

Oxidative Stress Measurements Made Easy: CellROX Reagents with Simple Workflow for High Content Imaging

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Oxidative stress results from an imbalance between production of reactive oxygen species (ROS) and the ability of cells to scavenge them. ROS play an important role in the progression of several diseases including inflammation, atherosclerosis, aging and age-related degenerative disorders. Oxidative stress can be caused by many different pathways, intrinsic and extrinsic, mediated either by mitochondrial respiration or by membrane-bound NADPH oxidases. Detection of ROS by high content imaging is advantageous over other techniques as it gives spatial information and enables multiplexing with other parameters of cell health. **Molecular Probes™ CellROX™ Deep Red, Orange and Green reagents** are new fluorogenic and photostable probes to measure oxidative stress in cells. Unlike dihydrodichloro fluoresceins, these probes can be directly added to the complete media with serum and thus simplify the workflow (Figure 1).

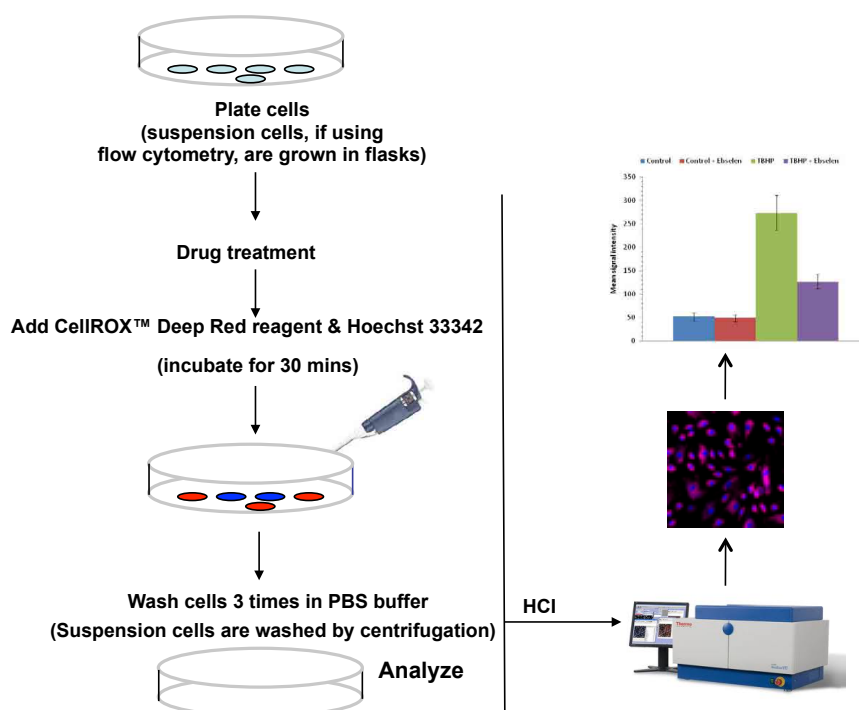


Figure 1: Simple workflow for CellROX Reagent staining. As highlighted in the figure, there are significant changes to the workflow when staining for flow cytometry experiments.

CellROX Green Reagent is a nucleic acid binding dye, and upon oxidation, it binds predominantly to DNA and thus, its signal is

localized primarily in the nucleus and mitochondria. In contrast, the signals from CellROX Deep Red and CellROX Orange Reagents are

localized primarily in the cytoplasm. The fluorescence resulting from **CellROX Reagents** can be easily measured by high-content imaging and analysis. The staining workflow is simple and the reagent can be applied to cells in complete growth media or buffer. CellROX Reagents are more photostable when compared to traditional ROS detection dyes like dichloro dihydro fluoresceins and dihydroethidium (Table 1).

	H2-DCFDA	DHE	CellROX Deep Red	CellROX Orange	CellROX Green
Add in Complete media	No	Yes	Yes	Yes	Yes
Fixable	No	No	Yes	No	Yes
Detergent resistant	No	No	No	No	Yes
Multiplexibility	Yes	No	Yes	Yes	Yes
GFP compatible	No	No	Yes	Yes	No
RFP compatible	Yes	No	Yes	No	Yes
Photostability	*	N/A	***	***	**

Table 1: Comparison of CellROX Reagents to traditional probes for oxidative stress.

In addition, some of the reagents retain their signal after formaldehyde fixation and detergent permeabilization (Table 1), allowing for assay flexibility and improved workflows compared to those based on classic dyes for ROS detection. CellROX Reagents are selective to oxidative stress measurements as shown by the inhibitor experiments (Figure 2). The approximate fluorescence excitation and emission maxima are **CellROX Green reagent:** 508/525 nm (oxidized product bound to nucleic acid); **CellROX Orange reagent:** 545/565 nm (oxidized product); and **CellROX Deep Red Reagent:** 644/665 nm (oxidized product) nm.

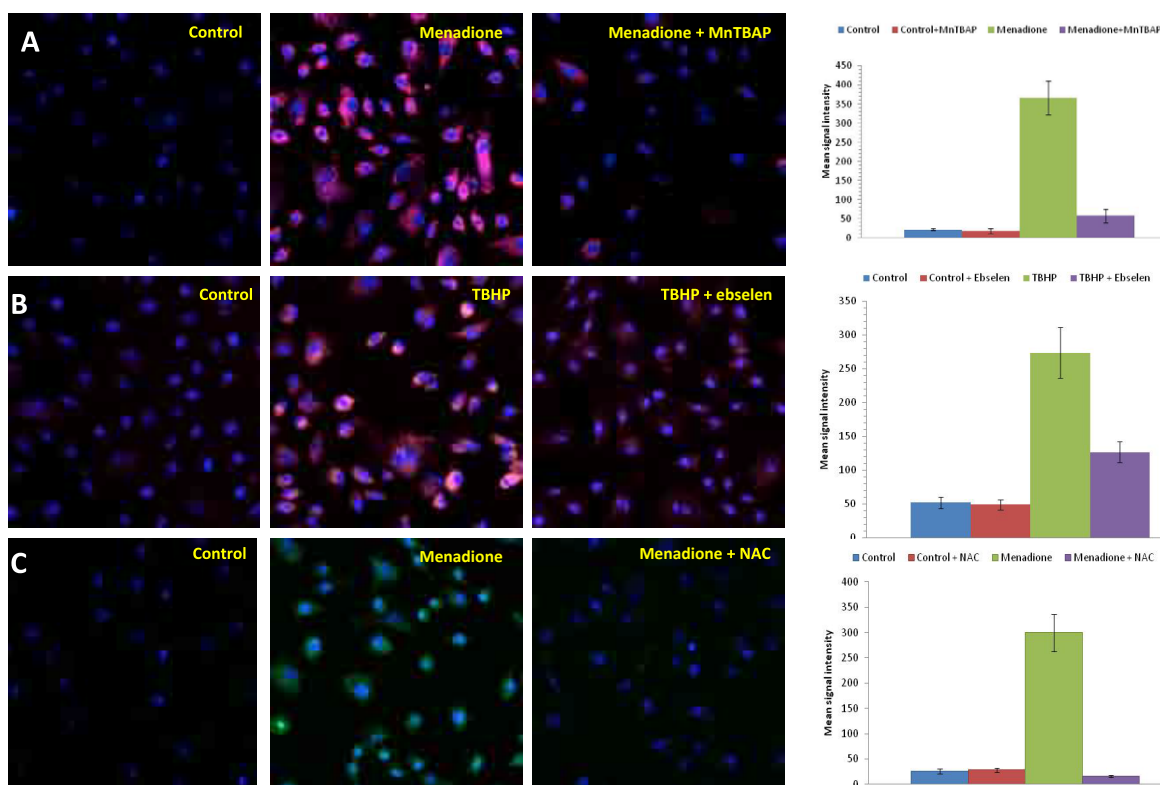


Figure 2: (A) BPAE cells were treated with or without 100µM menadione. Superoxide scavenger 100µM MnTBAP was added to some of the control and menadione-treated wells for the last 30 min of incubation. The cells were stained with CellROX Deep Red Reagent for 30 mins and (B) BPAE cells were treated with or without 200µM tert-butyl hydroperoxide (TBHP). The peroxide scavenger 10µM ebselen was added to some of the control and TBHP-treated cells. The cells were then stained with CellROX Orange Reagent and incubated for 30 mins. (C) BPAE cells were treated with or without 100µM menadione. The antioxidant 50µM N-Acetyl cysteine (NAC) was added to some of the control and menadione-treated wells for the last 30 min of incubation. The cells were then stained with CellROX Green Reagent. The cells were then washed and analyzed on a Thermo Scientific™ ArrayScan™ VTI HCA Reader. Reduction of the signal in antioxidant treated cells confirmed indication of oxidative stress by CellROX reagents.

Conclusion

- CellROX Deep Red, Green and Orange Reagents are new live cell probes with simple workflow for detection of cellular oxidative stress by traditional fluorescence microscopy, automated high content imaging and flow cytometry.
- CellROX Green Reagent is RFP-compatible, formaldehyde-fixable and detergent-resistant and is compatible with multiplexing with other compatible dyes and antibodies.
- CellROX Orange Reagent signal does not survive formaldehyde-fixation but is GFP-compatible and can be multiplexed with other compatible live cells dyes.
- CellROX Reagents work well in serum containing medium and can be read without washing the cells making them very useful for high content imaging-based assay workflows.

Products

1. Molecular Probes™ CellROX Deep Red Reagent, for oxidative stress detection (Cat no. C10422)
2. Molecular Probes™ CellROX Orange Reagent, for oxidative stress detection (Cat no. C10443)
3. Molecular Probes™ CellROX Green Reagent, for oxidative stress detection (Cat no. C10444)
4. Molecular Probes™ CellROX Reagent Variety pack, for oxidative stress detection (Cat no. C10448)

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