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

Catalog No：E-BC-F003

Specification：96T

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

Elabscience® Lipid Peroxide (LPO) 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
Tell: 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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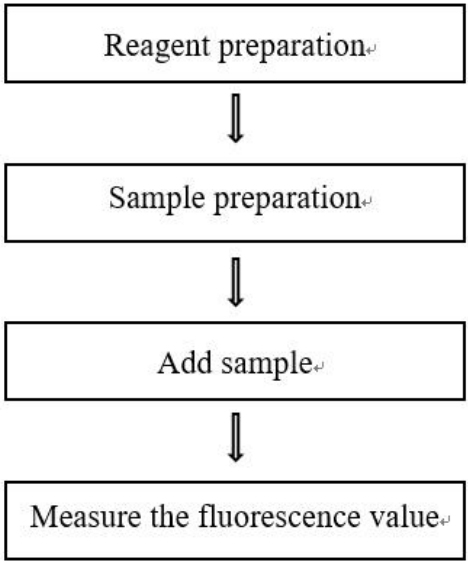
Assay summary



Intended use

This kit can be used to measure lipid peroxide (LPO) in cell samples.

Detection principle

Lipid peroxides (LPO) are substances produced by the action of reactive oxygen species (ROS) on unsaturated fatty acids. The LPO produced by the reaction will continue to oxidize the liposomes, thereby accumulating LPO in a chain reaction. Excess LPO decomposes into highly active aldehydes such as Acrolein, Malondialdehyde (MDA), and 4-hydroxy-2-nonenal (4-NHE), causing cell toxicity and cell death.

This kit provides a fluorescent probe C11-BODIPY 581/591 that can detect LPO production. This fluorescent probe can react with the lipid free radicals in the lipid peroxidation pathway, so as to detect the level of lipid peroxidation in cells. The probe fluoresces red under normal conditions through photoexcitation, and the fluorescence changes from red to green with the process of lipid peroxidation. Through the enhancement of green fluorescence intensity, the production of LPO can be detected with high sensitivity.

Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Buffer	50 mL × 2 vials	-20°C, 12 months
Reagent 2	5 mmol/L Probe	0.05 mL × 1 vial	-20°C, 12 months shading light
Reagent 3	100 mmol/L Positive Control	0.1 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:

Test tubes, Vortex Mixer, Centrifuge, Fluorescence Microplate reader, Fluorescence Microplate reader, Fluorescence Microscope, Flow Cytometry

Reagent preparation

① Equilibrate all the reagents to room temperature before use. Aliquot 5 mmol/L probe and storage at -20°C, and avoid repeated freeze/thaw cycles is advised.

② The preparation of buffer working solution:
Mix the buffer and double distilled water at the ratio of 1:9 thoroughly. (buffer working solution can be replaced by serum-free medium).

③ The preparation of probe working solution:
Dilute 5 mmol/L probe with buffer working solution to the desired concentration. The recommended concentration is about 2-5 μmol/L, which

can be adjusted according to the experimental results. The working solution should be prepared on spot and protected from light and it should be used up within 2 h.

④ The preparation of positive control working solution:
Dilute 100 mmol/L positive control with buffer working solution to the desired concentration. The recommended concentration is about 10-200 μmol/L, which can be adjusted according to the experimental results. The working solution should be prepared on spot and protected from light and it should be used up within 2 h.

The key points of the assay

① If using buffer working solution to wash and incubate cells, make sure to prepare a sufficient amount before the experiment.

② 5 mmol/L Probe should avoid repeated freeze/thaw cycles. before use, 5 mmol/L probe should be fully melted and centrifuge until the liquid reaches the bottom of the tube before opening the cover. Prepare the fresh needed amount 5 mmol/L probe working solution before use.

③ The fluorescent substance is easily quenched, and it is best to measure within 2 hours after incubation to prevent the fluorescence from weakening.

Operating steps

Parameter setting of instrument	
Green fluorescence	
Fluorescence Microplate reader	Excitation: 500 nm; Emission: 540 nm
Flow Cytometry	FITC
Fluorescence Microscope	FITC or GFP

① 2×10^5 cells were seeded into the plate wells. The cells can be cultured and treated according to the experimental needs.

② Suspension cells: Transfer the cells into 2 mL EP tubes, centrifuge at 300×g for 5 min, then remove the medium. Wash the cells with buffer working solution for 2-3 times.

Adherent cells: Remove the medium and wash the cells with buffer working solution for 2-3 times.

③ Set up different experimental groups, blank tube (normal cells only), control tube (normal cells only and loaded with probe), experiment tube (cells loaded with probe and treated with drug) and positive control tube (optional, cells loaded with probe and treated with positive control working solution).

④ Add buffer working solution to blank tube, add probe working solution to control, positive control and experiment tube. Usually every 2×10^5 cells add 200 to 500 μL of probe working solution. Incubate the cells at 37°C with shading light for 30-60 min. (The incubation time of this process was related to cell type and fluorescence probe concentration, and the volume of liquid added was consistent in all groups.)

⑤ Wash the cells were with buffer working solution for 2-3 times to remove the probes that did not enter the cells.

⑥ Add buffer working solution to blank tube and control tube. Add appropriate drug to experiment tube. Add positive control working solution to positive

control tube. In 2 mL EP tubes, usually every 2×10^5 cells add 200-500 μL of positive control working solution, and the recommended concentration of positive control working solution is 10-200 μmol/L, and the stimulation time is 30-60 min. (The incubation time of this process was related to cell type, drug concentration and positive stimulation concentration, and the volume of liquid added was consistent in each group).

⑦ Wash the cells were with buffer working solution for 2-3 times to remove the excess drug and positive control working solution.

⑧ Detection: Each tube of suspended cells need to add 100-200 μL buffer a working liquid to re-suspend cells, transfer to the detection carrier and for detection. Adherent cells can be detected directly with a slide.

Note: The experimental procedure can also be followed by drug treatment and positive stimulation and then incubation of the probe. Throughout the experiment, the buffer working solution can be replaced by serum-free culture medium.

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.