**Reactive Oxygen Probes - A Broad Range of Colors with Easier Labeling and Fixation: Novel CellROX® Reagents from Molecular Probes**

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**Introduction**

The generation of highly toxic radicals, called reactive oxygen species (ROS), is a natural consequence of aerobic respiration. Oxidatively produced ROS and other reactive free radicals can damage proteins, lipids and DNA. ROS have been implicated in human pathologies including cancer, neurodegeneration, cardiopulmonary disease and diabetes (1, 2). To combat oxidative stress, cells are equipped with antioxidant defense systems that correct imbalances in the concentrations of pro- and anti-oxidants. In other systems, ROS generation plays an important protective role in the early response of the innate immune system to pathogens (3). In addition, low concentrations of ROS may serve as secondary messengers in cell signaling (4). Reactive probes such as Antimycin A Fluor (AAF), hydroethidine (HER), and 5-(and-6)-carboxy-2′,7′-dichlorodihydrofluorescein diacetate (carboxy-H2DCFDA) have been widely used to detect ROS using flow cytometry and imaging platforms. All are readily by the fluorescence-labeled end in the cytosol of cells, and all demonstrate that labeling occurs in a pro-nuclease-free buffer. In contrast, the CellROX® ROS detection reagents from Molecular Probes® offer increased flexibility for multiplex experiments, with fluorescence emission from CellROX Orange® and SYTOX® Red, respectively. In addition, the CellROX® reagents are easier to use than traditional dyes. They are inactivated under normal growth conditions for 30-40 minutes prior to analysis using a flow cytometer with a 488 nm, 532 nm, or 633 nm laser for detection of the CellROX Green®, CellROX Orange, and CellROX® Deep Red Reagents, respectively. Suspensions of cells are not required before analysis. In contrast, a wash step is necessary in the workflow of adherent cells. Fluorescence emission from excitation of CellROX® reagents is collected using 530/30 bandpass (BP), 580/42 BP, and 605/40 BP filters for excitation from CellROX Green®, CellROX Orange, and CellROX® Deep Red, respectively.

**Methods**

In this study we investigated the use of novel fluorescent ROS probes, the CellROX® ROS detection reagents, to monitor ROS using a flow cytometry platform. Use of the reagents was compared in multiple cell lines and conditions and compared to results using the preacquired fluorescent ROS probe, carboxy-H2DCFDA.

**Results**

Cells treated with tert-butyl hydroperoxide or menadione (both inducers of ROS generation) and stained with the CellROX® ROS detection reagents demonstrated increased fluorescence compared to controls. Treatment of cells under oxidant stress with the antioxidant, N-acetylcyesteine, resulted in diminished staining with the CellROX® ROS detection reagents demonstrating the specificity of the reagents. Detection of ROS by the CellROX® reagents was increased compared to ROS detection carboxy-H2DCFDA. CellROX Orange® and CellROX® Deep Redferther additional choices for multiple fluorescence cytometry not previously available.

**Conclusions**

The CellROX® ROS detection reagents are bright and stable ROS sensors that offer significant advantages over other ROS sensors as they are compatible with labeling in different media and can be used with flow cytometry.

**Mechanisms of Action**

Fluorescent Non-fluorescent

ROS

Fluorescence

Reduced

Oxidized

Figure 1. CellROX® Deep Red chemical change upon oxidation. The cell permeable CellROX® reagents are essentially non-fluorescent while in a reduced state. They exhibit high levels of signal upon oxidation, providing a reliable measure of reactive oxygen species (ROS) in live cells.

**References**


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