℃, 12 months, shading light
Reagent 3	300 μmol/L Oligomycin	0.2 mL × 1 vial	0.2 mL × 1 vial	-20℃, 12 months, shading light
Reagent 4	500 μmol/L FCCP	0.2 mL × 1 vial	0.2 mL × 1 vial	-20℃, 12 months, shading light
Reagent 5	200 μmol/L Antimycin A	0.2 mL × 1 vial	0.2 mL × 1 vial	-20℃, 12 months, shading light
Reagent 6	100U/mL GOD	0.2 mL × 1 vial	0.2 mL × 1 vial	-20℃, 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 a temperature control function;

Ex/Em= 405 nm/675 nm).

Reagents:

Complete medium

Reagent preparation

① Equilibrate all the reagents to 25℃ before use. Aliquot probe storage at -20°C protected from light, and avoid repeated freeze/thaw cycles is advised.

② The preparation of probe working solution:
Dilute the probe 5-15 times with complete medium. The probe working solution should be used up within 8 h.

③ The preparation of oligomycin working solution:
Dilute the 300 μmol/L oligomycin to 20-60 μmol/L with complete medium. The oligomycin working solution should be used up within 8 h.

④ The preparation of FCCP working solution:
Dilute the 500 μmol/L FCCP to 20-40 μmol/L with complete medium. The FCCP working solution should be used up within 8 h.

⑤ The preparation of antimycin A working solution:
Dilute the 200 μmol/L antimycin A to 10-20 μmol/L with complete medium. The antimycin A working solution should be used up within 8 h.

⑥ The preparation of GOD working solution:
Dilute the 100U/mL GOD to 5 U/mL with double distilled water. The GOD working solution should be used up within 8 h. The GOD working solution is used as a positive control, it consumes oxygen during the process of converting glucose, it is necessary to determine whether additional glucose is needed based on the components of the culture medium.

The key points of the assay

① Preheate the reagents to 37°C 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).

Operating steps

The fluorescence microplate reader needs to be set at 37°C in advance to avoid mixing during the detection process.

Adherent cells:

① Set up blank well, control well, oligomycin well, FCCP well, antimycin A well, GOD well. It is recommended to do three duplicate wells for each well. For all wells except the GOD well, add 100 μL of cell suspension for inoculation (the cell concentration is approximately 5×10^5 cells/mL, about 5×10^ 4 cells/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 to blank wells, add 100 μL of probe working solution to another wells.

⑤ Incubate the culture plate for 30 min in the microplate reader (37°C).

⑥ Blank well/control well: Add 10 μL of complete medium to the wells.
Oligomycin well: Add 10 μL of oligomycin working solution to the wells.
FCCP well: Add 10 μL of FCCP working solution to the wells.
Antimycin A well: Add 10 μL of antimycin A working solution to the wells.
GOD well: Add 10 μL of GOD working solution to the wells.

⑦ Add 2 drops (about 80 μL) of sealing solution to each well immediately.

⑧ 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

① Resuspend cells with culture medium or probe working solution, the cell concentration is approximately 5×10^6 cells/mL. Set up blank well, control well, oligomycin well, FCCP well, antimycin A well, GOD well. It is recommended to do three duplicate wells for each well.

② Blank well: Add 100 μL of cell culture suspension for inoculation to the wells (about 5×10^5 cells/well).

Control well/Oligomycin well/FCCP well/antimycin A well: Add 100 μL of cell working suspension to the wells (about 5×10^5 cells/well).

GOD well: Add 100 μL of probe working solution to the wells.

③ Incubate the culture plate for 30 min in the microplate reader (37°C).

④ Blank well/control well: Add 10 μL of complete medium to the wells.

Oligomycin well: Add 10 μL of oligomycin working solution to the wells.

FCCP well: Add 10 μL of FCCP working solution to the wells.

Antimycin A well: Add 10 μL of antimycin A working solution to the wells.

GOD well: Add 10 μL of GOD working solution to the wells.

⑤ Add 2 drops (about 80 μL) of sealing solution to each well immediately.

⑥ Measure the kinetics using the fluorescence microplate reader at 37°C bottom reading (recommended filter settings: Ex/Em: 405 nm/675 nm, 2 min interval for more than 90 min).

Calculation

The sample:

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

[Note]:

The curve was drawn according to the fluorescence value (F) and time (min), and the time period T_1-T_2 when the fluorescence value was linear with time was selected to calculate the OCR. The fluorescence value of each well measured at T_1 is F_1, and that of each well measured at T_2 is F_2. ΔT is the fluorescence value change time

T_2-T_1, min.

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.