TECHNICAL MANUAL

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

- **SKU CODE:** MAES0298
- **SIZE:** 96 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Intended use

This kit can be used to measure reactive oxygen species (ROS) in living cell samples.

2. Detection principle

Reactive oxygen species (ROS) refers to the general term for oxygen-containing substances in the body or natural environment that are active in nature. These include excited oxygen molecules (singlet oxygen), three oxygen-containing free radicals (superoxide anion radicals, hydroxyl radicals, and hydroperoxy radicals), two peroxides (hydrogen peroxide and lipid peroxide), and nitrogen-containing oxides.

Dihydroethidium can cross the cell membrane and enter cells to bind with DNA or RNA, forming hydroethidium which produces red fluorescence. Dihydroethidium is primarily oxidized by superoxide anion-type reactive oxygen species in cells.

Dihydroethidium itself exhibits blue fluorescence with a maximum excitation wavelength of 370 nm and maximum emission wavelength of 420 nm. After oxidation, dihydroethidium binds to RNA or DNA to produce red fluorescence with excitation wavelengths of 300 nm or 518 nm and emission wavelength of 610 nm.

3. 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 Probe	0.15 mL × 1 vial	-20°C, 12 months, protect from light
Reagent 3	100 mmol/L Positive Control	0.2 mL × 1 vial	-20°C, 12 months, protect from light
	Black Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	

4. Materials prepared by users

Instruments: Fluorescence Microplate reader, Fluorescence microscope, Flow Cytometry

5. Reagent preparation

1. Equilibrate all reagents to room temperature before use. Centrifuge the 10 mmol/L probe and 100 mmol/L positive control at 300×g for 2 min before use. The 10 mmol/L probe should be stored in aliquots at -20°C and repeated freeze/thaw cycles should be avoided.
2. **Preparation of buffer working solution:** Before testing, prepare sufficient buffer working solution according to the number of test wells. For example, to prepare 10 mL of buffer working solution, mix 1 mL of buffer with 9 mL of double distilled water and mix well. The buffer working solution can be replaced with serum-free medium.
3. **Preparation of probe working solution:** Dilute the 10 mmol/L probe with buffer working solution. The recommended working concentration is 1-10 μmol/L, which can be adjusted according to experimental requirements. The probe working solution should be prepared fresh and used within 2 hours.
4. **Preparation of positive control solution:** Dilute the 100 mmol/L positive control with buffer working solution. The recommended working concentration is 10-200 μmol/L, which can be adjusted according to experimental requirements. The positive control solution should be prepared fresh and used within 2 hours.

6. The key points of the assay

1. If using buffer working solution for washing and incubating cells, prepare sufficient volume before testing.
2. Avoid repeated freezing and thawing of the probe. Before use, allow to fully thaw, centrifuge until liquid reaches the bottom of the tube, then open the cap. The probe working solution should be prepared fresh.
3. The fluorescent product is easily quenched. It is best to measure within 2 hours after incubation to prevent fluorescence weakening.

7. 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. Seed 2×10^5 cells into plate wells. The cells can be cultured and treated according to experimental needs.

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

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

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

4. Add buffer working solution to blank tube, add probe working solution to control, positive control and experiment tubes. Usually add 200 to 500 µL of probe working solution per 2×10^5 cells in 2 mL EP tube. The recommended probe working concentration is 1-10 µmol/L. Incubate cells at 37°C protected from light for 30-60 min. (The incubation time depends on cell type and fluorescence probe concentration, and the volume of liquid added should be consistent in all groups.)

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

6. Add buffer working solution to blank tube and control tube. Add positive control solution to positive control tube. In 2 mL EP tubes, usually add 200-500 µL of positive control solution per 2×10^5 cells. The recommended positive control solution concentration is 10-200 µmol/L, and stimulation time is 30-90 min. Add appropriate drug to experiment tube. The experimental group should select drug treatment conditions according to experimental needs. (The incubation time depends on cell type, drug concentration and positive stimulation concentration, and the volume of liquid added should be consistent in each group.)

7. Wash cells with buffer working solution 2-3 times to remove excess drug and positive control solution. Each tube of suspended cells needs 100-200 µL buffer working solution added to re-suspend cells, then transfer to detection carrier for detection. Adherent cells can be detected directly with a slide.

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

8. 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.

Assay Genie 100% money-back guarantee!

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