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

Catalog number: 22900
Unit size: 200 Tests

Component	Storage	Amount
Component A: Amplite™ ROS Green	Freeze (<-15 °C), Minimize light exposure	1 vial
Component B: Assay Buffer	Freeze (<-15 °C)	20 mL
Component C: DMSO	Freeze (<-15 °C)	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. Cell Meter™ Fluorimetric ROS Assay Kit uses our unique ROS sensor to quantify ROS in live cells. 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 Cell Meter™ 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 A GLANCE

Protocol summary

1. Prepare cells in growth medium
2. Add Amplite™ ROS Green working solution (100 µL/well for a 96- well plate or 25 µL/well for a 384-well plate)
3. Stain the cells at 37°C for 60 minutes
4. Treat the cells with test compounds to induce ROS
5. Monitor the fluorescence increase (bottom read mode) at Ex/Em= 490/525 nm (Cutoff = 515 nm) or fluorescence microscope with FITC filter set

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

KEY PARAMETERS

Instrument:	Fluorescence microplate reader
Excitation:	490 nm
Emission:	525 nm
Cutoff:	515 nm
Recommended plate:	Black wall/clear bottom
Instrument specification(s):	Bottom read mode
Instrument:	Fluorescence microscope
Excitation:	FITC filter
Emission:	FITC filter
Recommended plate:	Black wall/clear bottom

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

Note 20 µL of 500X Amplite™ ROS Green stock solution is enough for 1 plate. For storage, seal tubes tightly.

PREPARATION OF WORKING SOLUTION

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

Note This Amplite™ ROS Green 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 Green 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 = 490/525 nm (Cutoff = 515 nm) or observe cells using a fluorescence microscope with FITC filter set.

EXAMPLE DATA ANALYSIS AND FIGURES

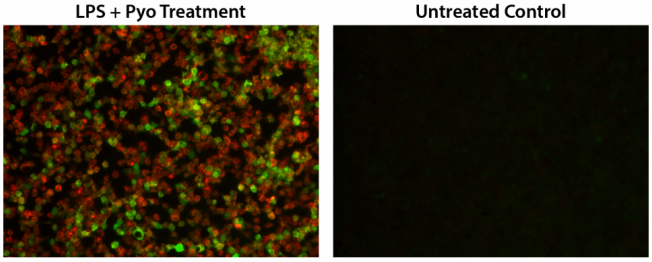

Figure 1. Fluorescence images of simultaneous detection of intracellular nitric oxide (NO) and total ROS in RAW 264.7 macrophage. Cells were co-stained with Nitrixyte™ Orange (Red) and Amplite™ ROS Green (Green). The cells were then treated with or without 20 µg/mL of lipopolysaccharide (LPS), 1 mM L-arginine (L-

Arg) and 50 µM Pyocyanin (Pyo) at 37°C for 16 hours. The fluorescence signals were measured using fluorescence microscope equipped with TRITC (Nitrixyte™ Orange, Red) and FITC (Amplite™ ROS Green, Green) filter sets, simultaneously.

DISCLAIMER