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

Catalog No：E-BC-F084

Specification：48T/96T

Measuring instrument：Fluorescence Microplate Reader

(Ex/Em=490 nm/535 nm)

Elabscience® Glycolysis 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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Intended use

This kit can be used to measure glycolysis stress fluorometric ability in cell samples under different conditions.

Detection principle

Glycolysis is an important ATP generation pathway in eukaryotic cells. Glycolytic stress testing can directly measure the extracellular acidification rate (ECAR) to evaluate the changes of glycolytic process in living cells, and can obtain key parameters for a comprehensive assessment of glycolytic pathway, including: glycolysis, glycolytic capacity, glycolytic reserve and non-glycolytic acidification.

This kit uses 2-deoxy-D-glucose (2-DG) and oligomycin to facilitate in-depth analysis of cellular glycolytic flux: 2-DG quantifies nonglycolytic extracellular acidification (ECA) by blocking glycolysis through competitive hexokinase inhibition; Oligomycin inhibits ATP synthetase and therefore prevents aerobic ATP production, forcing the cell to increase glycolytic flux to meet ATP demand. Such compensatory glycolysis levels achievable under the test conditions could reveal glycolytic perturbations that are not apparent under basal conditions and help assess glycolytic capacity with limited ATP demand.

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℃, 12 months
Reagent 2	Probe	0.03 mL × 1 vial	0.03 mL × 2 vials	-20℃, 12 months, shading light
Reagent 3	Glucose	0.09 g × 1 vial	0.09 g × 2 vials	-20℃, 12 months
Reagent 4	Oligomycin	0.06 mL × 1 vial	0.06 mL × 2 vials	-20℃, 12 months, shading light
Reagent 5	2-Deoxy Glucose	0.3 mL × 1 vial	0.6 mL × 1 vial	-20℃, 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 (Ex/Em= 490 nm/535 nm), Incubator

Regents:

DMSO

Consumable items:

0.22 μm filter membrane, Syringe

Reagent preparation

① Equilibrate all the reagents to 25°C before use.

② The preparation of glucose stock solution:
Dilute one vial of glucose with 0.5 mL of double distilled water, mix fully. Aliquoted storage at 2-8°C for a month.

③ The preparation of oligomycin stock solution:
Dilute one vial of oligomycin with 2.94 mL of saline solution, mix fully. Aliquoted storage at -20°C for 2 weeks.

④ The preparation of respiratory buffer solution:
Before testing, please prepare sufficient respiratory buffer solution according to the sample wells. For example, prepare 255 μL of respiratory buffer solution (mix well 5 μL of glucose stock solution, and 250 μL of saline solution).The prepared solution was filtered through a 0.22 μm filter membrane. Aliquoted storage at 4°C for 3 weeks.

⑤ The preparation of probe stock solution:
Dilute one vial of probe with 270 μL of DMSO, mix fully. Aliquoted storage at -20°C for 2 weeks.

⑥ The preparation of measuring solution:
Before testing, please prepare sufficient measuring solution according to the sample wells. For example, prepare 100 μL of measuring solution (mix well 5 μL of probe stock solution, and 95 μL of respiratory buffer solution). The measuring solution should be prepared on spot. The amount of measuring solution can be referred to the table below for the detection of the fluorescence microplate reader (the concentration of measuring solution can be adjusted according to the experimental results in the actual experiment, and the volume of probe can be appropriately increased).

	1 well	10 wells	50 wells	100 wells
Respiratory buffer solution (μL)	95	950	4750	9500
Probe stock solution (μL)	5	50	250	500
Measuring solution（μL）	100	1000	5000	10000

⑦ The preparation of glucose-free working solution:

According to the actual requirements of the experiment, before testing, please prepare sufficient glucose-free working solution according to the test wells. For example, prepare 100 μL of glucose-free working solution (mix well 95 μL of saline solution and 5 μL of probe stock solution). The glucose-free working solution should be prepared on spot, and the prepared solution should be used up on the same day.

The key points of the assay

① Cells in the logarithmic growth phase are recommended.

② The microplate reader and reagents added to the system need to be incubated to 37°C in advance.

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 discard the supernatant. The recommended cell density is 2×10^5/mL, such as 2×10^5 cells, resuspension with 1 mL of saline solution.

Design the sample wells, oligomycin control wells, 2-DG control wells and blank wells. Add 100 μL of cell suspension to the black clear-bottom culture plate to each well (add 100 μL of saline solution to the blank wells).

Adherent cells: Cell culture is performed according to the experimental design to ensure that the cells are healthy and do not overgrow. Design the sample wells, oligomycin control wells, 2-DG control wells and blank wells. The cells are seeded into the black clear-bottom culture plate, and the recommended cell density is 2×10^5/mL, add 100 μL of cell suspension to each well. Place the cells in a 37℃ carbon dioxide incubator after cell inoculated. Remove the cell culture medium after overnight incubation, add 100 μL of saline solution to each well (blank well was the well without cells inoculated).

① Place the microplate in a CO_2 -free culture environment, incubate at 37°C for 30 min protected from light.

② The temperature of the fluorescent microplate reader was set to 37°C.

③ Oligomycin control wells: add 10 μL of oligomycin stock solution to the corresponding wells.

2-DG control wells: add 10 μL of 2-Deoxyglucose to the corresponding wells.

④ Sample wells: add 100 μL of measuring solution to the corresponding wells.

Blank wells: add 100 μL of measuring solution to the corresponding wells.

Oligomycin control wells: add 90 μL of measuring solution to the corresponding wells.

2-DG control wells: add 90 μL of glucose-free working solution to the corresponding wells.

⑤ Set the excitation wavelength of 490 nm and emission wavelength of 535 nm.

Detected by fluorescent microplate reader every 2-5 min, and the total time was about 100-120 min. The curve of fluorescence value change with time was drawn, and the linear segment was selected to calculate the extracellular acidification rate (ECAR).

Calculation

The sample:

The cell sample:

$$\text{ECAR (Flourescence units/min)} = \frac{\Delta F_{sample} - \Delta F_{blank}}{\Delta T}$$

[Note]:

ECAR was calculated from time periods T_1 to T_2 when the fluorescence value was linear with time. The fluorescence value of each well examined at T_1 was F_1, and the fluorescence value of each well examined at T_2 was F_2.

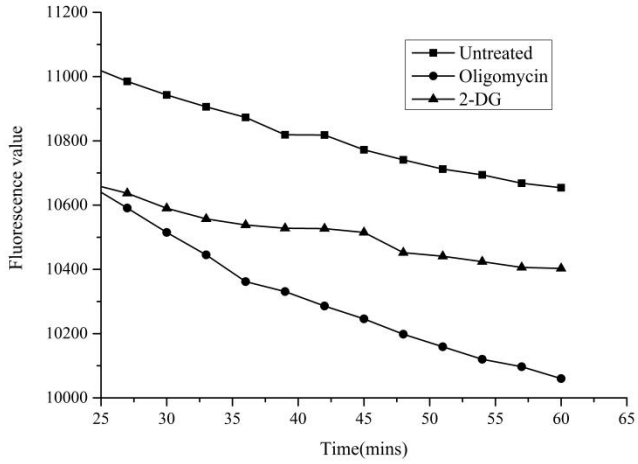
ΔF_{sample}：The change of the fluorescence values，F_1-F_2

ΔF_{blank}：The change of the fluorescence values of blank wells，F_1-F_2

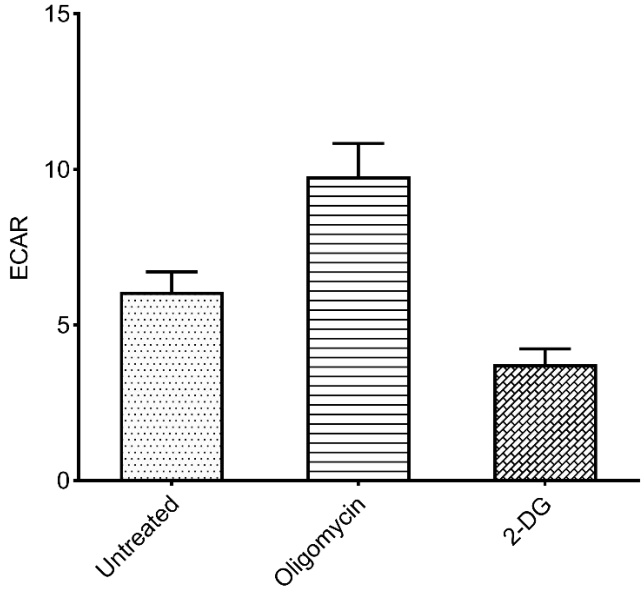
ΔT：The change time of fluorescence value, T_2-T_1，min

Appendix I Key data

1. Trend plots of fluorescence values over time in untreated group, Oligomycin treated and 2-DG treated group



2. ECAR in untreated group, Oligomycin treated and 2-DG treated group



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.