Cell Meter™ Fluorimetric Intracellular Total ROS Activity Assay Kit*Optimized for Flow Cytometry*

Catalog number: 22904
Unit size: 100 Tests

Component	Storage	Amount
Component A: Amplite™ ROS Green	Freeze (<-15 °C), Minimize light exposure	1 vial
Component B: Assay Buffer	Freeze (<-15 °C)	1 bottle (10 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 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. 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).

AT A GLANCE

Protocol summary

1. Prepare cells at the density of 0.5 - 1 × 10^6 cells/mL
2. Add 1 µL 500X Amplite™ ROS Green into 0.5 mL cell suspension
3. Stain the cells at 37 ºC for 1 hour
4. Treat the cells to induce ROS
5. Analyze cells using flow cytometer with FL1 channel (Ex/Em = 490/520 nm)

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

KEY PARAMETERS

Instrument:	Flow cytometer
Excitation:	488 nm laser
Emission:	530/30 nm filter
Instrument specification(s):	FITC channel

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 100 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 For storage, seal tubes tightly.

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. 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. Add 1 µL of 500X Amplite™ ROS Green stock solution into 0.5 mL cell suspension.

3. Incubate at 37 ºC for 1 hour.

   Note 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. 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 using a flow cytometer with FL1 channel (Ex/Em = 490/520 nm). Gate on the cells of interest, excluding debris.

EXAMPLE DATA ANALYSIS AND FIGURES

Tel: 408-733-1055 | Fax: 408-733-1304 | Email: support@aatbio.com
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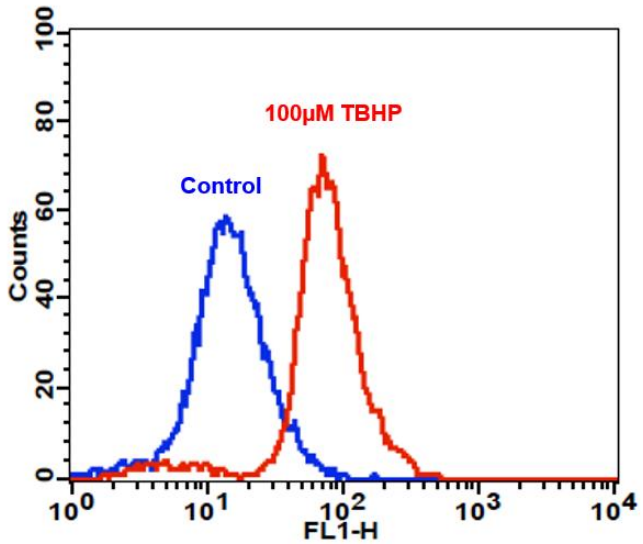

Figure 1. Detection of intracellular ROS in Jurkat cells upon TBHP treatment using Cell Meter™ Fluorimetric Intracellular Total ROS Activity Assay Kit. Cells were incubated with Amplite™ ROS Green at 37 °C for 1 hour. The cells were then treated without (Blue) or with (Red) 100 µM TBHP at 37 °C for 30 minutes. The fluorescence signal was monitored at FL1 channel using a flow cytometer (BD FACSCalibur).

DISCLAIMER

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