Cell Meter™ Fluorimetric Intracellular Total ROS Activity Assay Kit

Green Fluorescence Optimized for Flow Cytometry

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 22904 (100 assays)	Keep in freezer, Avoid light	Flow Cytometer

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.

Cell Meter™ Fluorimetric ROS Assay Kit uses our unique Amplite™ ROS Green sensor to quantify ROS in live cells. Amplite™ ROS Green is cell-permeable. It generates the green fluorescence when it reacts with ROS, and can be easily read at Ex/Em = 490/520 nm. 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. This kit is optimized for flow cytometry applications, its signal can be detected with Ex/Em = 490/520 nm (FL1 channel).

Kit Components

Components	**Amount**
Component A: Amplite™ ROS Green	1 vial
Component B: Assay Buffer	1 bottle (10 mL)
Component C: DMSO	200 µL

Assay Protocol for Flow Cytometer

Brief Summary

Prepare cells (0.5 - 1 × 10^6 cells/mL) → Add 1 µL 500X Amplite™ ROS Green into 0.5 mL cell suspension → Stain the cells at 37 ºC for 1 hour → Treat the cells to induce ROS → Analyze cells with a flow cytometer

Note: Thaw all the components at room temperature before use.

1. For each sample, prepare cells in 0.5 mL Assay Buffer (Component B) or buffer of your choice at a density of 5×10^5 to 1×10^6 cells/mL.
   Note: Each cell line should be evaluated on an individual basis to determine the optimal cell density for ROS induction.

2. Prepare Amplite™ ROS Green stock solution (500X): Add 100 µL of DMSO (Component C) into the vial of Amplite™ ROS Green (Component A), and mix well.
   Note: Unused 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 kept from light. Avoid repeated freeze-thaw cycles.

3. Add 1 µL of 500X Amplite™ ROS Green (Component A) into 0.5 mL cell suspension, and incubate at 37 ºC for 1 hour.
   Note 1: For adherent cells, gently lift the cells with 0.5 mM EDTA to keep the cells intact, and wash the cells once with serum-containing media prior to incubation with Amplite™ ROS Green.
   Note 2: The appropriate incubation time depends on the individual cell type and test compound used. Optimize the incubation time for each experiment.

4. Treat cells by adding 50 µL of 11X test compounds in the desired buffer (such as PBS or HBSS). For control wells (untreated cells), add the corresponding amount of buffer.

5. Incubate the cells at 37 ºC for a desired period of time to induce ROS, protected from light.
   Note: We treated Jurkat cells with 100 µM TBHP (tert-Butyl hydroperoxide) at 37 ºC for 30 minutes to induce ROS. See Figure 1 for details.

6. Monitor the fluorescence intensity at the FL1 channel (Ex/Em = 490/520 nm) using a flow cytometer. Gate on the cells of interest, excluding debris.

Data Analysis

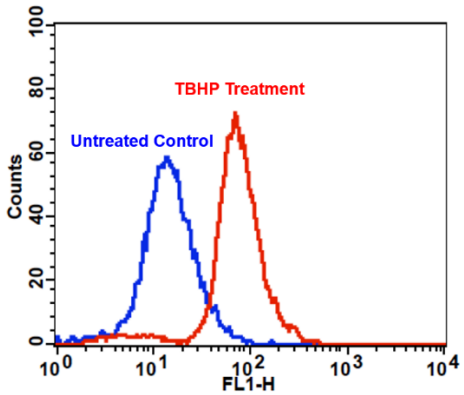

Figure 1. Detection of intracellular ROS in Jurkat cells upon TBHP treatment using Cell Meter™ Fluorimetric Intracellular Total ROS Activity Assay Kit (Cat#22904). Cells were incubated with Amplite™ ROS Green at 37 ºC for 1 hour. TBHP Treatment: The cells were treated with 100 µM TBHP at 37 ºC for 30 minutes. Untreated Control: Cells were incubated with Amplite™ ROS Green at 37 ºC for 1 hour without TBHP treatment. The fluorescence signal was monitored at FL1 channel using a flow cytometer (BD FACSCalibur).

References

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