Elite™ Mitochondrial ROS Activity Assay Kit (Deep Red Fluorescence)

CATALOG NUMBER: CA-R933, 200 assays

Description

Mitochondrial reactive oxygen species (ROS) are key regulators of cellular signaling and stress responses. Under oxidative stress, ROS levels can rise dramatically, leading to damage of cellular components. Elevated mitochondrial ROS has been implicated in a wide range of pathologies, including cardiovascular disease, diabetes, stroke, osteoporosis, neurodegeneration, inflammation, and cancer.

The Elite™ Mitochondrial ROS Assay Kit enables sensitive, one-step quantification of mitochondrial ROS—particularly superoxide and hydroxyl radicals—in live cells. The proprietary Elite™ ROS Deep Red dye is membrane-permeable and selectively accumulates in mitochondria, where it fluoresces upon reaction with ROS. The assay is optimized for high-throughput screening (HTS) and compatible with fluorescence microscopy, microplate readers (Ex/Em = 650/675 nm), and flow cytometry (FL2 or APC channel).

Key Features

- Targeted detection: Selective for mitochondrial ROS
- Bright signal: High fluorescence intensity with minimal background
- HTS-compatible: 96- and 384-well formats; automation-ready
- Flexible readout: Plate reader, microscope (TRITC filter), or flow cytometry

Kit Components

Component	Description	Quantity
A	Elite™ ROS Deep Red	1 vial
B	Assay Buffer	20 mL
C	DMSO	100 µL

Storage Conditions:

- Component A: -20 °C, protected from light
- Component B&C: 4 °C
- Shelf life: 6 months from receipt when stored properly

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Assay Protocol Overview

Microplate Format (96- or 384-Well)

1. **Cell Preparation**
   - Adherent cells:
     - 96-well: 10,000-40,000 cells/well in 90 µL
     - 384-well: 2,500-10,000 cells/well in 20 µL
   - Suspension cells:
     - 96-wll: 50,000-100,000 cells/well in poly-D-lysine-coated plates
     - Centrifuge at 800 rpm for 2 min (brake off) before staining
2. **Stain Preparation**
   - Reconstitute Component A with 40 µL DMSO (Component C) to make 500x stock
   - Dilute 20 µL of stock into 10 mL Assay Buffer (Component B) to prepare stain solution
   - Use within 2 hours at room temperature
3. **ROS Inductionn & Staining**
   - Add test compounds:
     - 96-well: 10 µL of 10x solution
     - 384-well: 5 µL of 5x solution
   - Incubate for 15-30 minutes at 37°C (e.g., 100 µM TBHP for Hela cells)
   - Add stain solution
     - 96-well: 100 µL/well
     - 384-well: 25 µL/well
   - Incubate 30-60 min at 37°C, 5% CO_2
   - Measure fluorescence at Ex/Em = 650/675nm (cut off = 665nm) using bottom-read mode or image with TRITC filter

Flow Cytometry Protocol

1. **Cell Preparation**
   - Resuspend cells at 0.5-1 x 10^6 cells/mL in growth medium
2. **Stain Preparation**
   - Reconstitute Component A with 40 µL DMSO to make 500x stock
   - Add 1 µL/mL cells (or 5 µL/mL for 100x dilution)
   - Incubate 30-60 min at 37°C, 5% CO_2
   - Analyze fluorescence in FL2 or APC channel

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Representative Data

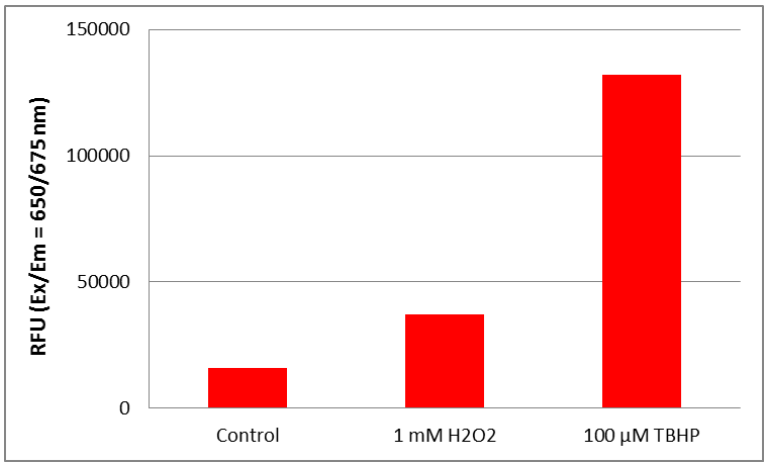

Figure 1. Quantification of mitochondrial ROS in HeLa cells using Elite™ ROS Deep Red.
HeLa cells were seeded overnight at 15,000 cells per well in 90 µL growth medium on a Costar 96-well plate (black wall, clear bottom). Cells were either left untreated (control) or treated with 1 mM hydrogen peroxide (H_2O_2) or 100 µM tert-butyl hydroperoxide (TBHP) for 30 minutes at 37 °C. Following treatment, 100 µL/well of Elite™ ROS Deep Red stain solution was added, and cells were incubated for 1 hour at 37 °C in a 5% CO_2 atmosphere. Fluorescence intensity was measured using bottom-read mode on a FlexStation microplate reader (Molecular Devices) at excitation/emission = 650/675 nm (cutoff = 665 nm)

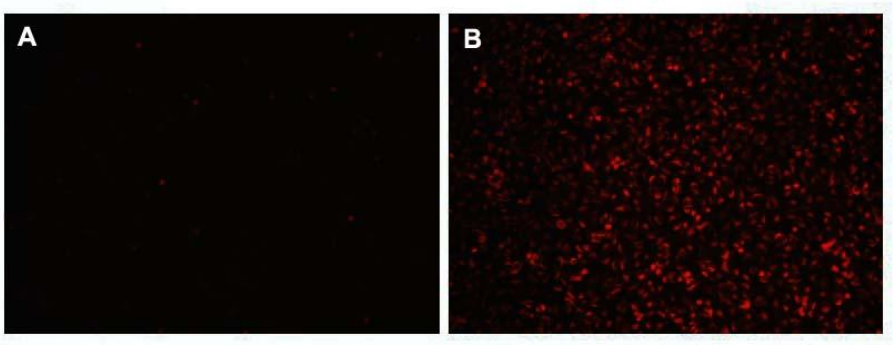

Figure 2. Fluorescence imaging of mitochondrial ROS in HeLa cells using Elite™ ROS Deep Red.
HeLa cells were cultured overnight at 15,000 cells per well in a Costar 96-well plate (black wall, clear bottom). Cells were stained with Elite™ ROS Deep Red following treatment conditions:
A: Untreated control cells
B: Cells treated with 100 µM tert-butyl hydroperoxide (TBHP) for 30 minutes at 37 °C prior to staining

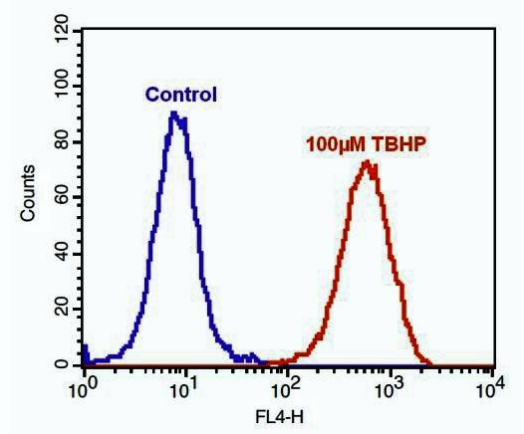

Figure 3. Flow cytometric detection of mitochondrial ROS in Jurkat cells using Elite™ ROS Deep Red.

Jurkat cells were incubated at 37 °C for 30 minutes either without treatment (blue trace) or with 100 μM tert-butyl hydroperoxide (TBHP; red trace) to induce oxidative stress. Cells were then stained with Elite™ ROS Deep Red and incubated for 1 hour at 37 °C in a 5% CO_2 atmosphere. Fluorescence intensity was measured using a FACSCalibur flow cytometer in the FL2 channel. TBHP-treated cells exhibited a marked increase in fluorescence, indicating elevated mitochondrial ROS levels