

# Vybrant® MitoFOX™ Green Mitochondrial Lipid Peroxidation Kit (V36001)

## Quick Facts

### Storage upon receipt:

- $\leq -20^{\circ}\text{C}$
- Desiccate
- Protect from light

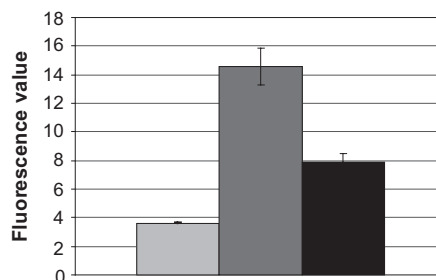
**Ex/Em of reaction product:** 496/524 nm

## Introduction

Generation of reactive oxygen species (ROS) and the subsequent oxidative modification of biomolecules such as lipids, proteins, and nucleic acids are inevitable events for aerobic organisms. While small amounts of ROS can be beneficial for inducing gene expression and cell proliferation, excess amounts of ROS resulting in oxidative stress are implicated in a variety of disease states including atherosclerosis, carcinogenesis, ischemic reperfusion injury, and several neurodegenerative disorders.<sup>1</sup> Polyunsaturated fatty acids and their esters are major targets for ROS, and their oxidation in membranes plays an important role in oxidative stress *in vivo*.

The MitoFOX Green reagent is the first probe from Molecular Probes designed to directly detect lipid peroxidation in the mitochondria of live cells. The weakly fluorescent MitoFOX Green reagent localizes to the mitochondria and exhibits bright green fluorescence (excitation and emission maxima of 496 and 524 nm, respectively) upon oxidation by lipid hydroperoxides. This reagent is approximately 200-fold more sensitive than diphenyl-1-pyrenylphosphine (DPPP), a UV-excitable indicator of lipid peroxidation. Furthermore, the MitoFOX Green reagent can be used in combination with other live cell probes, such as MitoTracker® Red CMXRos or dihydroethidium, and is fixable with aldehyde-based fixatives. Scientists at Molecular Probes have used this indicator in a staurosporine model for apoptosis, to detect lipid peroxidation in mitochondria that preceded other events associated with the execution of apoptosis.

The Vybrant MitoFOX Green Mitochondrial Lipid Peroxidation Assay Kit contains the MitoFOX Green reagent, DMSO for dissolving the reagent, and a solution of cumene hydroperoxide. Cumene hydroperoxide is a lipophilic hydroperoxide and can be used as a positive control for inducing ROS production. In mitochondria, the MitoFOX Green reagent does not significantly respond to hydrogen peroxide, a commonly used oxidant. The



■ no oxidant   ■ cumene hydroperoxide   ■ cumene hydroperoxide+BHT

**Figure 1.** Live Jurkat cells were exposed to 500 nM MitoFOX Green reagent and then oxidatively stressed with 100  $\mu\text{M}$  cumene hydroperoxide in the absence or presence of 50  $\mu\text{M}$  butylated hydroxytoluene (BHT), a lipophilic antioxidant. A 3.5X increase in fluorescence was observed when the cells were treated with cumene hydroperoxide; this oxidative effect was substantially reduced in the presence of BHT (right).

oxidized MitoFOX Green reagent can be visualized by any instrument that can detect fluorescein, including flow cytometers (Figure 1), microscopes, fluorometers, and fluorescence-based microplate readers. This reagent should prove useful to researchers investigating the role of mitochondrial lipid peroxidation in various disease states.

## Materials

### Kit Contents

- **MitoFOX Green reagent** (Component A), 10 vials
- **Dimethylsulfoxide (DMSO)** (Component B), 400  $\mu\text{L}$
- **Cumene hydroperoxide** (Component C) 1.0 mL of a 10 mM (0.15%) solution in deionized water ( $\text{dH}_2\text{O}$ )

### Storage and Handling

Upon receipt, store the kit at  $\leq -20^{\circ}\text{C}$ . The MitoFOX Green reagent (Component A) should be kept desiccated and protected from light. When stored properly, the kit components should be stable for at least six months from the date of purchase.

## Assay Protocol

### Stock Solution Preparation

**1.1 Prepare a stock solution of the MitoFOX Green reagent.** Dissolve one vial of the MitoFOX Green reagent (Component A) in 25  $\mu\text{L}$  of the provided DMSO (Component B). If stored at

≤-20°C and protected from light, this 2 mM stock solution should remain stable for several weeks.

**1.2 Prepare a working solution of cumene hydroperoxide (optional).** Dilute the provided 10 mM stock solution 100-fold in 1X PBS to make a 100 µM working solution. Prepare only enough working solution to meet the immediate experimental needs, and use within 24 hours.

### **Experimental Protocol**

The following protocol is provided as a guide and is suitable for both adherent and non-adherent cell lines. Optimal loading concentration, time, and temperature will need to be determined empirically. In general, it is desirable to use the minimum dye concentration required to yield fluorescence signals with an adequate signal-to-noise ratio.

**2.1 Prepare cells.** Prepare cells according to the normal procedures for the cell line of interest.

**2.2 Prepare a working solution of the MitoFOX Green reagent.** Dilute the 2 mM MitoFOX Green/DMSO stock solution (prepared in step 1.1) into suitable buffer, such as Hanks' balanced salt solution (HBSS) or PBS. A suggested starting concentration range is 50–500 nM. Prepare a fresh working solution before each use.

**2.3 Label the cells with the MitoFOX Green reagent.** Incubate the cells with the diluted MitoFOX Green reagent for 15–30 minutes at 37°C.

**2.4 Wash cells.** After incubating, wash the cells with buffer to remove excess reagent. Replace the staining solution with fresh buffer or medium.

**2.5 Prepare a positive control (optional).** For a positive control, stimulate cells with 100 µM cumene hydroperoxide (prepared in step 1.2) for an additional 15–30 minutes at 37°C.

**2.6 Detect signal.** The fluorescence excitation and emission maxima of the oxidized MitoFOX Green reagent are 496 and 524 nm, respectively. Because these maxima are very similar to those of fluorescein, detection systems designed for fluorescein or FITC can be used. Cells should show distinct mitochondrial labeling; cells treated with cumene hydroperoxide should show enhanced fluorescence. Please note that the MitoFOX Green reagent is susceptible to light-induced auto-oxidation; thus, prolonged exposure to excitation light can result in a strong fluorescence signal that is not due to lipid peroxidation.

---

## **References**

1. Free Rad Biol Med 31, 164 (2001).

---

## **Product List** *Current prices may be obtained from our Web site or from our Customer Service Department.*

<b>Cat #</b>	<b>Product Name</b>	<b>Unit Size</b>
V36001	Vybrant® MitoFOX™ Green Mitochondrial Lipid Peroxidation Assay Kit.....	1 kit

---

## Contact Information

Further information on Molecular Probes' products, including product bibliographies, is available from your local distributor or directly from Molecular Probes. Customers in Europe, Africa and the Middle East should contact our office in Leiden, the Netherlands. All others should contact our Technical Assistance Department in Eugene, Oregon.

Please visit our Web site — [www.probes.com](http://www.probes.com) — for the most up-to-date information.

### **Molecular Probes, Inc.**

29851 Willow Creek Road, Eugene, OR 97402  
Phone: (541) 465-8300 • Fax: (541) 335-0504

**Customer Service:** 6:00 am to 4:30 pm (Pacific Time)  
Phone: (541) 335-0338 • Fax: (541) 335-0305 • [order@probes.com](mailto:order@probes.com)

**Toll-Free Ordering for USA and Canada:**  
Order Phone: (800) 438-2209 • Order Fax: (800) 438-0228

**Technical Assistance:** 8:00 am to 4:00 pm (Pacific Time)  
Phone: (541) 335-0353 • Toll-Free (800) 438-2209  
Fax: (541) 335-0238 • [tech@probes.com](mailto:tech@probes.com)

### **Molecular Probes Europe BV**

Poortgebouw, Rijnsburgerweg 10  
2333 AA Leiden, The Netherlands  
Phone: +31-71-5233378 • Fax: +31-71-5233419

**Customer Service:** 9:00 to 16:30 (Central European Time)  
Phone: +31-71-5236850 • Fax: +31-71-5233419  
[eurorder@probes.nl](mailto:eurorder@probes.nl)

**Technical Assistance:** 9:00 to 16:30 (Central European Time)  
Phone: +31-71-5233431 • Fax: +31-71-5241883  
[eurotech@probes.nl](mailto:eurotech@probes.nl)

Molecular Probes' products are high-quality reagents and materials intended for research purposes only. These products must be used by, or directly under the supervision of, a technically qualified individual experienced in handling potentially hazardous chemicals. Please read the Material Safety Data Sheet provided for each product; other regulatory considerations may apply.

Several of Molecular Probes' products and product applications are covered by U.S. and foreign patents and patents pending. Our products are not available for resale or other commercial uses without a specific agreement from Molecular Probes, Inc. We welcome inquiries about licensing the use of our dyes, trademarks or technologies. Please submit inquiries by e-mail to [busdev@probes.com](mailto:busdev@probes.com). All names containing the designation ® are registered with the U.S. Patent and Trademark Office.

Copyright 2004, Molecular Probes, Inc. All rights reserved. This information is subject to change without notice.