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

Catalog No：E-BC-F069

Specification：48T/96T

Measuring instrument：Fluorescence Microplate reader

(Ex/Em = 490nm/535nm)

Elabscience® Extracellular Acidification Rate (ECAR) 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
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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 extracellular acidification rate (ECAR) of cell samples and analyzing the degree of glycolysis.

Detection principle

During cellular metabolism, energy generation requires either aerobic or anaerobic respiration. When cells undergo anaerobic respiration, a large number of acidic substances are produced, which are pumped by the cell membrane into the extracellular environment, leading to the acidification of the extracellular environment. Therefore, ECAR is an important indicator to evaluate the acidic substances produced by anaerobic respiration during cell metabolism, and its change can reflect the degree of cell metabolism.

The kit uses a specific probe (Ex/Em: 490 nm/535 nm) that changes the fluorescence value when the pH of the extracellular environment changes. The acidified extracellular environment will reduce the fluorescence value of the probe to detect the extracellular acidification rate.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Saline solution	30 mL × 1 vial	60 mL × 1 vial	-20°C, 12 months
Reagent 2	Probe	Liquid × 1 vial	Liquid × 2 vials	-20°C, 12 months shading light
	Black Clear-bottom Culture Plate	2 pieces		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 (Ex/Em=490 nm/535 nm), Incubator (37℃)

Reagents:

DMSO

Reagent preparation

① Equilibrate all the reagents to 25℃ before use.

② The preparation of high concentration probe stock solution:
Dilute one vial of probe with 270 μL of DMSO, mix well to dissolve.
Aliquoted storage at -20℃ for 2 weeks.

③ The preparation of working solution:
Before testing, please prepare sufficient working solution according to the sample wells. The working solution should be prepared on spot.

	1 well	10 wells	50 wells	100 wells
Saline solution (μL)	95	950	4750	9500
High concentration probe stock solution (μL)	5	50	250	500
Working solution（μL）	100	1000	5000	10000

The key points of the assay

① Saline solution and working solution should be preheated to 37℃ in advance, and the fluorescence microplate reader should be set to 37℃ in advance.

② The detection value of ECAR was related to the number of cells per well. When the fluorescence value per unit time did not change significantly, the number of cells could be adjusted.

③ Saline solution contains 20 mM glucose. If it affects the experimental setup, it is recommended to use HBSS buffer or serum-free medium instead of saline solution.

Operating steps

The preparation of sample:

Suspension cells: Cell culture is performed according to the experimental design to ensure that the cells are healthy and do not overgrow. Centrifuge at 500×g for 5 min at 4℃ and collect the cell precipitation. Resuspend 2×10^5 cells with 1 mL of saline solution. Add corresponding drugs to stimulate cells according to the experimental design.

Sample well: Add 100 μL of cell suspension to the well of a black clear-bottom culture plate.

Blank well: Add 100 μL of saline solution to the well of a black clear-bottom culture plate.

Adherent cells: Cell culture is performed according to the experimental design to ensure that the cells are healthy and do not overgrow. Seed the cells into black clear-bottom culture plate, add 100 μL of cell suspension to each well (the recommended cell density is 2×10^5 /mL). Cultured overnight in a 5% CO_2 incubator at 37℃. After culture, remove the culture medium carefully. Add 100 μL of saline solution into each well (the blank well not seeded cells). Add corresponding drugs to stimulate cells according to the experimental design.

① Incubate at 37℃ for 30 min protected from light, and set the temperature of the fluorescence microplate reader to 37°C.

② Add 100 μL of working solution to each well. Measure the kinetics using the fluorescence microplate reader (recommended filter settings: Ex/Em: 490 nm/535 nm, 2 - 3 min interval for 100 -120 min). The curve was drawn according to the fluorescence value (F) and time (min), the linear part was selected to calculate the extracellular acidification rate (ECAR).

Calculation

The sample:

$$ECAR = \frac{\Delta F_{sample} - \Delta F_{blank}}{\Delta T}$$

[Note]

The time period T_1~T_2 where the fluorescence intensity is linear with time is selected to calculate ECAR. The fluorescence intensity of each well measured at T_1 is F_1, and that of each well measured at T_2 is F_2.

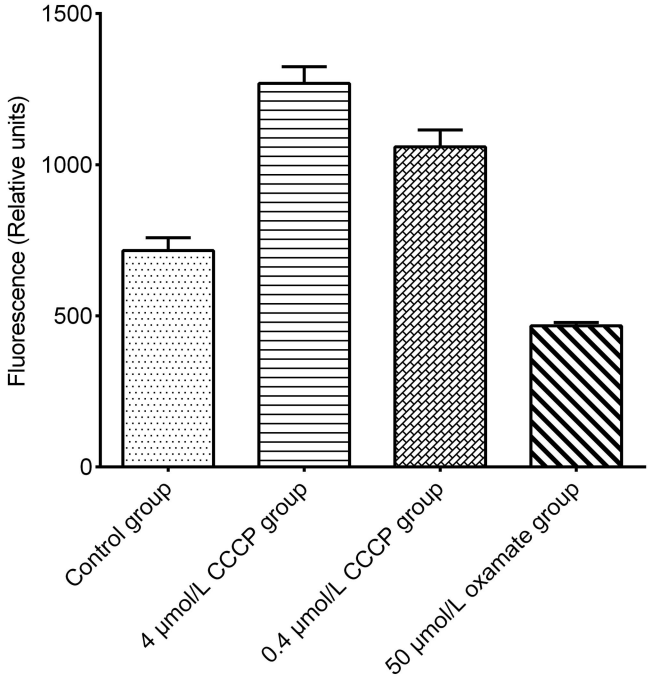
ΔF_{sample}: The absolute fluorescence intensity of sample well, F_1-F_2.

ΔF_{blank}: The absolute fluorescence intensity of blank well, F_1-F_2.

ΔT: The change time of fluorescence intensity T_2-T_1, min.

Appendix I Performance Characteristics

The comparison of ΔF between the drug treatment group (CCCP was a uncoupling drug, oxamate was a inhibit glycolysis drug) and the sample group:



Appendix II Publications

1. Xu W , Geng Q , Jie Y ,et al.A subcellular selective APEX2-based proximity labeling used for identifying mitochondrial G-quadruplex DNA binding proteins[J].Nucleic Acids Research, 2024(1):1.DOI:10.1093/nar/gkae1259.
2. Zhang S , Wang J , Chen Y ,et al.CAFs-derived lactate enhances the cancer stemness through inhibiting the MST1 ubiquitination degradation in OSCC[J].Cell & Bioscience, 2024, 14(1).DOI:10.1186/s13578-024-01329-y.
3. Zhao X , Zhao F , Yan L ,et al.Long non-coding ribonucleic acid SNHG18 induced human granulosa cell apoptosis via disruption of glycolysis in ovarian aging[J].Journal of Ovarian Research, 2024, 17(1).DOI:10.1186/s13048-024-01510-4.
4. Tan J , Tang Y , Li B ,et al.Exosomal lncRNA Mir100hg derived from cancer stem cells enhance glycolysis and promote metastasis of melanoma through miR-16-5p and miR-23a-3p[J].Experimental Cell Research, 443(1)[2025-04-27].

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.