

## OxiVision™ Ratiometric Mitochondrial Lipid Peroxidation Sensor

Catalog number: 21511  
Unit size: 5x100 ug

Component	Storage	Amount (Cat No. 21511)
OxiVision™ Ratiometric Mitochondrial Lipid Peroxidation Sensor	Freeze (< -15 °C), Minimize light exposure	5 X 100 µg

### OVERVIEW

A wide range of diseases is considered to result from mitochondrial oxidative damage that is associated with the lipid peroxidation of mitochondrial inner membranes. There are no specific methods to assess mitochondrial lipid peroxidation in live cells. To address this unmet need, AAT Bioquest has developed the OxiVision™ Ratiometric Mitochondrial Lipid Peroxidation Sensor, a fluorescent mitochondria-targeted probe that detects lipid peroxidation in live cells. OxiVision™ Ratiometric Mitochondrial Lipid Peroxidation Sensor enters cells rapidly, and selectively accumulates in mitochondria. It has a high specificity for the detection of mitochondrial lipid peroxidation. Mitochondrial lipid peroxidation results in a great change of fluorescence intensity ratio at 520 nm/590 nm, which can conveniently be monitored by fluorimetry, fluorescence microscopy or a fluorescence microplate reader. The fluorescence intensities are collected with FITC and Cy3/TRITC filter sets respectively at 520 nm/590 nm.

### AT A GLANCE

#### Important Note

Before initial use, thaw OxiVision™ Ratiometric Mitochondrial Lipid Peroxidation Sensor at room temperature and briefly centrifuge to collect the dried pellet.

#### Protocol Summary

1. Prepare and treat cells as needed in growth medium
2. Stain cells with OxiVision™ Ratiometric Mitochondrial Lipid Peroxidation Sensor working solution
3. Incubate samples at 37 °C in a 5% CO<sub>2</sub> incubator for 30–60 minutes
4. Monitor fluorescence intensity with FITC and Cy3 filters

### KEY PARAMETERS

#### Fluorescence microscope

Emission	FITC and Cy3/TRITC Filter
Excitation	FITC and Cy3/TRITC Filter
Recommended plate	Black wall/clear bottom

### PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Protect from light and avoid repeated freeze-thaw cycles

#### OxiVision™ Ratiometric Mitochondrial Lipid Peroxidation Sensor Stock Solution

1. Prepare 2 to 5 mM stock solution in DMSO. For example, add 25 µL of DMSO into one vial to create a 5 mM OxiVision™ Ratiometric Mitochondrial Lipid Peroxidation Sensor stock solution.

**Note:** Prepare single-use aliquots of the stock solution and store

Tel: 408-733-1055 | Fax: 408-733-1304 | Email: support@aatbio.com

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at ≤ -20°C. Protect from light and avoid repeated freeze-thaw cycles.

### PREPARATION OF WORKING SOLUTION

#### OxiVision™ Ratiometric Mitochondrial Lipid Peroxidation Sensor Working Solution

1. Prepare a 500 to 1000 nM OxiVision™ Ratiometric Mitochondrial Lipid Peroxidation Sensor working solution. For example, add 2 µL of 5 mM OxiVision™ Ratiometric Mitochondrial Lipid Peroxidation Sensor stock solution to 10 mL of cell culture medium or HHBS buffer (AAT cat# 20011).

**Note:** Protect the working solution from light by covering it with foil or placing it in the dark.

**Note:** For best results, use the solution within 2 hours of its preparation.

**Note:** 10 mL of working solution is enough for 100 tests.

### SAMPLE EXPERIMENTAL PROTOCOL

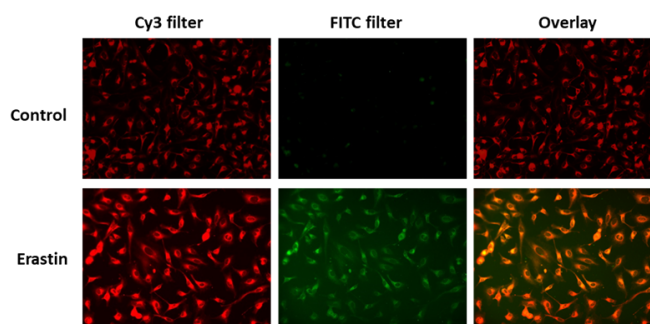
1. Plate cells as needed in a 96-well black wall, clear bottom plate.
2. Add treatment to induce lipid peroxidation.
3. Add 100 µL of OxiVision™ Ratiometric Mitochondrial Lipid Peroxidation Sensor working solution to cells.
4. Incubate cells at 37 °C in a 5% CO<sub>2</sub> incubator for 30–60 minutes, protected from light.

**Note:** The optimal concentration and incubation time may vary by cell line; we recommend testing with different concentrations.

5. *Optional:* Remove the dye working solution and wash cells twice with HHBS buffer if background fluorescence is observed.

6. Add HHBS buffer and analyze the cells using a fluorescence microscope with a FITC and Cy3 filter set.

### EXAMPLE DATA ANALYSIS AND FIGURES



**Figure 1.** Fluorescence response of OxiVision™ Ratiometric Mitochondrial Lipid Peroxidation Sensor (1000 nM) in HeLa cells with or without Erastin (10  $\mu$ M) treatment at 37 °C in a 5% CO<sub>2</sub> incubator for 30 minutes. The fluorescence intensities were monitored with fluorescence microscopy using FITC and Cy3 filters.

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