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

Catalog No：E-BC-F062

Specification：48T/96T

Measuring instrument：Flow cytometer, Fluorescence microscope

Elabscience® Singlet Oxygen($^1O_2$) 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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Intended use

This kit can be used to measure Singlet Oxygen ($^1O_2$) of cell samples

Detection principle

Singlet oxygen ($^1O_2$) is a type of reactive oxygen species (ROS) generated through cellular metabolism, redox reactions, photosensitization, and other pathways. It can damage biological cellular components such as lipids, proteins, and nucleic acids, making it a subject of widespread attention across multiple fields. Although it is a potent oxidizing agent, intracellular antioxidant enzymes and antioxidants can inactivate it through corresponding mechanisms. Therefore, singlet oxygen and other ROS naturally generated in normal cells do not cause significant damage to organelles.

This assay kit detects intracellular $^1O_2$ via fluorescence. The probe crosses the cell membrane into the cell, where it binds to $^1O_2$ and emits fluorescence. Detection is performed using instruments such as flow cytometers and fluorescence microscopes.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Buffer	50 mL × 1 vial	50 mL × 2 vials	-20°C, 12months,
Reagent 2	Probe	Powder × 1 vial	Powder × 2 vials	-20°C, 12 months, shading light

Note: All the reagents should be stored according to the table. The reagents from different kits can not be mixed or used interchangeably. For liquid reagents with small volumes or powders, centrifuge them before use to prevent loss.

Instruments

Flow cytometer (FITC), Fluorescence microscope(Ex/Em=504/525nm), Cell Incubator (37°C, 5%CO_2)

Materials required but not provided

Methanol

Reagent preparation

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

② The Preparation of Probe Storage Solution：

Before testing, prepare a sufficient amount of Probe Storage Solution according to the test wells. For example, prepare 75 μL of a Probe Storage Solution (mix 75 μL of methanol and 1 vial of Probe thoroughly). Probe Storage Solution should be freshly prepared before use. Stable for 7 days when stored at -20°C protected from light.(The Probe Storage Solution is highly volatile and must be aliquoted, sealed with sealing film, and stored at -20°C protected from light.)

③ The preparation of Working solution：

The Working solution must be shaken well before use. Dilute the Probe Storage Solution 200 times with buffer to prepare working solution. The working solution should be freshly prepared before use. Stable for 4 h when stored at -20°C protected from light.

Operating steps

Detection Instrument Parameter Settings	
Flow cytometer	FITC
Fluorescence microscope	E_x/E_m=504/525 nm

Flow Cytometry Analysis

① Suspended cells: Centrifuge to collect cells, discard the supernatant. Digest adherent cells to prepare them as suspended cells for subsequent procedures. Wash cells twice with Buffer, centrifuge and discard the supernatant. Resuspend cells in Buffer. Set up experimental groups, including blank tubes and sample tubes. Maintain a cell density of no less than 2 × 10^5 cells/mL.

② Blank tube: Add 300 μL of Buffer.
Sample tube: Add 300 μL of Working solution

③ After incubation at 37 °C with 5% CO_2 for 15–30 minutes, a minimum of 1 × 10^4 cells per tube were collected and analyzed by flow cytometry using the FITC channel.

Fluorescence Microscopy Detection (Adherent Cells)

① Using a 24-well plate as an example, configure the wells according to experimental requirements (including blank and sample wells).It is recommended that the cell seeding density be no less than 2 × 10^4 cells/mL. After seeding, incubate overnight at 37°C in a 5%CO_2 incubator.

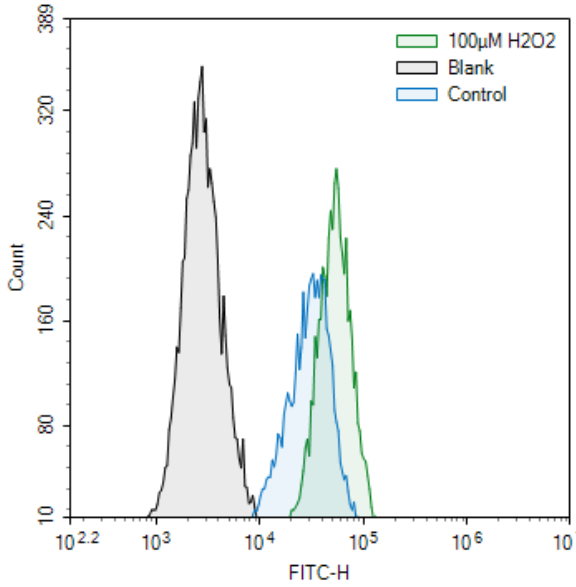
② Remove the medium, wash the cells twice with Buffer, and discard the supernatant.

③ Blank well: Add 300 μL of Buffer.
Sample well: Add 300 μL of Working solution

④ Incubate at 37°C in a 5% CO_2 incubator for 15–30 min (optimal incubation times vary by cell type. Use 15 min as the initial incubation time and optimize based on the cells used to achieve optimal results). Observe staining effects under a fluorescence microscope (Ex/Em=504/525 nm).

Appendix I Example Analysis

Treat HeLa cells with hydrogen peroxide at a final concentration of 100 μmol/L for 30 minutes to induce increased production of singlet oxygen. Proceed with the staining procedure as described, then analyze samples using flow cytometry with the FITC channel. Load 5 × 10^4 HeLa cells per sample. The results are shown in the figure below.:



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.