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

Catalog No：E-BC-F005

Specification：96T

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

Elabscience® Reactive Oxygen Species (ROS) Fluorometric Assay Kit (Red)

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：

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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 reactive oxygen species (ROS) in live cell samples.

Detection principle

Reactive oxygen species (ROS) refers to the general term of substances that are composed of oxygen in the body or in the natural environment and contain oxygen and are active in nature: there is mainly a kind of excited oxygen molecule, namely singlet oxygen molecule or singlet oxygen molecule. Three oxygen-containing free radicals, namely superoxide anion radicals, hydroxyl radicals, and hydroperoxyradicals, two peroxides, namely hydrogen peroxide and lipid peroxide, and a nitrogen-containing oxide.

Dihydroethidium(DHE) can cross the cell membrane and enter the cell to bind to DNA or RNA, forming hydroethidium to produce red fluorescence. Dihydroethidium is mainly oxidized by superoxide anion type reactive oxygen species in cells.Dihydroethidium itself is blue fluorescence with a maximum excitation wavelength of 370 nm and a maximum emission wavelength of 420 nm. After dehydrogenation, dihydroethidium binds to RNA or DNA to produce red fluorescence with an excitation wavelength of 300 nm or 518 nm and an emission wavelength of 610 nm.

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 DHE	0.15 mL ×1 vial	-20°C, 12 months, shading light
Reagent 3	100 mmol/L ROS Positive Control	0.2 mL ×1 vial	-20°C, 12 months, shading light
	Black Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	
	Sample Layout Sheet	1 piece	

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 & Consumables:

Double distilled water

Reagent preparation

① Equilibrate all reagents to room temperature before use. The 10 mmol/L DHE and the 100 mmol/L ROS Positive Control were centrifuged at 300×g for 2 min before use. The 10 mmol/L DHE should be aliquoted storage at -20°C, and avoid repeated freeze/thaw cycles is advised.

② The preparation of Buffer Working Solution：

Before testing, please prepare sufficient Buffer Working Solution according to the test wells. For example, prepare 10 mL of Buffer Working Solution (mix well 1 mL of Buffer and 9 mL of double distilled water). The Buffer Working Solution can be replaced with serum-free medium.

③ The preparation of DHE Solution：

Dilute the 10 mmol/L DHE with the Buffer Working Solution. The recommended working concentration is 1–10 μmol/L, with 5 μmol/L being preferred; the concentration can be adjusted based on experimental results. The DHE Solution should be prepared fresh and used within 2 hours.

④ The preparation of ROS Positive Control：

Dilute the 100 mmol/L ROS Positive Control with the Buffer Working Solution. The recommended working concentration is 10–200 μmol/L, with 5 μmol/L being preferred; the concentration can be adjusted based on experimental results. The ROS Positive Control should be prepared fresh and used within 2 hours.

The key points of the assay

① If use Buffer Working Solution washing and incubating cells, please prepare sufficient it before testing.

② Avoid the DHE repeated freezing and thawing. Before use, it is necessary to fully melt, centrifuge until the liquid reaches the bottom of the tube and then open the cap. The DHE Solution should be prepared on spot.

③ The fluorescent product is easy to quench, and it is best to measure within 2 h after incubation to prevent fluorescence weakening.

Operating steps

Parameter setting of instrument	
Fluorescence Microplate reader	Excitation: 300 or 518 nm; Emission: 610 nm
Flow Cytometry	PE
Fluorescence Microscope	Cy3 Filter, TexasRed or RFP

① According to the experimental design and grouping requirements, perform cell culture.

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 Select an appropriate volume of DHE Solution according to the cell culture vessel. Usually every 5×10^4 cells add 200 to 500 μL of DHE Solution in 2 mL EP tube, the recommended DHE Solution concentration is 1-10 μmol/L. Incubate the cells at 37°C protected from 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).

④ Wash the cells were with Buffer Working Solution for 2-3 times to remove the probes that did not enter the cells.

⑤ Positive stimulation is applied to the positive control group (optional): the positive control group is treated with the ROS Positive Control. An appropriate volume of the ROS Positive Control should be selected based on the cell culture vessel. Typically, 200–500 μL of the ROS Positive Control is added per 5×10^4 cells. The recommended concentration of the ROS Positive Control is 10–200 μmol/L, and the

stimulation time is 30–90 minutes.

⑥ Wash the cells were with Buffer Working Solution for 2-3 times to remove the excess drug and ROS Positive Control. Each tube of suspended cells need to add 100-200 μL Buffer Working Solution 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 DHE. 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.