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

Catalog No：E-BC-F089

Specification：96T

Measuring instrument：Fluorescence Microplate Reader, Fluorescence Microscope, Flow Cytometry

Elabscience® Cholesterol 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
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 cholesterol uptake ability in cell samples.

Detection principle

Cholesterol is the main steroid compound in mammals and plays an important role in basic cellular life activities. Moderate intake of cholesterol can provide a good guarantee for the oxidation stability and physical stability of liposomes, and has a preventive effect on cardiovascular diseases, but excessive intake of cholesterol has the risk of causing hyperlipidemia, tumor and other diseases. Therefore, it is of great significance to study the ability of cells to take up cholesterol.

This kit can measure cholesterol uptake ability in cell samples by convenient fluorescence method. Cholesterol substrate in this kit can enter cells through the endocytosis mediated by low density lipoprotein receptor on the cell membrane, and the ability of cells to take up cholesterol can be measured by fluorescence microscopy, fluorescence microplate reader, flow cytometry and other instruments to detect fluorescence intensity.

Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Buffer Solution	40 mL ×2 vials	-20℃, 12 months
Reagent 2	Probe	0.48 mL ×1 vial	-20℃, 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

Reagents:

Culture medium

Reagent preparation

Equilibrate all the reagents to 25℃ before use. The probe should be aliquoted storage at -20 ℃ for a month protected from light, and avoid repeated freeze/thaw cycles is advised.

Operating steps

Parameter setting of instrument	
Fluorescence Microplate reader	Ex: 485 nm; Em: 535 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 Preparation of cell suspension, set up the blank tube and sample tube. The number of cells in each tube is at least 1×10^5, and the number of cells in the tube is the same.

② Blank tube: Remove the supernatant and resuspend cells with 0.5 mL of culture medium.

Sample tube: Remove the supernatant and resuspend cells with 0.496 mL of culture medium and 0.004 mL of probe.

③ Incubate at 37℃ for 1 h, centrifuge at 500×g for 5 min and then remove supernatant.

④ Resuspend cells with 0.5 mL of buffer solution in each tube.

⑤ **Fluorescence microplate reader**: Add 0.2 mL of cell suspension to each well of a black microplate. Measure the fluorescence intensity at the excitation wavelength of 485 nm and the emission wavelength of 535 nm with fluorescence microplate reader. The cholesterol uptake capacity of cells was determined by subtracting the fluorescence value of the blank well from the fluorescence value of the sample well.

Flow cytometry: It is recommended to detect 4×10^5 cells, the flow cytometry set the excitation wavelength of 488 nm, or select FITC filter for detection.

Fluorescence microscope: Take 0.01~0.02 mL of cell suspension, drop on the slide, gently cover the cover slide, use a fluorescence microscope to observe

and photograph.

Confocal microscopy: Ex: 488 nm, Em: 500-550 nm;

General fluorescence microscope: GFP or FITC filters.

Adherent cell

① The cells were seeded into plate wells or petri dishes. The number of cells in each well is at least 1×10^5.

② Blank well: Remove the supernatant and add 0.5 mL of culture medium. Incubate the plate for 1 hour in a 5% CO_2 incubator at 37℃.

Sample well: Remove the supernatant and add 0.496 mL of culture medium and 4 μL of probe. Incubate the plate for 1 hour in a 5% CO_2 incubator at 37℃.

③ Remove the supernatant.

④ **Fluorescence microplate reader**: Collect the cells with the pancreatic enzymes, then centrifuge and discard the supernatant. Wash cells with buffer solution for 2 times. Add 0.25 mL buffer solution to resuspend cells. Add 0.2 mL of cell suspension to each well of a black microplate. Measure the fluorescence intensity at the excitation wavelength of 485 nm and the emission wavelength of 535 nm with fluorescence microplate reader. The cholesterol uptake capacity of cells was determined by subtracting the fluorescence value of the blank well from the fluorescence value of the sample well.

Flow cytometry: Collect the cells with the pancreatic enzymes, then centrifuge and discard the supernatant. Wash cells with buffer solution for 2 times. Add buffer solution to resuspend cells. It is recommended to detect 4×10^5 cells, the flow cytometry set the excitation wavelength of 488 nm, or select FITC filter for detection.

Fluorescence microscope: After add the buffer solution, use fluorescence microscope to detect.

Confocal microscopy: Ex: 488 nm, Em: 500-550 nm;

General fluorescence microscope: GFP or FITC filters.

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.