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Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Elite™ Mitochondrial ROS Activity Assay Kit (Orange Fluorescence)

CATALOG NUMBER: CA-R922, 200 assays

Description

Reactive oxygen species (ROS) are natural byproducts of the normal metabolism of oxygen and play important roles in cell signaling and homeostasis. 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.

This Elite™ Mitochondrial ROS Assay Kit uses our unique ROS sensor to quantify mitochondrial ROS. Elite™ ROS Orange uses a membrane-permeable non-fluorescent ROS sensor, which quickly penetrates the mitochondrial membrane and generates very strong fluorescence signal when it reacts with ROS. The kit is an optimized "mix and read" assay format that is compatible with HTS liquid handling instruments.

This kit provides a sensitive, one-step fluorimetric assay to detect mitochondrial ROS (**especially superoxide and hydroxyl radicals**) 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. The intensity of the signal can be easily measured using a fluorescence microplate reader at Ex/Em = 540/570 nm, or a fluorescent microscope with TRITC filter or a flow-cytometry in FL2 channel. This kit can be used to either quantity the ROS activities in cells or screen the ROS inhibitors.

Kit Components

- **Component A:** Elite™ ROS Orange — 1 vial
- **Component B:** Assay Buffer — 1 bottle (20 ml)
- **Component C:** DMSO — 1 vial (100 µl)

Storage

Keep **Component A** in freezer (-20 °C) and avoid exposure to light; **Component B&C** at 4 °C. All components are stable for 6 months after receipt if stored properly.

Materials Required (but not supplied) for the Microplate-Format Assay

- A 96-well or 384-well microplate (tissue culture microplate with black wall and clear bottom is recommended) and a fluorescence microplate reader

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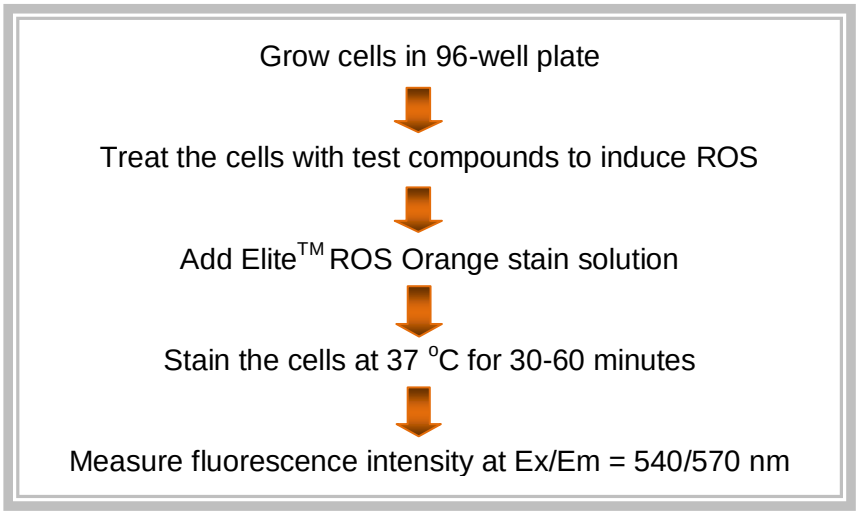
Assay Protocol (for 96-Well or 384-Well Microplate)

Brief Summary of the Assay



1. Preparation of 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. **Preparation of Elite™ ROS Orange stain solution:**

2.1. Prepare Elite™ ROS Orange stock solution (500X): Add 40 µL of DMSO (**Component C**) into the vial of Elite™ ROS Orange (**Component A**), and mix them well.

Note: 20 µL of reconstituted Elite™ ROS Orange 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 Elite™ ROS Orange stain solution: Add 20 µL of 500X DMSO reconstituted Elite™ ROS Orange 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. Run ROS assay:

4.1. 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.

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 100 µM tert-butyl hydroperoxide (TBHP)).

4.3. Add 100 µL/well (96-well plate) or 25 µL/well (384-well plate) of Elite™ ROS Orange stain solution (from Step 2.2) into the cell plate.

4.4. Incubate the cells in a 5% CO_2, 37 °C incubator for 30 min to 1 hour, and monitor the fluorescence increase at Ex/Em = 540/570 nm (cut off = 550 nm) with bottom read mode or take images with TRITC filter set.

Data Analysis

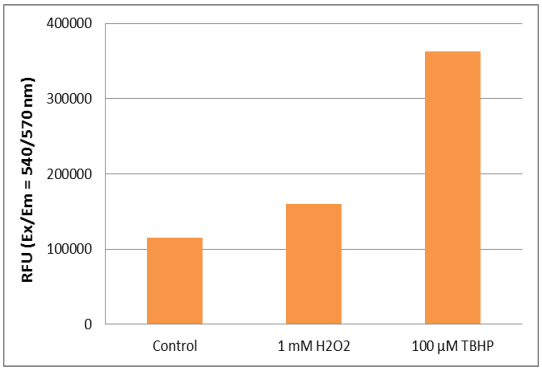


Figure 1. Detection of ROS in Hela cells. Hela cells were seeded overnight at 15,000 cells/90µL/well in a Costar black wall/clear bottom 96-well plate. The cells were treated (1 mM H_2O_2 or 100 µM tert-butyl hydroperoxide) or untreated (control) for 30 min at 37 ^{o}C. The Elite™ ROS Orange stain solution (100µL/well) was added and incubated in a 5% CO2, 37 °C incubator for 1 hour. The fluorescence signal was monitored at Ex/Em = 540/570 nm (cut off = 550 nm) with bottom read mode using FlexStation (Molecular Devices).

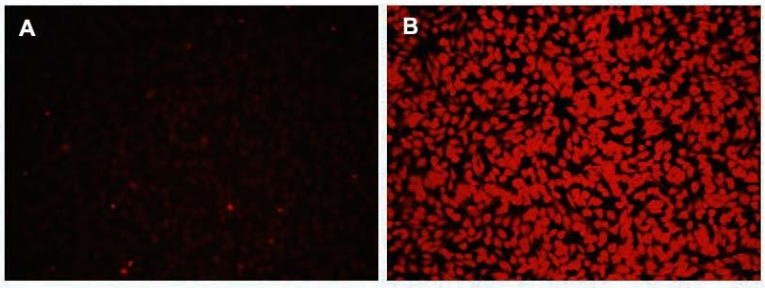
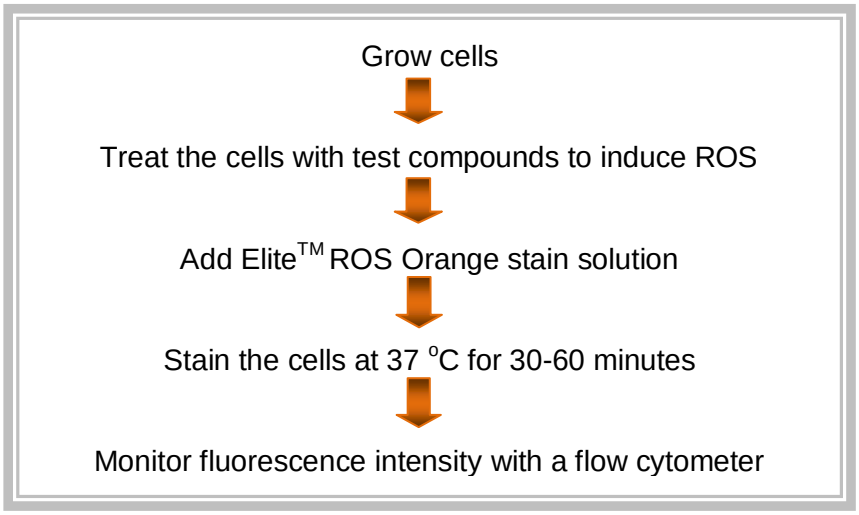


Figure 2. Images of Hela cells stained with the Elite™ ROS Orange sensor in a Costar black wall/clear bottom 96-well plate. A: Untreated control cells. B: Cells treated with 100 µM tert-butyl hydroperoxide (TBHP) for 30min before staining.

Protocol for Flow-Cytometry Analysis

Brief Summary of the Assay



1. **Preparation of cells:**

   Prepare cells in growth medium at the density of 5x 10^5 to 10x 10^5 cells/mL.

   Note: *each cell lines should be evaluated on the individual basis to determine the optimal cell density for apoptosis induction.*

2. **Preparation of Elite™ ROS Orange stock solution (1000x):**

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

   Note: 1 µL of reconstituted Elite™ ROS Orange 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: 1000x reconstituted Elite™ ROS Orange stock solution can be diluted by 5 folds to 200x in DMSO for convenience. 200x Elite™ ROS Orange 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.

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 100 µM tert-butyl hydroperoxide (TBHP)).

   3.3. Add 1 µL/ml cells of 1000x Elite™ ROS Orange stock solution (from Step 2) or 5 µL/mL cells of 200x Elite™ ROS Orange stock solution to cells.

   3.4. Incubate the cells in a 5% CO2, 37 °C incubator for 30 min to one hour, and monitor the fluorescence intensity using a flow cytometry in FL2 channel.

Data Analysis

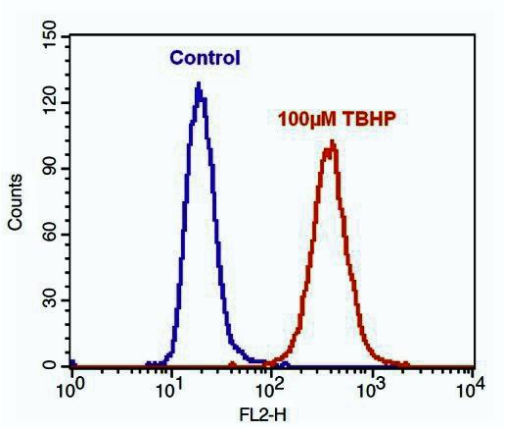


Figure 3. Detection of Elite™ ROS Orange in Jurkat cells. Jurkat cells were treated without (Blue) or with 100μM tert-butyl hydroperoxide (TBHP) (Red) for 30min at 37 °C, and then loaded with Elite™ ROS Orange in a 5% CO2, 37 °C incubator for 1 hour. The fluorescent intensities were measured with a FACSCalibur flow cytometer using FL2 channel.