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

Catalog No：E-BC-F067

Specification：96T

Measuring instrument：Fluorescence Microplate reader, Fluorescence microscope, Flow Cytometry

Elabscience® Fatty Acid Uptake 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.

Table of contents

Intended use

This kit can be used to measure fatty acid uptake ability in cell samples.

Detection principle

Fatty acid is an important source of energy for the body. Fatty acid intake capacity has been associated with diseases such as obesity and diabetes, and is therefore an important target for the treatment of diseases such as obesity, II diabetes, and fatty liver. Hyperactive cancer cells have a strong fatty acid uptake capacity, so the fatty acid uptake capacity is also used as an important indicator of cancer drug development, and the fatty acid pathway is also attracted attention as one of the targets of cancer drug development.

This kit provides a convenient fluorescence assay to measure the cell's ability to take up fatty acids. Fatty acid substrate can enter the cell through the fatty acid transporter on the cell membrane, and the ability of cells to take up fatty acid can be detected by fluorescence microscopy, fluorescence microplate reader, flow cytometry and other instruments to detect the fluorescence intensity.

Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Buffer	50 mL × 2 vials	-20°C, 12 months
Reagent 2	10 mmol/L Substrate Solution	0.2 mL × 1 vial	-20°C, 12 months, shading light
	Black Microplate	96 wells	No requirement
	Plate Sealer	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, Fluorescence microscope, Flow Cytometry

Reagent preparation

① Equilibrate all the reagents to room temperature before use. The 10 mmol/L substrate solution should be aliquoted storage at -20°C protected from light, and avoid repeated freeze/thaw cycles is advised.

② The preparation of 50 μmol/L substrate working solution:
Before testing, please prepare sufficient 50 μmol/L substrate working solution according to the test wells. For example, prepare 2000 μL of 50 μmol/L substrate working solution (mix well 1990 μL of buffer and 10 μL of 10 mmol/L substrate solution). The 50 μmol/L substrate working solution should be prepared on spot protected from light and used up within 1 day.

③ Dosage reference for substrate working solution and buffer:

	Adherent cell				**Suspension cells**
Well plate	6-well plate	24-well plate	96-well plate	35 mm petri dish	1.5 mL EP tube
Substrate working solution	1.5 mL/well	0.3 mL/well	0.1 mL/well	1.5 mL/well	0.5 mL/tube
Buffer	1.5 mL/well	0.3 mL/well	0.1 mL/well	1.5 mL/well	0.5 mL/tube

Operating steps

Parameter setting of instrument	
Fluorescence Microplate reader	Ex: 485 nm; Em: 515 nm
Flow Cytometry	Ex: 488 nm , FITC
Fluorescence Microscope	Confocal microscope: Ex: 488 nm; Em: 500-550 nm General fluorescence microscope: FITC or GFP

Suspension cells

① Centrifuge and collect the cells, discard the supernatant medium and resuspend the cells with buffer. Set up the experimental groups, it was suggested to set up a negative cell control group. The number of cells in each group was not less than 5×10^4, centrifuge the cells at 500× g for 3 min and discard the supernatant.

② Resuspend the negative cell control group with buffer, resuspend the fatty acid uptake cell group with substrate working solution.

③ Culture the cells at 37°C in a 5% CO_2 incubator for 30 min (the incubation time could be adjusted according to the number of cells and the degree of uptake).

④ Centrifuge at 500 × g for 3 min, discard the supernatant. Wash the cells of each group twice with buffer.

⑤ Centrifuge at 500 × g for 3 min and discard the supernatant. Resuspend the cells in each group with buffer.

⑥ Fluorescence microplate reader detection: Take 0.1 mL of cell suspension and add to 96-well black microplate. Detect the fluorescence value of each well by setting the excitation wavelength at 485 nm and emission wavelength at 515 nm.

Flow cytometry detection: The number of cells used for detection is at least

1 × 10^4 and set the excitation wavelength at 488 nm for flow cytometry detection.

Fluorescence microscopy detection: Resuspend cells and transfer to the carrier for fluorescence microscopy detection. Confocal microscopy: Ex: 488 nm, Em: 500-550 nm and select GFP or FITC filters for ordinary fluorescence microscopy.

Adherent cell

① Seed the cells into the plate and it was suggested to set up a negative cell control group. The density of cells in each group was not less than 1×10^5 cells /mL and the cells in each group adhered to the wall.

② Discard the culture medium for each group, wash the cells twice with buffer.

③ Add buffer to the negative cell control group, add substrate working solution to the fatty acid uptake cell group. Culture the cells at 37°C, 5% CO_2 incubator for 30 min (the incubation time could be adjusted according to the number of cells and the degree of uptake).

④ Discard the supernatant, wash the cells in each group twice with buffer.

⑤ Fluorescence microplate reader detection: Strip the cells with cell lifter or trypsin, discard the supernatant and resuspend the cells in each group with buffer. Take 0.1 mL of cell suspension and add to 96-well black microplate. Detect the fluorescence value of each well by setting the excitation wavelength at 485 nm and emission wavelength at 515 nm.

Flow cytometry detection: Strip the cells with cell lifter or trypsin, discard the supernatant and resuspend the cells in each group with buffer. The number of cells used for detection is at least 1 × 10^4 and set the excitation wavelength at 488 nm for flow cytometry detection.

Fluorescence microscopy detection: After adding the buffer and detect with

fluorescence microscope or make the slide for observation. Confocal microscopy: Ex: 488 nm, Em: 500-550 nm, and select GFP or FITC filters for ordinary fluorescence microscopy.

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.