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

Catalog number: 22903
Unit size: 200 Tests

Component	Storage	Amount
Component A: ROS Brite™ 670	Freeze (< -15 °C), Minimize light exposure	1 vial
Component B: Assay Buffer	Freeze (< -15 °C)	1 bottle (20 mL)
Component C: DMSO	Freeze (< -15 °C)	1 vial (100 µL)

OVERVIEW

Reactive oxygen species (ROS) are natural byproducts of the normal metabolism of oxygen and play important roles in cell signaling. 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 Intracellular Total ROS Activity Assay Kit uses our proprietary ROS Brite™ 670 sensor to quantify ROS in live cells. The cell-permeable and non-fluorescent ROS Brite™ 670 exhibits a strong fluorescence signal upon reaction with ROS. ROS Brite™ 670 sensor is localized in the cytoplasm. The fluorescence signal of ROS Brite™ 670 sensor can be measured by fluorescence microscopy, high-content imaging, microplate fluorometry, or flow cytometry. The Cell Meter™ Fluorimetric Intracellular Total ROS Activity Assay Kit provides a sensitive, one-step fluorimetric assay to detect intracellular ROS (especially superoxide and hydroxyl radical) in live cells within 1 hour incubation. The assay can be performed in a convenient 96-well or 384-well microtiter-plate format using either a fluorescence microplate reader or a fluorescent microscope with Cy5 filter.

AT A GLANCE

Protocol A summary (Fluorescence microplate reader, fluorescence microscope)

1. Prepare cells in growth medium
2. Treat the cells with test compounds to induce ROS
3. Add ROS Brite™ 670 working solution (100 µL/well for a 96-well plate or 25 µL/well for a 384-well plate)
4. Stain the cells at 37 °C for 30 - 60 minutes
5. Monitor the fluorescence increase (bottom read mode) at Ex/Em= 650/675 nm (Cutoff = 665 nm) or fluorescence microscope with Cy5 filter set

Protocol B summary (Flow cytometer)

1. Prepare cells in growth medium
2. Treat cells with test compounds to induce ROS
3. Incubate ROS Brite™ 670 with the cells for 30 - 60 minutes
4. Monitor the fluorescence intensities using flow cytometer with APC channel

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

KEY PARAMETERS

Flow cytometer

Excitation	640 nm laser
Emission	660/20 nm filter
Instrument specification(s)	APC channel

Fluorescence microscope

Excitation	Cy5 filter
Emission	Cy5 filter
Recommended plate	Black wall/clear bottom

Fluorescence microplate reader

Excitation	650 nm
Emission	675 nm
Cutoff	665 nm
Recommended plate	Black wall/clear bottom
Instrument specification(s)	Bottom read mode

CELL PREPARATION

For guidelines on cell sample preparation, please visit https://www.aatbio.com/resources/guides/cell-sample-preparation.html

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.

ROS Brite™ 670 stock solution (500X)
Add 40 µL of DMSO (Component C) into the vial of ROS Brite™ 670 (Component A) and mix well to make 500X ROS Brite™ 670 stock solution. Protect from light.

Note 20 µL of 500X ROS Brite™ 670 stock solution is enough for 1 plate. For flow cytometer, 500X ROS Brite™ 670 stock solution can be diluted by 5 folders to 100X in DMSO for convenience. For storage, seal tubes tightly.

PREPARATION OF WORKING SOLUTION

Add 20 µL of 500X ROS Brite™ 670 stock solution into 10 mL of Assay Buffer (Component B) and mix well to make ROS Brite™ 670 working solution.

Note This ROS Brite™ 670 working solution is stable for at least 2 hours at room temperature.

SAMPLE EXPERIMENTAL PROTOCOL

For Protocol A:

1. Treat cells with 10 µL of 10X test compounds (96-well plate) or 5 µL of 5X 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.

2. To induce ROS, incubate the cell plate at room temperature or in a 5% CO_2, 37 °C incubator for a desired period of time (for example: 30 minutes treatment for Hela cells with 100 µM tert-butyl hydroperoxide (TBHP)).

3. Add 100 µL/well (96-well plate) or 25 µL/well (384-well plate) of ROS Brite™ 670 working solution into the cell plate.

4. Incubate the cells in a 5% CO_2, 37 °C incubator for 30 min to 60 minutes.

5. Monitor the fluorescence increase with a fluorescence microplate reader (bottom read mode) at Ex/Em = 650/675 nm (Cutoff = 665 nm) or observe cells using a fluorescence microscope with Cy5 filter set.

For Protocol B:

1. Prepare cells at the density from 5×10^5 to 1×10^6 cells/mL. *Note:* Each cell line should be evaluated on the individual basis to determine the optimal cell density for apoptosis induction.

2. Treat cells with test compounds in your desired buffer (such as PBS or HHBS). For control wells (untreated cells), add the corresponding amount of compound buffer.

3. To induce ROS, incubate the cell plate at room temperature or in a 5% CO_2, 37 °C incubator for at least 30 minutes or a desired period of time (30 minutes for Hela cells treated with 100 µM tert-butyl hydroperoxide (TBHP)).

4. Add 1 µL/mL cells of 500X ROS Brite™ 670 stock solution or 5 µL/mL cells of 100X ROS Brite™ 670 stock solution to cells medium.

5. Incubate the cells in a 5% CO_2, 37 °C incubator for 30 to 60 minutes.

6. Monitor the fluorescence intensity using a flow cytometer with APC channel.

EXAMPLE DATA ANALYSIS AND FIGURES

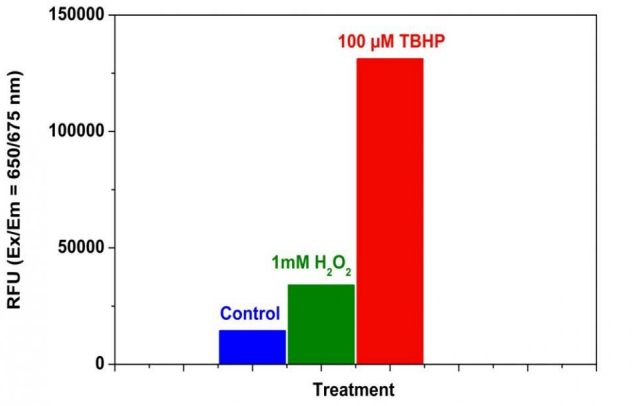

Figure 1. Detection of ROS in HeLa cells with Cell Meter™ Fluorimetric Intracellular Total ROS Activity Assay Kit. HeLa cells were seeded overnight at 15,000 cells/90 µL/well in a Costar black wall/clear bottom 96-well plate. The cells were untreated (control) or treated with 1 mM H_2O_2 or 100 µM tert-butyl hydroperoxide (TBHP) for 30 minutes at 37 °C. The ROS Brite™ 670 working solution (100 µL/well) was added and incubated in a 5% CO2, 37 °C incubator for 1 hour. The fluorescence signal were monitored at Ex/Em = 650/675 nm (Cutoff = 665 nm) with bottom read mode using FlexStation (Molecular Devices).

DISCLAIMER

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