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

| *Ordering Information* | *Storage Conditions* | *Instrument Platform* |
|---|---|---|
| Product Number: 22901 (200 assays) | Keep in freezer<br>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 Red is cell-permeable. It generates the red 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 1 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 fluorescent microscope at Ex/Em = 520/605 nm. It can be used to either quantity the ROS activities in cells or screen the ROS inhibitors.

### Kit Key Features

| | |
|---|---|
| ***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 Red | 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 Red 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= 520/605 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 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 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 your experiment.

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

**2. Prepare Amplite™ ROS Red stain solution:**

2.1 Prepare Amplite™ ROS Red stock solution (500X): Add 40 µL of DMSO (Component C) into the vial of Amplite™ ROS Red (Component A), and mix them well.
*Note: 20 µL of reconstituted Amplite™ ROS Red stock solution is enough for 1 plate. 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.*

2.2 Prepare Amplite™ ROS Red working solution: Add 20 µL of 500X DMSO reconstituted Amplite™ ROS Red 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 Red 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 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.

4.2 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 1mM $H_2O_2$).

4.3 Monitor the fluorescence increase at Ex/Em = 520/605 nm with bottom read mode.

## Data Analysis

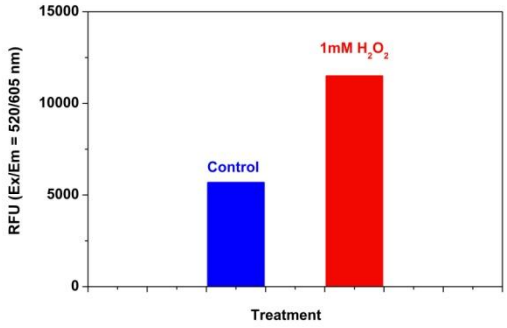


**Figure 1.** Detection of ROS in Jurkat cells. Jurkat cells were seeded on the same day at 300,000 cells/100µL/well in a Costar black wall/clear bottom 96-well plate. The ROS assay loading solution (100µL/well) was added and incubated in a 5% $CO_2$, 37 °C incubator for 1 hour. The cells were treated with or without 1mM $H_2O_2$ for 2 hours. The fluorescence signal was monitored at Ex/Em = 520/605 nm (cut off = 590 nm) with bottom read mode using FlexStation (Molecular Devices)

## References

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