OxiVision Green™ Hydrogen Peroxide Sensor

Ordering Information	*Storage Conditions*
Product Number: 21505 (1 mg)	Store at -20 °C, desiccated and protected from light Expiration date is 12 months from the date of receipt

Introduction

Despite the importance of H_2O_2 to human health and disease, the molecular mechanisms of its production, accumulation, trafficking, and function are insufficiently understood due to the lack of sensitive and specific H_2O_2 sensors that can be used in live cells. The limitations of currently available H_2O_2-responsive probes include interfering background fluorescence from other ROS, the need for an external activating enzyme, lack of water solubility or compatibility, and/or excitation profiles in the ultraviolet region. OxiVision Green™ hydrogen peroxide sensor is non-fluorescent and displays no absorption in the visible region. The addition of H_2O_2 triggers a prompt fluorescence increase with concomitant growth of a visible wavelength absorption band. This probe has a large dynamic range due to its binary absorption/emission response. The fluorescence response of OxiVision Green™ hydrogen peroxide sensor is H_2O_2-selective. OxiVision Green™ hydrogen peroxide sensor exhibits a >100-fold selectivity for H_2O_2 over similar ROS such as $O_2^{\bullet-}$, NO, or OCl^-.

Chemical and Physical Properties

Molecular Weight: ~600
Solvent: dimethylsulfoxide (DMSO)
Spectral Properties: Excitation = 490 nm; Emission = 514 nm

Protocol for One 96-well Plate

Following is our recommended protocol for H_2O_2 assay in solution and live cells. This protocol only provides a guideline, and should be modified according to your specific needs.

Brief Summary

Prepare 10 µM OxiVision Green™ hydrogen peroxide sensor in 20 mM HEPES buffer (50 µL) → Add H_2O_2 standards or test samples (50 µL) → Incubate at room temperature for 15-60 min → Read fluorescence intensity at Ex/Em = 490 nm/525 nm

1. Prepare OxiVision Green™ hydrogen peroxide sensor working solution:

1.1 Prepare a 2 to 5 mM stock solution of OxiVision Green™ hydrogen peroxide sensor in high-quality, anhydrous DMSO. The stock solution should be used promptly; any remaining solution should be aliquoted and frozen at -20ºC.
Note: Avoid repeated freeze-thaw cycles.

1.2 Prepare a 2X OxiVision Green™ hydrogen peroxide sensor working solution: On the day of the experiment, either dissolve OxiVision Green™ hydrogen peroxide sensor solid in DMSO or thaw an aliquot of the sensor stock solution to room temperature. Prepare a 2X working solution at the concentration ranging from 2 to 20 µM in 20 mM Hepes buffer or buffer of your choice, pH 7. It is recommended to use OxiVision Green™ hydrogen peroxide sensor at the final concentration of 5 µM to measure H_2O_2 concentration in solution.

2. Run H_2O_2 Assay in supernatants:

2.1 Add 50 µL of 2X OxiVision Green™ hydrogen peroxide sensor working solution (from Step 1.2) to each well of the H_2O_2 standard, blank control, and test samples to make the total H_2O_2 assay volume of 100 µL/well.

Note: For a 384-well plate, add 25 µL of sample and 25 µL of 2X OxiVision Green™ hydrogen peroxide sensor working solution into each well.

2.2 Incubate the reaction at room temperature for 15 to 60 minutes, protected from light.
2.3 Monitor the fluorescence increase with a fluorescence plate reader at Ex/Em = 490/525 nm.
2.4 The fluorescence in blank wells (with the assay buffer only) is used as a control, and is subtracted from the values for those wells with the H_2O_2 reactions.

3. Run H_2O_2 Assay in Live Cells:

OxiVision Green™ hydrogen peroxide sensor can be loaded passively into living cells and report the micromolar changes in intracellular H_2O_2 concentrations. The following is a suggested microscope imaging protocol which can be modified according to your specific research needs.

3.1 The OxiVision Green™ hydrogen peroxide sensor working solution should be prepared as Step 1.2. It is recommended to use PBS or Hanks Balanced Salt Solution (HBSS) with 20 mM Hepes buffer instead of 20 mM Hepes buffer only.
3.2 Treat the cells as desired.
3.3 Incubate the cells with OxiVision Green™ hydrogen peroxide sensor working solution for 5 to 60 min or a desired period of time. Wash the cells with PBS buffer twice.
3.4 Monitor the fluorescence increase at Ex/Em = 490/525 nm with a fluorescence plate reader with bottom read mode. Or image the fluorescence change by a fluorescence microscopy using the FITC channel.

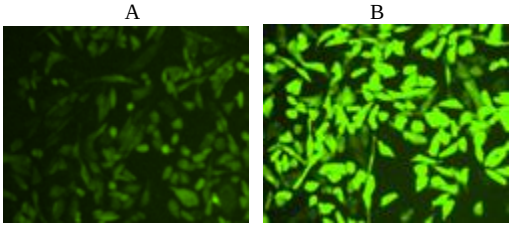

Figure 1. Images of live CHO-K1 cells in a 96-well Costar black plate. The CHO-K1 cells were stained with OxiVision Green™ hydrogen peroxide sensor. A: Control cells. B: Cells treated with H_2O_2 at the final concentration of 100 µM or 5 min at room temperature.

Assay Protocol for Flow Cytometry Analysis

Brief Summary

Prepare cells in growth medium → Stain the cells at 37 °C for 30 minutes → Treat the cells with test compounds to induce H_2O_2 → Monitor the fluorescence intensity with a flow cytometer

1. Prepare cells:

Prepare cells at the density from 5×10^5 to 1×10^6 cells/mL.
Note: Each cell line should be evaluated on the individual basis to determine the optimal cell density for apoptosis induction.

2. Prepare OxiVision Green™ hydrogen peroxide sensor stock solution:

Prepare a 2 to 5 mM stock solution of OxiVision Green™ hydrogen peroxide sensor in high-quality, anhydrous DMSO. The stock solution should be used promptly; any remaining solution should be aliquoted and frozen at -20 ºC.
Note: Avoid repeated freeze-thaw cycles.

3. Run H_2O_2 Assay:

3.1 Stain cells with OxiVision Green™ peroxide sensor in full medium or in your desired buffer at 37 ºC for 30 minutes, protected from light.
3.2 Treat cells with test compounds in full medium or in your desired buffer at 37 ºC for desired period of time. For control samples (untreated cells), add the corresponding amount of compound buffer.
3.3 Monitor the fluorescence intensity at FITC channel (Ex/Em = 490/530 nm) using a flow cytometer. Gate on the cells of interest, excluding debris.