molecular probes®
by life technologies™

CellROX® Oxidative Stress Reagents

Catalog nos. C10422, C10443, C10444, C10448

Table 1 Contents and storage

Material	Catalog no.	Amount	Excitation/Emission	Concentration	Storage*
CellROX® Deep Red Reagent	C10422	5 × 50 µL	640/665 nm (Deep Red)	2.5 mM stabilized solution in DMSO	• ≤–20°C • Protect from light • Desiccate
CellROX® Orange Reagent	C10443		545/565 (Orange)		
CellROX® Green Reagent	C10444		485/520 (Green)		
CellROX® Variety Pack	C10448	1 × 50 µL CellROX® Deep Red Reagent 1 × 50 µL CellROX® Orange Reagent 1 × 50 µL CellROX® Green Reagent			

*When stored as directed, the product is stable for 6 months from the date of receipt.

Introduction

CellROX® Oxidative Stress Reagents are fluorogenic probes designed to reliably measure reactive oxygen species (ROS) in live cells. The cell-permeable reagents are non-fluorescent or very weakly fluorescent while in a reduced state and upon oxidation exhibit strong fluorogenic signal. CellROX® Green Reagent is a DNA dye, and upon oxidation, it binds to DNA; 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 in the cytoplasm. The fluorescence resulting from CellROX® Oxidative Stress Reagents can be measured using traditional fluorescence microscopy, high-content imaging and analysis, microplate fluorometry, or flow cytometry. These reagents can be detected using the appropriate benchtop instrument such as the Attune® Acoustic Focusing Cytometer, Tali® Image-based Cytometer, and FLoid™ Cell Imaging Station (See Table 2, page 2).

The staining workflow is simple (Figure 2, page 2), and the reagent can be applied to cells in complete growth medium or buffer. All of the CellROX® Oxidative Stress Reagents are very photostable when compared to traditional ROS detection dyes. In addition, some of the reagents retain their signal after formaldehyde fixation and detergent permeabilization (Table 2, page 2), allowing for assay flexibility and improved workflows compared to those based on classic dyes for ROS detection.

CellROX® Oxidative Stress Reagents specifically detect ROS as shown by inhibition of menadione-induced ROS in endothelial cells (Figure 3, page 3). These probes have been used to evaluate ROS generated by various agents, including lipopolysaccharide, menadione, angiotensin II, and nefazodone in several different live-cell models.

Figure 1 Fluorescence excitation and emission spectra of the oxidized CellROX® Oxidative Stress Reagents. **(A)** CellROX® Deep Red Reagent, **(B)** CellROX® Orange Reagent, **(C)** CellROX® Green Reagent.

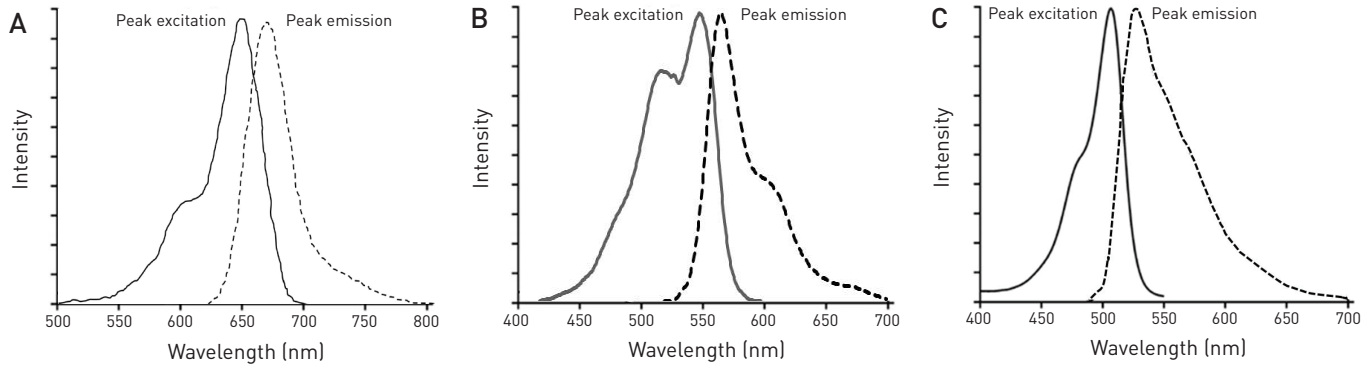


Figure 2 Workflow for CellROX® Oxidative Stress Reagents.

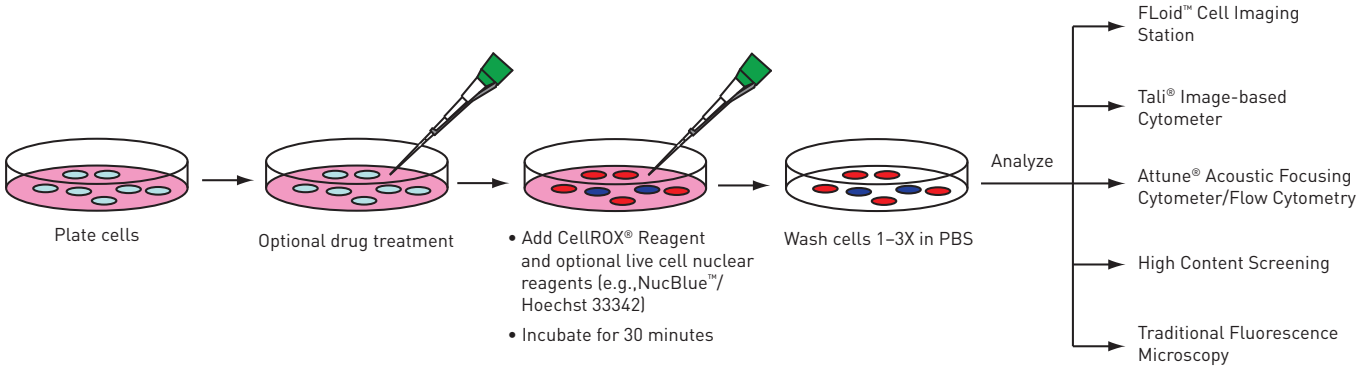


Table 2 Comparison between ROS detection methods

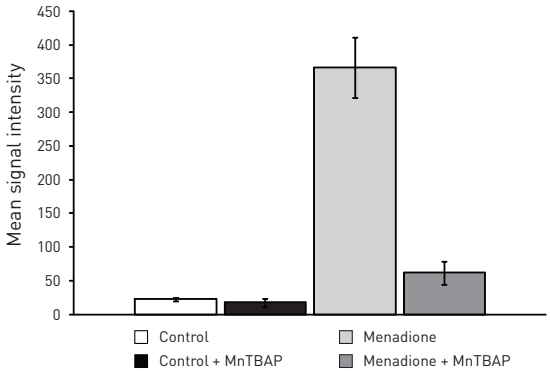
	CellROX® Deep Red Reagent	CellROX® Orange Reagent	CellROX® Green Reagent	H_2-DCFDA*	Dihydroethidium (DHE)
Live cell compatible	Yes	Yes	Yes	Yes	Yes
Labeling in complete medium	Yes	Yes	Yes	No	Yes
Formaldehyde fixable	Yes	No	Yes	No	No
Detergent resistant	No	No	Yes	No	No
Compatible platforms	• Imaging • HCS† • HTS‡ • Flow cytometry • Attune® Acoustic Focusing Cytometer	• Imaging • HCS • Flow cytometry • Tali® Image-based Cytometer	• Imaging • HCS • HTS • Flow cytometry • Tali® Image-based Cytometer • FLoid™ Cell Imaging Station • Attune® Acoustic Focusing Cytometer	• Imaging • HCS • HTS • Flow cytometry • FLoid™ Cell Imaging Station	• Imaging • HCS

* H_2-DCFDA: Dihydrodichlorofluorescein, diacetate; † High-content screening; ‡ High-throughput screening.

Figure 3 Quantitation and statistical analysis of oxidative stress based on staining with CellROX® Oxidative Stress Reagents (***a = The values are significantly different from controls with P ≤ 0.0001 ; ***b = values were significantly different from drug treated cells with P ≤ 0.0001).

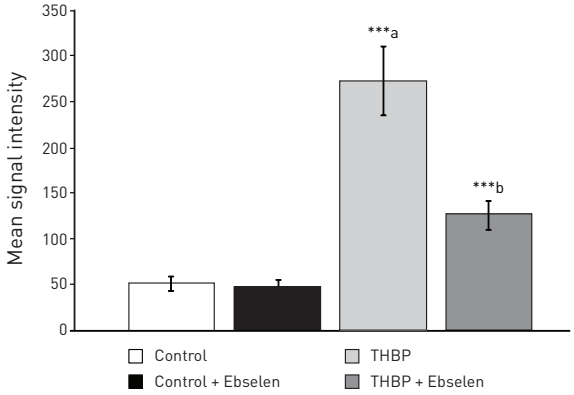
(A) CellROX® Deep Red Reagent. Bovine pulmonary artery endothelial (BPAE) cells were plated in a 96-well plate. The cells were treated with or without 100 µM of menadione for 1 hour at 37°C. 100 µM of superoxide scavenger, MnTBAP, was added to some of the control and menadione-treated wells for the last 30 minutes of incubation. The cells were then stained with 5 µM of CellROX® Deep Red Reagent and Hoechst 33324 by adding the probe to the complete medium and incubating the cells at 37°C for 30 minutes. The cells were then washed with PBS and analyzed on a Thermo Fisher Cellomics ArrayScan® VTI.

A



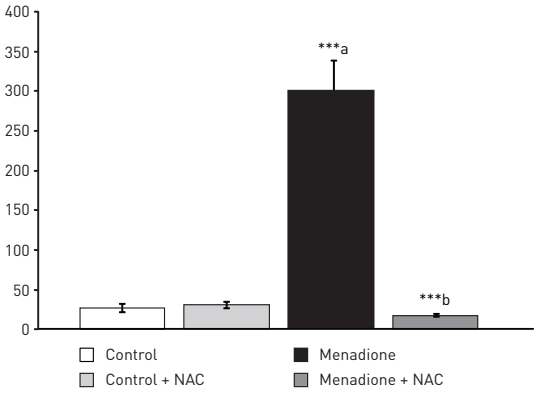
(B) CellROX® Orange Reagent. Bovine pulmonary artery endothelial (BPAE) cells were plated in 96-well plates. The cells were treated with or without 200 µM of tert-butyl hydroperoxide (TBHP) for 2 hours at 37°C. 10 µM of ebselen was added to some of the control and TBHP-treated wells. The cells were then stained with 5 µM of CellROX® Orange Reagent and Hoechst 33342 by adding the probe to the complete medium and incubating the cells at 37°C for 30 minutes. The cells were then washed with PBS and analyzed on a Thermo Fisher Cellomics ArrayScan® VTI. Ebselen treatment inhibited ROS caused by TBHP, confirming that the signal was due to ROS induced by the compound.

B



(C) CellROX® Green Reagent. Bovine pulmonary artery endothelial (BPAE) cells were plated on glass bottom 35-mm MaTtek dishes. BPAE cells were treated with or without 100 µM menadione for 1 hour at 37°C. 50 µM N-acetyl cysteine was added to some of the control and menadione-treated wells. The cells were then stained with 5 µM CellROX® Orange Reagent and Hoechst 33342 by adding the probe to the complete media and incubating at 37°C for 30 minutes. The cells were then washed with PBS and then imaged on a Zeiss Axiovert inverted microscope using a 40X objective. N-acetyl cysteine treatment inhibited ROS caused by TBHP, confirming that the signal was due to ROS induced by the compound.

C



Before Starting

Materials required but not provided

- Cells and culture medium
- Phosphate buffered saline (PBS, pH 7.2–7.6)
- *Optional:* Fixative (i.e., 3.7% formaldehyde in PBS)
- *Optional:* Permeabilization solution (i.e., 0.5% Triton® X-100)

Caution

DMSO is known to facilitate the entry of organic molecules into tissues. Handle reagents containing DMSO using equipment and practices appropriate for the hazards posed by such materials. Dispose of the reagents in compliance with all pertaining local regulations. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Always wear suitable laboratory protective clothing and gloves when handling this reagent.

Experimental Protocols

The following protocol was developed with BPAE, HepG2, U-2 OS, and RAW microphage cells with an optimized CellROX® Reagent concentration of 5 µM, but the assay can be adapted for any cell type. Growth medium, cell density, cell type variations, and other factors may influence labeling. In initial experiments, we recommend testing a concentration range of the reagent to determine the optimal experimental conditions and the concentration for your cell type.

The CellROX® Reagents are sensitive to exposure to light and air; care should be taken not to keep the vials open for long periods of time. Discard any unused material once the vial is opened.

CellROX® Reagent Staining and Detection

Following protocol for staining cells can be adapted for CellROX® Deep Red, CellROX® Orange, and CellROX® Green Reagents, but specific conditions should be determined separately for each reagent.

1. Treat the cells with the test compound or drug and incubate for the recommended time.

   Note: You do not need to remove the medium after test compound or drug treatment.

2. Add the CellROX® Reagent at a final concentration of 5 µM to the cells and incubate for 30 minutes at 37°C.

3. Remove medium and wash cells three times with PBS.

4. *Optional:* If you are using the CellROX® Deep Red or the CellROX® Green Reagent, you can preserve the cells with a formaldehyde-based fixative at this stage. Fixation with 3.7% formaldehyde for 15 minutes is recommended. Analyze the signal within 24 hours for the CellROX® Green Reagent and within 2 hours for the CellROX® Deep Red Reagent.

   Note: You can use the Live Cell Imaging Solution (Cat. no A14291DJ) to keep the cells healthier during analysis with longer duration.

5. *Optional:* You can stain the cells with NucBlue™ Live Cell Stain, a nuclear counterstain, or another counterstain at this time.

6. *Optional:* If you are using the CellROX® Green Reagent, you can permeabilize the cells with 0.5% Triton® X-100 for 10 minutes, if cell permeabilization is required for multiplexing with another reagent.

   Alternatively, you can use the Image-iT® Fixation/Permeabilization Kit (Cat. no. R37602) to fix and permealize the cells.

References

1. Trends Biochem Sci 35, 505 (2010); **2.** Oxid Med Cell Longev 2, 259 (2010); **3.** Free Rad Res 10, 1239 (2010).

Product List **Current prices may be obtained at www.invitrogen.com or from our Customer Service Department.**

Catalog no.	Product Name	Unit Size
C10422	CellROX® Deep Red Reagent *for oxidative stress detection*	5 × 50 µL
C10443	CellROX® Orange Reagent *for oxidative stress detection*	5 × 50 µL
C10444	CellROX® Green Reagent *for oxidative stress detection*	5 × 50 µL
C10448	CellROX® Reagent Variety Pack *for oxidative stress detection*	1 kit
Related Products		
A14291DJ	Live Cell Imaging Solution	500 mL
C10423	CellEvent® Caspase-3/7 Green Detection Reagent *2 mM solution in DMSO*	100 µL
C6827	CM-H_2DCFDA (5-(and-6)-chloromethyl- 2′,7′-dichlorodihydrofluorescein diacetate, acetyl ester) *mixed isomers* *special packaging*	5 × 50 µg
D399	H_2DCFDA (2′,7′-dichlorodihydrofluorescein diacetate (2′,7′-dichlorofluorescin diacetate)	100 mg
D11347	dihydroethidium (hydroethidine) *special packaging*	10 × 1 mg
D23844	DAF-FM diacetate (4-amino-5-methylamino- 2′,7′-difluorofluorescein diacetate) *special packaging*	10 × 50 mg
I10291	Image-iT® DEAD Green™ viability stain	25 µL
I36007	Image-iT® LIVE Green Reactive Oxygen Species Detection Kit	1 kit
M36008	MitoSOX® Red mitochondrial superoxide indicator *for live-cell imaging*	5 × 50 µg
T10096	ThiolTracker™ Violet (Glutathione Detection Reagent) *for 5 microplates*	each
R37602	Image-iT® Fixation/Permeabilization Kit	1 kit
R37603	BackDrop™ Background Suppressor *for live cells*	1 kit
R37605	NucBlue™ Live Cell Stain *Hoechst 33342 special formulation*	1 kit
R37606	NucBlue™ Fixed Cell Stain *DAPI special formulation*	1 kit

Related Platforms

Attune® Acoustic Focusing Cytometer
(Cat. no. 4469120)

Tali® Image-based Cytometer
(Cat. no. T10796)

FLoid™ Cell Imaging Station
(Cat. no. 4471136)

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