Cell Meter™ Fluorimetric Intracellular Total ROS Activity Assay Kit

Green Fluorescence

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 22900 (200 assays)	Keep in freezer Avoid exposure to light	Fluorescence microplate readers

Introduction

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 Green is cell-permeable. It generates the green fluorescence when it reacts with ROS. The kit is an optimized "mix and read" assay format that is compatible with HTS liquid handling instruments.

The Amplite™ Fluorimetric ROS Assay Kit provides a sensitive, one-step fluorimetric assay to detect intracellular ROS in live cells with one hour 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 fluorescence microscope at Ex/Em = 490/520 nm.

Kit Key Features

Broad Application:	Can be used for quantifying ROS in live cells.
Continuous:	Easily adapted to automation without a separation step.
Convenient:	Formulated to have minimal hands-on time.
Non-Radioactive:	No special requirements for waste treatment.

Kit Components

Components	**Amount**
Component A: Amplite™ ROS Green	1 vial
Component B: Assay Buffer	20 mL
Component C: DMSO	200 µL

Assay Protocol for One 96-well Plate

Brief Summary

Prepare cells in growth medium → Add Amplite™ ROS Green working solution (100 µL/well for a 96-well plate or 25 µL/well for a 384-well plate) → Stain the cells at 37 °C for 60 minutes → Treat the cells with test compounds to induce ROS → Monitor the fluorescence increase at Ex/Em= 490/525 nm

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

1. Prepare cells:

1.1 For adherent cells: Plate cells overnight in growth medium at 10,000 to 40,000 cells/well/100 µL for a 96-well plate or 2,500 to 10,000 cells/well/25 µL for a 384-well plate.

1.2 For non-adherent cells: Centrifuge the cells from the culture medium and then suspend the cell pellets in culture medium at 50,000-100,000 cells/well/100 µL for a 96-well poly-D lysine plate or 10,000-25,000

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cells/well/25 µL for a 384- well poly-D lysine plate. Centrifuge the plate at 800 rpm for 2 minutes with brake off prior to the experiment.

Note: Each cell line should be evaluated on an individual basis to determine the optimal cell density.

2. Prepare Amplite™ ROS Green stain solution:

2.1 Prepare Amplite™ ROS Green stock solution (500X): Add 40 µL of DMSO (Component C) into the vial of Amplite™ ROS Green (Component A), and mix them well.
Note: 20 µL of Amplite™ ROS Green stock solution *is enough for 1 plate. Un-used reconstituted Amplite™ ROS Green stock solution can be aliquoted and stored at < -20 °C for more than one month if the tubes are sealed tightly and protected from light. Avoid repeated freeze-thaw cycles.*

2.2 Prepare Amplite™ ROS Green working solution: Add 20 µL of 500X DMSO reconstituted Amplite™ ROS Green stock solution (from Step 2.1) into 10 mL of Assay Buffer (Component B), and mix them well. This working solution is stable for at least 2 hours at room temperature.

3. Stain the cells:

3.1 Add 100 µL/well (96-well plate) or 25 µL/well (384-well plate) of Amplite™ ROS Green working solution (from Step 2.2) into the cell plate.

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

4. Run ROS Assay:

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

4.2 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 1mM H_2O_2) to induce ROS.

4.3 Monitor the fluorescence increase at Ex/Em = 490/525 nm with bottom read mode.

Data Analysis

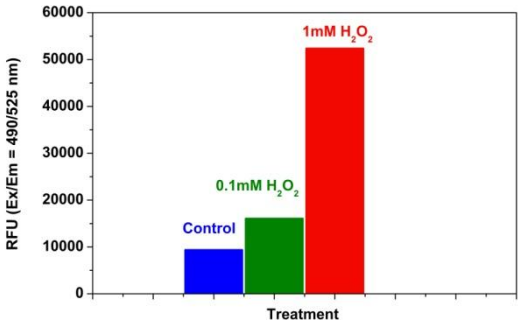

Figure 1. Detection of ROS in Jurkat cells. Jurkat cells were seeded on the same day at 300,000 cells/100µL/well in a Costar black wall/clear bottom 96-well plate. The ROS assay loading solution (100µL/well) was added and incubated in a 5% CO_2, 37 °C incubator for 1 hour. And then the cells were treated with 1mM, 0.1mM H_2O_2 or without H_2O_2 for 30 minutes. The fluorescence signal was monitored at Ex/Em = 490/525 nm (cutoff at 515 nm) with bottom read mode using FlexStation (Molecular Devices)

References

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