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

Catalog No：E-BC-F008

Specification：96T

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

Elabscience® Mitochondrial Superoxide 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.

Table of contents

Intended use

This kit can be used to measure superoxide in living cell mitochondrial samples.

Detection principle

The fluorescent probe provided in this kit specifically targets mitochondria in living cells, which are oxidized by mitochondrial superoxides to produce bright red fluorescence. The probe excited at 396 nm can specifically detect superoxide of supermitochondria, and the mitochondrial reactive oxygen species (ROS) can also be detected using the excitation wavelength of 510 nm.

Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Buffer Solution	20 mL ×1 vial	-20 ℃, 12 months, shading light
Reagent 2	Probe	0.08 mL ×1 vial	-20 ℃, 12 months, shading light
	Black Microplate	96 wells	No requirement
	Plate Sealer	4 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, Incubator (37℃)

Reagent preparation

① Equilibrate all reagents to 25℃ before use. The Probe should be storaged at -20 ℃ protected from light, and avoid repeated freeze/thaw cycles is advised.

② The preparation of measuring buffer solution:

Before testing, please prepare sufficient measuring buffer solution according to the test wells. For example, prepare 10 mL of measuring buffer solution (mix well 9 mL of double distilled water and 1 mL of buffer solution). The measuring buffer solution should be prepared on spot. Store at -20 ℃ for a week protected from light.

③ The preparation of working solution:

Dilute probe with measuring buffer solution (the recommended dilution is 200-2000 times). The working solution should be prepared on spot protected from light and used up within 1 day.

④ The amount of working solution and measuring buffer solution in different petri dishes:

Cell type	**Adherent cells**				**Suspension cells**
Well	6-well plate	24-well plate	96-well plate	35 mm petri dish	2 mLEP tube
The volume of working solution or measuring buffer solution	1.5 mL/well	0.3 mL/well	0.1 mL/well	1.5 mL/well	0.3 mL/tube

Operating steps

Detection of culture cell sample

Instrument parameter	
Fluorescence microplate reader	Superoxide: Ex/Em = 396 nm/610 nm ROS: Ex/Em = 510 nm/610 nm
Flow cytometry	Set Violet 610 or PE-Texas Red to detect
Fluorescence microscope	Texas Red、RFP

1. Suspension cell:

① Prepare the cell suspension. Set up control and sample tubes (for 2 mL EP tube), and the number of cells per tube is at least 2×10^5. Remove the medium, and wash the cells with measuring buffer solution for twice.

② Sample tube: Remove supernatant and add 0.2 mL of working solution to resuspension cells.
Control tube: Remove supernatant and add 0.2 mL of measuring buffer solution to resuspension cells.

③ Incubate at 37℃ for 10~60 min protected from light. Please adjust the concentration of working solution and incubation time according to the actual experimental situation.

④ Add 0.1 mL of cell suspension to 96-well plate. It can be detected by fluorescence microplate reader and flow cytometry, or use a fluorescence microscope to observe and photograph.

2. Adherent cell:

① Set up control and sample tubes, each tube cell number for at least 1×10^5, remove medium, wash 2 times with the measuring buffer solution.

② Sample tube: Add working solution.
Control tube: Add measuring buffer solution.

③ Incubate at 37℃ for 10~60 min protected from light. Please adjust the

concentration of working solution and incubation time according to the actual experimental situation.

④ It can be detected by fluorescence microplate reader and flow cytometry, or use a fluorescence microscope to observe and photograph.

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.