

Image-iT® Lipid Peroxidation Kit

for live cell analysis

Catalog number C10445

Table 1 Contents and storage

| Material | Amount | Concentration | Storage | Stability |
|---|-----------|------------------------|--|---|
| Image-iT® Lipid Peroxidation Sensor (Component A) | 5 × 25 µL | 10 mM solution in DMSO | <ul style="list-style-type: none"> • ≤−20°C • Protect from light | When stored as directed, the product is stable for 6 months from the date of receipt. |
| Cumene hydroperoxide (Component B) | 100 µL | 5.4 M | Room temperature | |

Excitation/Emission: In reduced state, the excitation and emission maxima of the Image-iT® Lipid Peroxidation Sensor is 581/591 nm; after oxidation, the probe shifts the excitation and emission to 488/510 nm.

Introduction

Lipid peroxidation generally refers to the oxidative degradation of cellular lipids by reactive oxygen species. Peroxidation of unsaturated lipids affects cell membrane properties¹, signal transduction pathways², apoptosis, and the deterioration of foods and other biological compounds³. Lipid hydroperoxides have been reported to accumulate in oxidatively stressed individuals, including HIV-infected patients⁴. Lipid peroxidation may play a role in aging, as well as pathological processes such as drug-induced phototoxicity and atherosclerosis⁴, and it is often the cause of free radical-mediated damage in cells.

The Image-iT® Lipid Peroxidation Kit is based on BODIPY® 581/591 C11 reagent and is a sensitive fluorescent reporter for lipid peroxidation. Upon oxidation in live cells, fluorescence shifts from red to green of the phenylbutadiene segment of the fluorophore⁵, providing a ratiometric indication of lipid peroxidation by traditional and high content microscopy as well as flow cytometry. This oxidation-dependent emission shift enables ratiometric fluorescence imaging of lipid peroxidation in live cells⁶. Other common applications of BODIPY® 581/591 C11 reagent include fluorometric assays of antioxidant efficacy in plasma⁷ and in lipid vesicles⁸. The oxidation and nitro-oxidation products of this BODIPY® fatty acid have been characterized by mass spectrometry⁹.

- Optimized kit for ratiometric detection of lipid peroxidation in live cells.
- Upon oxidation, the reagent shifts fluorescence emission peak from ~590 nm (red) to ~510 nm (green).
- Cumene hydroperoxide is included as a positive control to induce lipid peroxidation.
- The reagent is provided as a stable, ready-to-use DMSO solution with a simple protocol compatible with standard workflows in fluorescence microscopy.
- The lipid peroxidation sensor is provided as five single-use vials and contains enough reagent for five 96-well plates or 100 coverslips.

Figure 1 Fluorescence emission spectra of the Image-iT® Lipid Peroxidation Sensor, before and after lipid peroxidation.

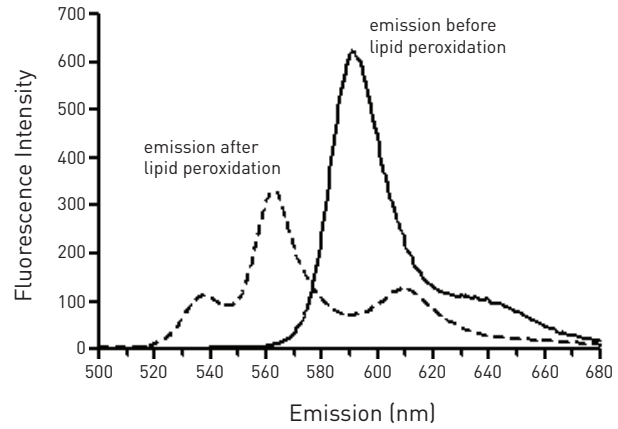


Figure 2 Workflow for Image-iT® Lipid Peroxidation Kit.

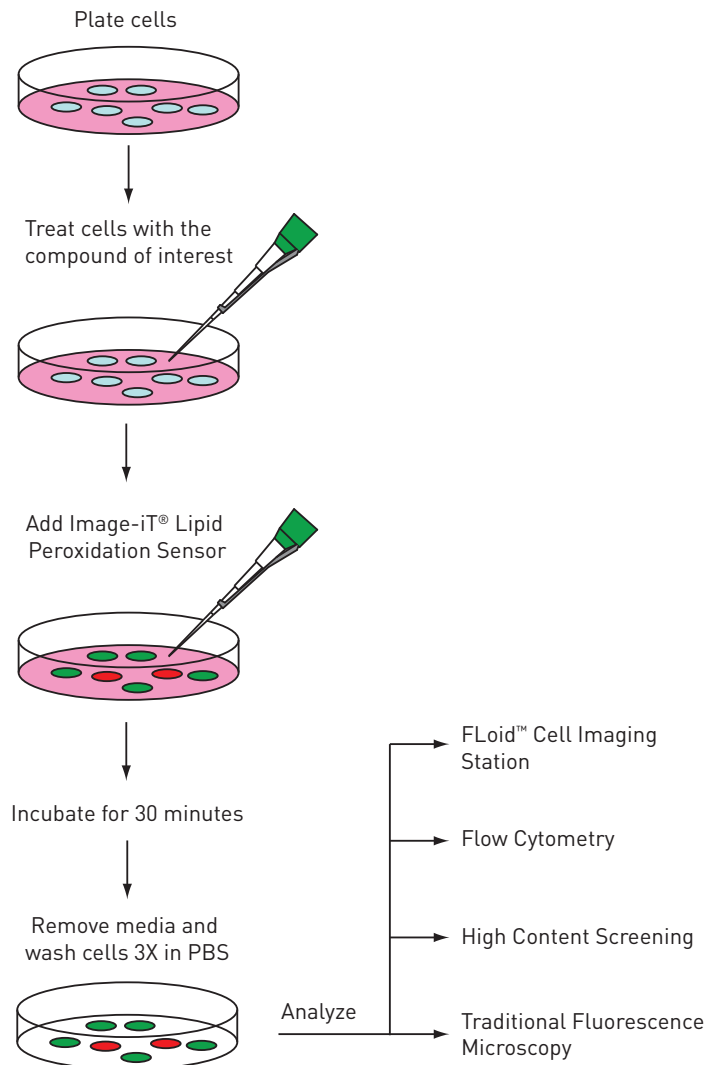
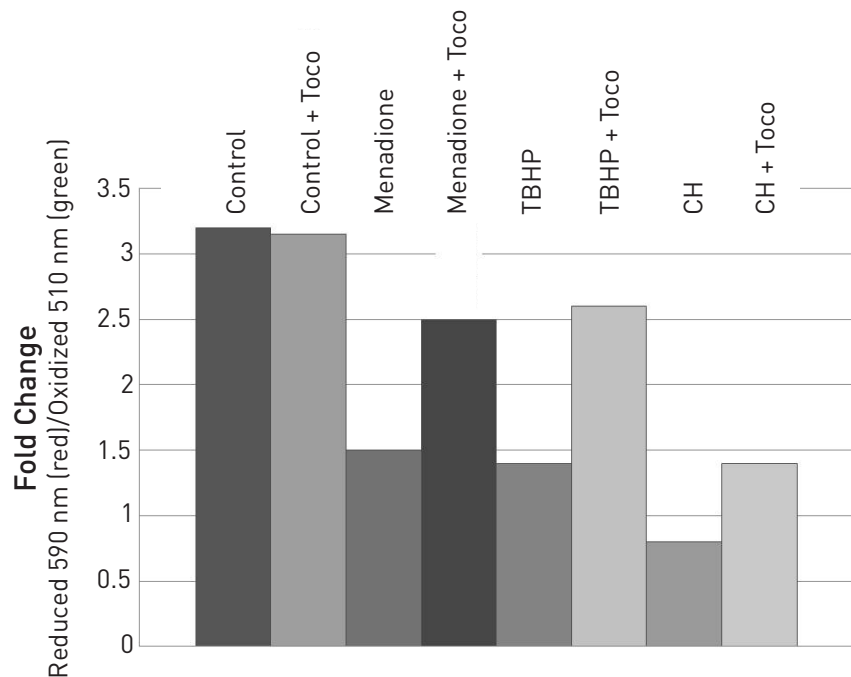


Figure 3 Human osteosarcoma cells (U-2 OS) were plated on 35-mm glass bottom dishes (MatTek) and stained with 10 μM Lipid Peroxidation Sensor for 30 minutes in complete growth medium at 37°C. For tocopherol treatment, the cells were pre-treated with 150 μM α -tocopherol for 30 minutes. The cells were then treated with vehicle (DMSO), 100 μM menadione, 200 μM tert-butyl hydroperoxide (TBHP), or 100 μM cumene hydroperoxide (CH) for 2 hours at 37°C. Cells were stained with Hoechst 33342 during last 30 minutes of compound incubation. The cells were then washed 3X with phosphate buffered saline (PBS) and then imaged on a Zeiss Axiovert inverted microscope using a 40X objective using filters for Hoechst, FITC, and Texas Red® channels. The signal was then quantitated using SlideBook™ 5.0 software and the ratios of the signal from the 590 to 510 channels were used to quantify lipid peroxidation in cells. In control cells, most of the signal is in red channel and the ratio of 590/510 is high. When the cells are treated with ROS producing reagents (i.e., menadione, TBHP, and CH), the ratios are lower. Tocopherol pre-treatment decreases the lipid peroxidation in cells as seen from increased ratios in tocopherol pre-treated samples.



Before Starting

Materials required but not provided

- Cells and culture medium
- Phosphate buffered saline (PBS, pH 7.2–7.6)

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.

Cumene hydroperoxide (Component B) is toxic. Use appropriate precautions when using this compound.

Prepare stock solution of cumene hydroperoxide

To prepare a 100 mM stock solution of cumene hydroperoxide, add 1 μL of cumene hydroperoxide (Component B, 5.4 M) to 54 μL of 100% ethanol.

Experimental Protocols

The following protocol was developed with BPAE, HepG2, U-2OS cells with an optimized Image-iT® Lipid Peroxidation Sensor concentration of 10 µM, but the assay can be adapted to any cell type. Growth media, cell density, cell type variations may influence labeling. In initial experiments, we recommend testing a concentration range of Image-iT® Lipid Peroxidation Sensor to determine the optimal dye concentration for your cell type.

- Labeling and detection**
1. Plate cells at a desired density and incubate them overnight at 37°C.
 2. Treat the cells with the compound of interest and incubate for the recommended time.
 3. Add Image-iT® Lipid Peroxidation Sensor (Component A) at a final concentration of 10 µM to the cells. Then incubate for another 30 minutes at 37°C.
 4. Remove media and wash cells three times with PBS.
 5. Read the fluorescence at two separate wavelengths; one at excitation/emission of 581/591 nm (Texas Red® filter set) for the reduced dye, and the other at excitation/emission of 488/510 nm (traditional FITC filter set) for the oxidized dye (see **Imaging and Analysis**, below).
 6. The ratio of the emission fluorescence intensities at 590 nm to 510 nm gives the read-out for lipid peroxidation in cells.

Inducing oxidative stress with cumene hydroperoxide

1. Plate cells at a desired density and incubate them overnight at 37°C.
2. Add cumene hydroperoxide (Component B) to the cells at a final concentration of 100 µM and incubate at 37°C for 2 hours.
3. Stain with Image-iT® Lipid Peroxidation Sensor (Component A) by adding the reagent at a final concentration of 10 µM for the last 30 minutes of cumene hydroperoxide incubation. Add Hoechst 33342 dye at this point if performing high content imaging.
4. Remove media and wash cells three times with PBS, and image cells within 2 hours of staining.

Note: We recommend that you keep the cells in Live Cell Imaging Solution (Cat. no A14291DJ) to keep them healthier.

Imaging and analysis

Perform fluorescence imaging using traditional Texas Red® (590 nm) and FITC (510 nm) emission filters. Determine lipid peroxidation by quantitating the fluorescence intensities and calculating the ratio of intensity in Texas Red® channel to the intensity in FITC channel.

If using high content imaging, use an additional channel (NucBlue™/Hoechst 33342) to identify cells. Automatic calculation of ratios can be set up. Refer to the appropriate instrument user guide to set up the ratio calculation.

References

1. Biochim Biophys Acta 1487, 61 (2000); 2. Circ Res 84, 229 (1999); 3. Plant Physiol 125, 1591 (2001); 4. J Biol Chem 269, 798 (1994);
5. Methods Enzymol 319, 603 (2000); 6. FEBS Lett 453, 278 (1999); 7. Anal Biochem 354, 290 (2006); 8. Biochemistry 45, 8135 (2006);
9. Free Radic Biol Med 36, 1635 (2004).

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

| Catalog no. | Product Name | Unit Size |
|-------------------------|---|------------|
| C10445 | Image-iT® Lipid Peroxidation Kit *for live cell analysis* | 1 kit |
| Related Products | | |
| A14291DJ | Live Cell Imaging Solution | 500 mL |
| C10422 | CellROX® Deep Red Reagent *for oxidative stress detection* | 5 × 50 µL |
| C10423 | CellEvent® Caspase-3/7 Green Detection Reagent *2 mM solution in DMSO* | 100 µL |
| C10443 | CellROX® Orange Reagent *for oxidative stress detection* | 5 × 50 µL |
| C10444 | CellROX® Green Reagent *for oxidative stress detection* | 5 × 50 µL |
| C10446 | Click-iT® Lipid Peroxidation Imaging Kit - Alexa Fluor® 488 | 1 kit |
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| D399 | H ₂ DCFDA (2',7'-dichlorodihydrofluorescein diacetate (2',7'-dichlorofluorescein 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 | ThioTracker™ Violet (Glutathione Detection Reagent) *for 5 microplates* | each |
| R37603 | BackDrop™ Background Suppressor *for live cells* | 1 kit |
| R37605 | NucBlue™ Live Cell Stain *Hoechst 33342 special formulation* | 1 kit |

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