Elite™ Intracellular Total ROS Activity Assay Kit (Red Fluorescence)

CATALOG NUMBER: CA-R911, 200 assays

Description

Reactive oxygen species (ROS) are natural byproducts of cellular metabolism and play essential roles in signaling and homeostasis. However, under oxidative stress, ROS levels can rise dramatically, leading to damage of cellular components. Elevated ROS has been implicated in a wide range of diseases, including cardiovascular disorders, diabetes, stroke, osteoporosis, neurodegeneration, inflammation, and cancer.

The Elite™ Intracellular ROS Assay Kit provides a sensitive, one-step fluorometric assay for detecting intracellular ROS—especially superoxide—in live cells. The proprietary Elite™ ROS Red dye is cell-permeable and fluoresces upon reaction with ROS, enabling quantification via fluorescence microscopy or microplate reader (Ex/Em = 520/605nm). The assay is optimized for high-throughput screening (HTS) and compatible with 96- and 384-well formats.

Key Features

- Sensitive & specific: Detects intracellular ROS with high signal-to-noise ratio
- Rapid protocol: One-hour incubation, no wash steps required
- HTS-ready: Compatible with automated liquid handling systems
- Flexible readout: Fluorescence microplate reader or microscope

Kit Components

Component	Description	Quantity
A	Elite™ ROS Red	1 vial
B	Assay Buffer	20 mL
C	DMSO	200 µL

Storage:

- 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

1. **Cell Preparation**
   - Adherent cells:
     - 96-well: 10,000-40,000 cells/well in 100 µL
     - 384-well: 2,500-10,000 cells/well in 25 µL
   - Suspension cells:
     - 96-well: 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
     - Aliquot and store unused stock at < -20°C (avoid repeated freeze-thaw cycles)
3. **Cell Staining**
   - Add stain solution:
     - 96-well: 100 µL/well
     - 384-well: 25 µL/well
   - Incubate at 37°C, 5% CO_2 for 1 hour
4. **ROS Inductionn & Measurement**
   - Add test compounds:
     - 96-well: 20 µL of 11X test compound solution
     - 384-well: 10 µL of 6X test compound solution
   - Incubate for 15-30 minutes (e.g., 1 mM H_2O_2 for 30 min in Hela cells)
   - Measure fluorescence at Ex/Em = 520/605nm (cut off = 590nm) using bottom-read mode using FlexStation (Molecular Devices)

Representative Data

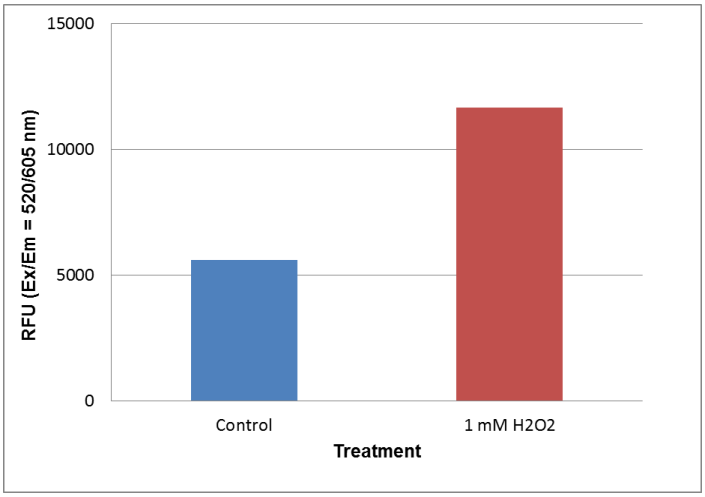

Figure 1. Detection of intracellular ROS in Jurkat cells using the Elite™ ROS Red Assay. Jurkat cells were seeded at 300,000 cells per well in 100 µL growth medium on a Costar 96-well plate (black wall, clear bottom). On the same day, cells were stained with 100 µL/well of ROS Red assay solution and incubated at 37 °C in a 5% CO_2 atmosphere for 1 hour. Following staining, cells were treated with or without 1 mM H_2O_2 for 2 hours. Fluorescence intensity was measured using bottom-read mode on a FlexStation microplate reader (Molecular Devices) at excitation/emission = 520/605 nm (cutoff = 590 nm).