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

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 22902 (200 assays)	Keep in freezer Avoid exposure to light	Fluorescence microplate readers, Microscope, Flow Cytometer

Introduction

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™ 570 sensor to quantify ROS in live cells. The cell-permeable and non-fluorescent ROS Brite™ 570 exhibits a strong fluorescence signal upon reaction with ROS. ROS Brite™ 570 sensor is localized in the cytoplasm. The fluorescence signal of ROS Brite™ 570 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 at Ex/Em = 540/570 nm or a fluorescent microscope with TRITC filter.

Kit Components

Components	Amount
Component A: ROS Brite™ 570	1 vial
Component B: Assay Buffer	20 mL
Component C: DMSO	100 µL

Assay Protocol for One 96-well Plate

Brief Summary

Prepare cells in growth medium → Treat the cells with test compounds to induce ROS →Add ROS Brite™ 570 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 30-60 minutes → Monitor the fluorescence increase at Ex/Em= 540/570 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/90 µL for a 96-well plate or 2,500 to 10,000 cells/well/20 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/90 µL for a 96-well poly-D lysine plate or 10,000-25,000 cells/well/20 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 ROS Brite™ 570 Assay Solution:

2.1 Prepare ROS Brite™ 570 stock solution (500X): Add 40 µL of DMSO (Component C) into the vial of ROS Brite™ 570 (Component A), and mix them well.
Note: 20 µL of reconstituted ROS Brite™ 570 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.

©2013 by AAT Bioquest®, Inc., 520 Mercury Drive, Sunnyvale, CA 94085. Tel: 408-733-1055
Ordering: sales@aatbio.com; Tel: 800-990-8053 or 408-733-1055; Fax: 408-733-1304
Technical Support: support@aatbio.com; Tel: 408-733-1055

2.2 Prepare ROS Brite™ 570 assay solution: Add 20 µL of 500X DMSO reconstituted ROS Brite™ 570 stock solution (from Step 2.1) into 10 mL of Assay Buffer (Component B), and mix them well. This assay solution is stable for at least 2 hours at room temperature.

3. Run ROS Assay:

3.1 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.

3.2 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 100µM tert-butyl hydroperoxide (TBHP)).

3.3 Add 100 µL/well (96-well plate) or 25 µL/well (384-well plate) of ROS Brite™ 570 working solution (from Step 2.2) into the cell plate.

3.4 Incubate the cells in a 5% CO_2, 37 °C incubator for 30 min to one hour, and monitor the fluorescence increase at Ex/Em = 540/570 nm (cut off = 550 m) with bottom read mode, or take images with TRITC filter set.

Data Analysis

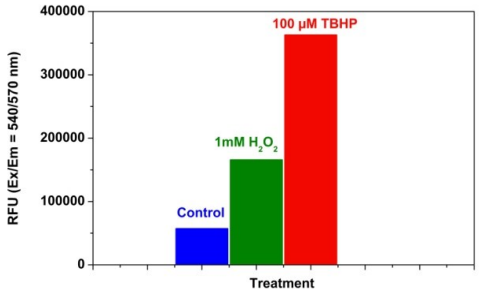

Figure 1. Detection of ROS in Hela cells. Hela cells were seeded overnight at 15,000 cells/90µL/well in a Costar black wall/clear bottom 96-well plate. The cells were untreated (control) or treated with1 mM H_2O_2 or 100µM tert-butyl hydroperoxide (TBHP) for 30min at 37 °C. The ROS Brite™ 570 assay solution (100µL/well) was added and incubated in a 5% CO_2, 37 °C incubator for 1 hour. The fluorescence signal were monitored at Ex/Em = 540/570 nm (cut off = 550 nm) with bottom read mode using FlexStation (Molecular Devices).

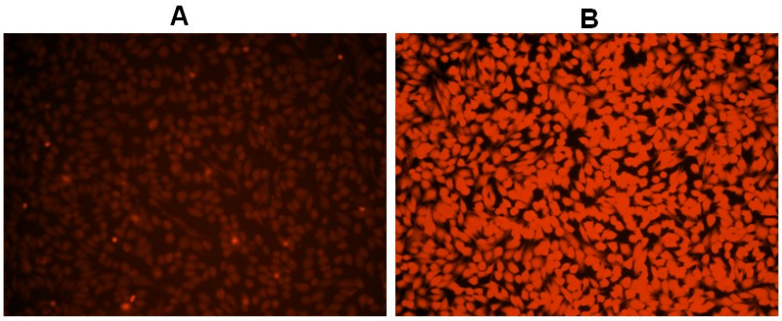

Figure 2. Images of Hela cells stained with the Cell Meter™ Fluorimetric Intracellular Total ROS Activity Assay Kit in a Costar black wall/clear bottom 96-well plate. A: Untreated control cells. B: Cells treated with 100 µM tert-butyl hydroperoxide (TBHP) for 30min before staining.

©2013 by AAT Bioquest®, Inc., 520 Mercury Drive, Sunnyvale, CA 94085. Tel: 408-733-1055
Ordering: sales@aatbio.com; Tel: 800-990-8053 or 408-733-1055; Fax: 408-733-1304
Technical Support: support@aatbio.com; Tel: 408-733-1055

Assay Protocol for Flow Cytometry Analysis

Brief Summary

Prepare cells in growth medium → Treat cells with test compounds to induce ROS →Incubate ROS Brite™ 570 with the cells for 30-60 min → Monitor the fluorescence intensities with a flow cytometer

1. Prepare cells:

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. Prepare ROS Brite™ 570 stock solution (1000X):

Add 40 µL of DMSO (Component C) into the vial of ROS Brite™ 570 (Component A), and mix them well.

Note 1: 1 µL of reconstituted ROS Brite™ 570 stock solution is for 1 ml cells. 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.

Note 2: 1000X reconstituted ROS Brite™ 570 stock solution can be diluted by 5 folds to 200X in DMSO for convenience. 200X ROS Brite™ 570 stock solution can also be aliquoted and stored at ≤ -20 °C for more than one month if the tubes are sealed tightly and kept from light.

3. Run ROS Assay:

3.1 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.

3.2 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 100µM tert-butyl hydroperoxide (TBHP)).

3.3 Add 1 µL/mL cells of 1000X ROS Brite™ 570 stock solution (from Step 2) or 5 µL/mL cells of 200X ROS Brite™ 570 stock solution to cells medium.

3.4 Incubate the cells in a 5% CO_2, 37 °C incubator for 30 min to one hour, and monitor the fluorescence intensity using a flow cytometry in FL2 channel.

Data Analysis

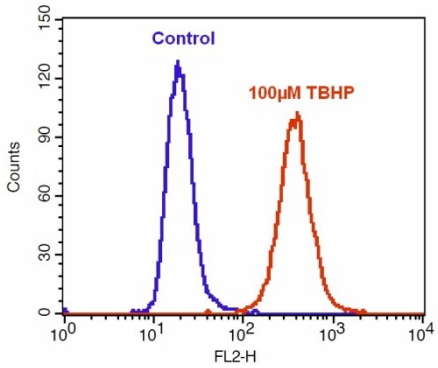

Figure 3. Detection of ROS in Jurkat cells. Jurkat cells were treated without (Blue) or with 100µM tert-butyl hydroperoxide (TBHP) (Red) for 30min at 37 °C, and then loaded with ROS Brite™ 570 in a 5% CO_2, 37 °C incubator for 1 hour. The fluorescent intensities were measured with a FACSCalibur flow cytometer using FL2 channel.

Warning: This kit is only sold to end users. Neither resale nor transfer to a third party is allowed without written permission from AAT Bioquest®. Chemical analysis of the kit components is strictly prohibited. Please call us at 408-733-1055 or e-mail us at info@aatbio.com if you have any questions.