Reactive Oxygen Species (ROS) Assay Kit (DHE)

Introduction

Reactive oxygen species (ROS), including superoxide anion, hydrogen peroxide and hydroxyl radicals, are natural by-products of cellular metabolism of oxygen. Physiological levels of ROS are involved in cell signaling, but excess ROS can exceed the antioxidant capacity of cells, cause DNA, protein and lipid damage, and disrupt the thiol redox balance, leading to apoptosis, necrosis, or abnormal signaling.

This kit can be used to detect superoxide in living cells. The principle is based on the fact that DHE can enter living cells and react with intracellular superoxide to produce ethidium, which binds DNA or RNA and shows red fluorescence. The fluorescent signal is proportional to the ROS level.

Components and Storage

Components \ Size	96 Assays	Storage
10X Assay buffer	2 x 50 mL	-20°C
Probe (10 mM)	0.15 mL	-20°C away from light
Positive control (100 mM)	0.2 mL	-20°C away from light
Shipping: Blue ice	**shelf life: 6 months**	

Protocol

1. Preparation before the experiment

   1) Dilute an appropriate amount of 10X Assay buffer in ddH_2O to make 1X Assay buffer.

   2) Dilute the Probe (10 mM) with a 1X Assay buffer at a recommended working concentration of 1-10 μM. 5 μM can be used as an initial concentration for the first use. Prepare fresh working solution every time, put it on ice and use within 2 h.

   3) Dilute Positive control (100 mM) with 1X Assay buffer at a recommended working concentration of 10-200 μM. 50 μM can be used as the initial concentration for the first use. Prepare fresh working solution every time, put it on ice and use within 2 h.

***Note:** When using for the first time, it is recommended to aliquot the Probe (10 mM) and Positive control (100 mM) to avoid repeated freeze-thaw.

2. Cell assay

1) For adherent cells, seed 1-4 x 10^4 cells per well in a 96-well plate and incubate overnight.

2) Remove the medium, wash cells 2-3 times with 1X Assay buffer.

***Note:** For suspension cells, on the day of assay, centrifuge cells at 300 g for 5 min, then discard the medium and wash cells 2-3 times with 1X Assay buffer.

3) Add 100 μL of Probe (5 μM) to each well, and incubate at 37°C for 0.5-1 h in the dark.

***Note:** Probe working concentration can be optimized in the range of 1-10 μM. Incubation time can also be optimized.

4) Wash cells 2-3 times with a 1X Assay buffer.

5) Refer to the table below to design the experiment. The working concentration of positive control can be optimized in the range of 10-200 μM.

Control group	-
Experimental group	Treat with the test drug for a certain time
Positive group	Treat the cells with positive control (50 μM) for 0.5-1.5 h

***Note:** The cells can also be treated with test drug or positive before loading the probe.

6) Wash cells 2-3 times with a 1X Assay buffer.

7) Add 100 μL of 1X Assay buffer per well. Then monitor fluorescence with a microscope at Ex/Em=518/610 nm, or directly using Cy3 or RFP filters. If using the multimode microplate reader, setting Ex/Em=300/610 nm is better.

***Note:** Detect as soon as possible after adding the 1X Assay buffer.

Notes

1. For your safety and health, please wear lab coats and gloves during the experiment.
2. For research use only. Not to be used in clinical diagnostic or clinical trials.

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