7040 Virginia Manor Road
Beltsville, MD 20705, USA
Web: www.abpbio.com;
Email: info@abpbio.com

Lipid Peroxidation Kit
Catalog Number: A067

Table 1. Kit Components and Storage

Material	Amount	Concentration	Storage	Stability
Lipid Peroxidation Sensor (Component A)	**125 µL**	**10 mM in DMSO**	• **-20 °C,** • **Protect from light**	**The product is stable for 1 year when stored as directed.**
Cumene hydroperoxide (Component B)	**100 µL**	**5.4 M**		

Number of assays: 500 assays.
Excitation/Emission: In reduced state, the excitation and emission maxima of the Lipid Peroxidation Sensor is 581/591 nm; after oxidation, the probe shifts the excitation and emission to 488/510 nm.

Introduction

Lipid peroxidation is the oxidative degradation of cellular lipids by free radicals from reactive oxygen species, which can degrade lipids containing carbon-carbon double bonds such as phospholipids and polyunsaturated fatty acids. Oxidation of lipids leads to the generation of lipid peroxides and can cause damage to cell membranes, resulting in changes to signal transduction pathways and eventually leading to cell death. Lipid peroxidation is involved in apoptosis and one of the main contributors to ferroptosis, an iron-dependent, non-apoptotic form of cell death. Oxidative stress from lipid peroxidation plays a role in aging, as well as pathologies such as cancer, atherosclerosis, and neurodegenerative diseases.

The Lipid Peroxidation Kit enables the detection of lipid peroxidation in live cells through oxidation of BODIPY™ 581/591 C11 reagent. This reagent localizes to membranes throughout live cells and upon oxidation by lipid hydroperoxides, displays a shift in peak fluorescence emission from ~590 nm to ~510 nm. Fluorescence from live cells shifts from red to green, providing a ratiometric indication of lipid peroxidation compatible with traditional and high content microscopy, as well as flow cytometry. This kit also includes cumene hydroperoxide as a positive control compound to induce lipid peroxidation in cells.

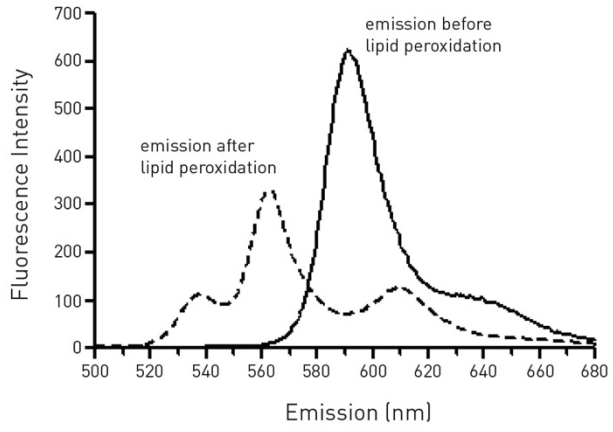

Figure 1. Fluorescence emission spectra of the Lipid Peroxidation Sensor, before and after lipid peroxidation.

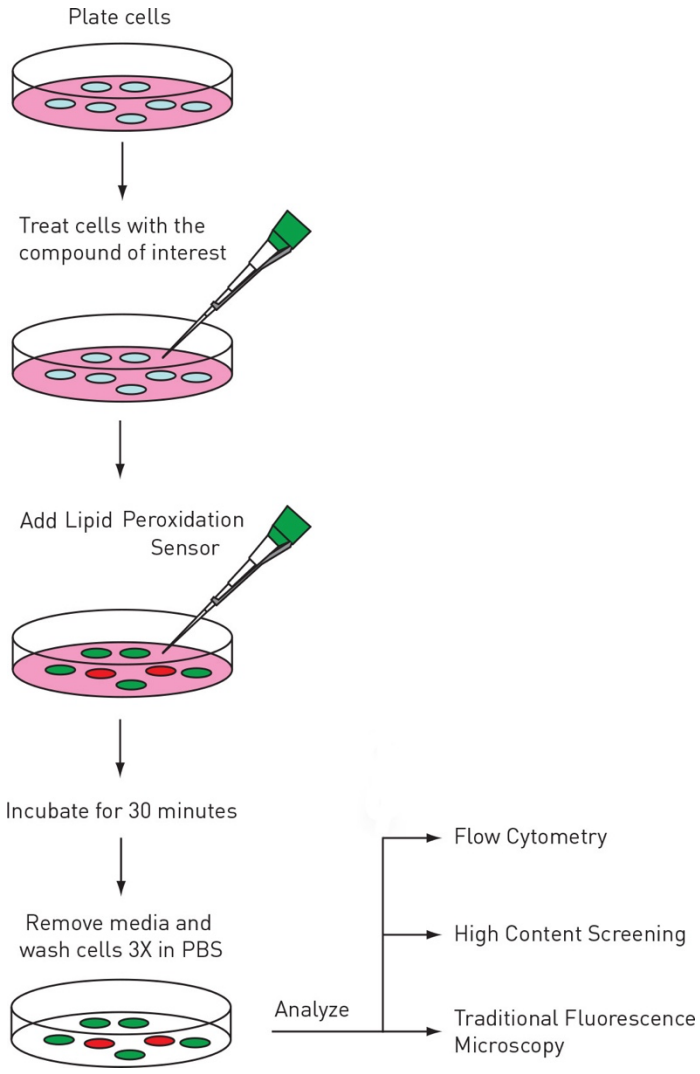

Figure 2. Workflow for Lipid Peroxidation Kit.

Prepare cumene hydroperoxide stock solution

- 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 Protocol

The following protocols provide general guidelines. Growth media, cell density, cell type variations may influence labeling. In initial experiments, we recommend testing a concentration range of Lipid Peroxidation Sensor to determine the optimal dye concentration for your cell type.

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 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.

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5. Read the fluorescence at to 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.
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 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.