



TECHNICAL MANUAL

Mitochondrial ROS Fluorometric Assay Kit

- **SKU CODE:** MAES0299
- **SIZE:** 96 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

1. Intended use

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

2. 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 mitochondria, and mitochondrial reactive oxygen species (ROS) can also be detected using an excitation wavelength of 510 nm.

3. Kit components & storage

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

4. Materials prepared by users

Instruments:

Fluorescence microplate reader, Fluorescence microscope, Flow cytometry, Incubator (37 °C)

5. Reagent preparation

1. Equilibrate all reagents to 25 °C before use. The Probe should be stored at -20 °C protected from light, and repeated freeze/thaw cycles should be avoided.
2. **The preparation of measuring buffer solution:** Before testing, 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 the spot. Store at -20 °C for one 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 the 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 6-well plate: 1.5 mL/well 24-well plate: 0.3 mL/well 96-well plate: 0.1 mL/well 35 mm petri dish: 1.5 mL/well 2 mL EP tube: 0.3 mL/tube

6. 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 twice.
2. Sample tube: Remove supernatant and add 0.2 mL of working solution to resuspend cells.
Control tube: Remove supernatant and add 0.2 mL of measuring buffer solution to resuspend cells.
3. Incubate at 37 °C 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.

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 °C 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.

7. Statement

1. This assay kit is for Research Use Only. Assay Genie assumes no responsibility for any problems or legal liabilities arising from the use of this kit for clinical diagnosis or any other purpose.
2. Please read the instructions carefully and calibrate the instruments before performing the experiments. Follow the instructions strictly throughout the procedure.
3. Appropriate protective measures must be taken, including wearing a lab coat and latex gloves.
4. If the concentration of the substance falls outside the detection range, perform an additional dilution or concentration step on the sample.
5. It is recommended to perform a pre-test if your sample type is not listed in the instruction manual.
6. Experimental results are closely related to reagent quality, operator technique, environmental conditions, and other factors. Assay Genie guarantees the quality of the kits only and is NOT responsible for sample consumption resulting from use of the assay kits. It is advisable to estimate the expected sample usage and reserve sufficient samples before starting the experiment.

Notes:

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.



Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.