Cell Meter™ Fluorimetric Intracellular Total ROS Activity Assay Kit*Red Fluorescence*

Catalog number: 22901
Unit size: 200 Tests

Component	Storage	Amount
Component A: Amplite™ ROS Red	Freeze (<-15 °C), Minimize light exposure	1 vial
Component B: Assay Buffer	Freeze (<-15 °C)	1 bottle (20 mL)
Component C: DMSO	Freeze (<-15 °C)	1 vial (200 µL)

OVERVIEW

Reactive oxygen species (ROS) are natural byproducts of the normal metabolism of oxygen and play important roles in cell signaling. However, during oxidative stress-related states, ROS levels can increase dramatically. The accumulation of ROS results in significant damage to cell structures. The role of oxidative stress in cardiovascular disease, diabetes, osteoporosis, stroke, inflammatory diseases, a number of neurodegenerative diseases and cancer has been well established. The ROS measurement will help to determine how oxidative stress modulates varied intracellular pathways. Amplite™ Fluorimetric ROS Assay Kit uses our unique ROS sensor to quantify ROS in live cells. Amplite™ ROS Red is cell-permeable. It generates the red fluorescence when it reacts with ROS. The kit is an optimized "mix and read" assay format. The Amplite™ Fluorimetric ROS Assay Kit provides a sensitive, one-step fluorimetric assay to detect intracellular ROS in live cells with 1-2 hours incubation. The assay can be performed in a convenient 96-well or 384-well microtiter-plate format and easily adapted to automation without a separation step. Its signal can be easily read using either a fluorescence microplate reader or a fluorescent microscope. It can be used to either quantify the ROS activities or screen the ROS inhibitors.

AT A GLANCE

Protocol summary

1. Prepare cells in growth medium
2. Add Amplite™ ROS Red working solution (100 µL/well for a 96-well plate or 25 µL/well for a 384-well plate)
3. Incubate the cells at 37°C for 1 hour
4. Treat the cells with test compounds to induce ROS
5. Monitor the fluorescence increase (bottom read mode) at Ex/Em= 520/605 nm (Cutoff = 590 nm) or fluorescence microscope with Ex/Em = 520/605 nm filter set

Important Thaw all the kit components at room temperature before starting the experiment.

KEY PARAMETERS

Instrument:	Fluorescence microplate reader
Excitation:	520 nm
Emission:	605 nm
Cutoff:	590 nm
Recommended plate:	Black wall/clear bottom
Instrument specification(s):	Bottom read mode

Instrument:	Fluorescence microscope
Excitation:	520 nm
Emission:	605 nm
Recommended plate:	Black wall/clear bottom
Instrument specification(s):	Texas Red filter set

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles.

1. Amplite™ ROS Red stock solution (500X):
Add 40 µL of DMSO (Component C) into the vial of Amplite™ ROS Red (Component A) and mix well to make 500X Amplite™ ROS Red stock solution. Protect from light.

Note 20 µL of 500X Amplite™ ROS Red stock solution is enough for 1 plate.

Note Unused portion can be aliquoted and stored at < -20 °C for more than one month if the tubes are sealed tightly and kept from light. Avoid repeated freeze-thaw cycles.

PREPARATION OF WORKING SOLUTION

Add 20 µL of 500X Amplite™ ROS Red stock solution into 10 mL of Assay Buffer (Component B) and mix well to make Amplite™ ROS Red working solution.

Note This Amplite™ ROS Red working solution is stable for at least 2 hours at room temperature.

PREPARATION OF CELL SAMPLES

For guidelines on cell sample preparation, please visit
https://www.aatbio.com/resources/guides/cell-sample-preparation.html

SAMPLE EXPERIMENTAL PROTOCOL

1. Add 100 µL/well (96-well plate) or 25 µL/well (384-well plate) of Amplite™ ROS Red working solution into the cell plate.

2. Incubate the cells in a 5% CO_2, 37°C incubator for one hour.

3. Treat cells with 20 µL of 11X test compounds (96-well plate) or 10 µL of 6X test compounds (384-well plate) in your desired buffer (such as PBS or HHBS). For control wells (untreated cells), add the corresponding amount of compound buffer.

4. To induce ROS, incubate the cell plate at room temperature or in a 5% CO_2, 37°C incubator for at least 15 minutes or a desired period of time (30 minutes for Hela cells treated with 1 mM H_2O_2).

5. Monitor the fluorescence increase with a fluorescence microplate reader (bottom read mode) at Ex/Em = 520/605 nm (Cutoff = 590 nm) or observe cells using a fluorescence microscope with Ex/Em = 520/605 nm filter set (Texas Red filter).

EXAMPLE DATA ANALYSIS AND FIGURES

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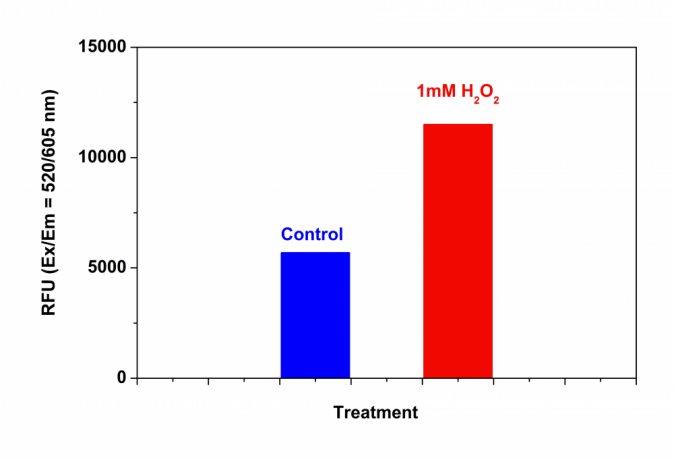

Figure 1. Detection of ROS in Jurkat cells with Cell Meter™ Fluorimetric Intracellular Total ROS Activity Assay Kit. Jurkat cells were seeded on the same day at 300,000 cells/100µL/well in a Costar black wall/clear bottom 96-well plate. The ROS assay loading solution (100 µL/well) was added and incubated in a 5% CO2, 37 °C incubator for 1 hour. The cells were treated with or without 1 mM H_2O_2 for 2 hours. The fluorescence signal was monitored at Ex/Em = 520/605 nm (Cutoff = 590 nm) with bottom read mode using FlexStation (Molecular Devices).

DISCLAIMER