Cell Meter™ Intracellular Fluorimetric Hydrogen Peroxide Assay Kit *Green Fluorescence*

Catalog number: 11503
Unit size: 200 Tests

Component	Storage	Amount
Component A: OxiVision Green™ hydrogen peroxide sensor	Freeze (< -15 °C), Minimize light exposure	1 vial
Component B: H2O2	Freeze (< -15 °C), Minimize light exposure	1 vial (3% stabilized solution, 200 µL)
Component C: Assay Buffer	Freeze (< -15 °C)	1 bottle (20 mL)
Component D: DMSO	Freeze (< -15 °C)	1 vial (200 µL)

OVERVIEW

Hydrogen peroxide (H2O2) is a reactive oxygen metabolic by-product that serves as a key regulator for a number of oxidative stress-related states. It is involved in a number of biological events that have been linked to asthma, atherosclerosis, diabetic vasculopathy, osteoporosis, a number of neurodegenerative diseases and Down's syndrome. Perhaps the most intriguing aspect of hydrogen peroxide biology is the recent report that antibodies have the capacity to convert molecular oxygen into hydrogen peroxide to contribute to the normal recognition and destruction processes of the immune system. Measurement of this reactive species will help to determine how oxidative stress modulates varied intracellular pathways. This Cell Meter™ Hydrogen Peroxide Assay Kit uses our unique OxiVision™ Green hydrogen peroxide sensor to quantify hydrogen peroxide in live cells. OxiVision™ Green is cell-permeable, and generates the green fluorescence when it reacts with hydrogen peroxide. The kit is an optimized 'mix and read' assay format that is compatible with HTS liquid handling instruments.

AT A GLANCE

Protocol Summary for Solution Assay

1. Prepare and add OxiVision Green™ hydrogen peroxide sensor working solution (50 µL)
2. Add H_2O_2 standards or test samples (50 µL)
3. Incubate at room temperature for 15 - 60 minutes
4. Read fluorescence intensity at Ex/Em = 490/525 nm (Cutoff = 515 nm)

Important Thaw all the kit components at room temperature before starting the experiment.

Protocol Summary for Live Cell Assay

1. Prepare cells in growth medium
2. Stain cells with OxiVision Green™ hydrogen peroxide sensor working solution and incubate for your desired period of time
3. Treat cells with test compounds
4. Monitor fluorescence intensity at Ex/Em = 490/525 nm (Cutoff = 515 nm) with bottom read mode

Important 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 that can be modified to meet specific research needs.

KEY PARAMETERS

Fluorescence microscope

Excitation	FITC channel
Emission	FITC channel
Recommended plate	Black wall/clear bottom

Fluorescence microplate reader

Excitation	490 nm
Emission	525 nm
Cutoff	515 nm
Recommended plate	Black wall/clear bottom
Instrument specification(s)	Bottom read mode

CELL PREPARATION

For guidelines on cell sample preparation, please visit https://www.aatbio.com/resources/guides/cell-sample-preparation.html

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. Avoid repeated freeze-thaw cycles.

1. OxiVision Green™ hydrogen peroxide sensor stock solution (250X)
Add 50 µL of DMSO (Component D) into the vial of OxiVision Green™ hydrogen peroxide sensor (Component A) to make 250X OxiVision Green™ hydrogen peroxide sensor stock solution.

Note Protect from light.

2. H_2O_2 standard solution (20 mM)
Add 22.7 µL of 3% H_2O_2 (0.88 M, Component B) into 977 µL of Assay Buffer (Component C) to make 20 mM H_2O_2 standard solution.

Note The diluted H_2O_2 standard solution is not stable. The unused portion should be discarded.

PREPARATION OF STANDARD SOLUTION

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/11503

H2O2 standard
Add 50 µL of 20 mM H2O2 standard solution into 950 µL of Assay Buffer (Component C) to get 1000 µM H2O2 standard solution. Take 1000 µM H2O2 standard solution and perform 1:3 serial dilutions to get serially diluted H2O2 standards (HS1 - HS7) with Assay Buffer (Component C).

PREPARATION OF WORKING SOLUTION

Add 20 µL of 250X OxiVision Green™ hydrogen peroxide sensor stock solution into 5 mL of Assay Buffer (Component C) to make OxiVision Green™ hydrogen peroxide sensor working solution.

SAMPLE EXPERIMENTAL PROTOCOL

Run H_2O_2 assay in supernatants reactions

Table 1. Layout of H_2O_2 standards and test samples in a solid black 96-well microplate. HS= H_2O_2 Standards (HS1 - HS7, 300 to 0.3 µM); BL=Blank Control; TS=Test Samples

BL	BL	TS	TS
HS1	HS1	...	...
HS2	HS2	...	...
HS3	HS3		
HS4	HS4		
HS5	HS5		
HS6	HS6		
HS7	HS7		

Table 2. Reagent composition for each well.

Well	Volume	Reagent
HS1 - HS7	50 µL	Serial Dilutions (300 to 0.3 µM)
BL	50 µL	Assay Buffer (Component C)
TS	50 µL	test sample

1. Prepare H_2O_2 standards (HS), blank controls (BL), and test samples (TS) according to the layout provided in Tables 1 and 2. For a 384-well plate, use 25 µL of reagent per well instead of 50 µL.

2. Add 50 µL of OxiVision Green™ hydrogen peroxide sensor working solution to each well of H_2O_2 standard, blank control, and test samples to make the total H_2O_2 assay volume of 100 µL/well. For a 384-well plate, add 25 µL of OxiVision Green™ hydrogen peroxide sensor working solution into each well instead, for a total volume of 50 µL/well.

3. Incubate the reaction at room temperature for 15 to 30 minutes, protected from light.

4. Monitor the fluorescence increase with a fluorescence plate reader at Excitation = 490 ± 10, Emission = 520 ± 10 nm (optimal Ex/Em = 490/525 nm, Cutoff = 515 nm).

Run H_2O_2 assay in live cells

1. Activate the cells as desired.

2. Wash the cells with PBS buffer, incubated the cells with 100 µL/well OxiVision Green™ hydrogen peroxide sensor working solution for 5 to 60 minutes or your desired time. For a 384-well plate, add 25 µL/well of OxiVision Green™ hydrogen peroxide sensor working solution.

3. Monitor the fluorescence increase with a fluorescence plate reader (bottom read mode) at Ex/Em = 490/525 nm (Cutoff = 515 nm) Or image the fluorescence change with a fluorescence microscope using FITC channel.

EXAMPLE DATA ANALYSIS AND FIGURES

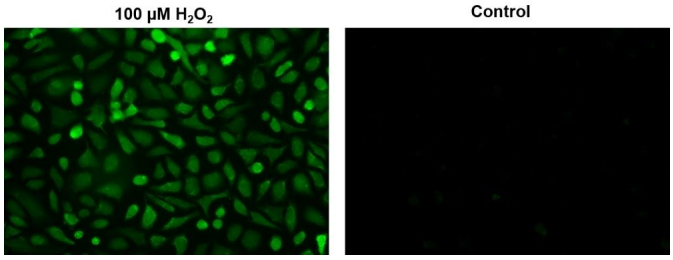

Figure 1. Fluorescence images of intracellular hydrogen peroxide in HeLa cells using Cell Meter™ Intracellular Fluorimetric Hydrogen Peroxide Assay Kit (Cat#11503). HeLa cells were treated with (left) or without (right) 100 µM hydrogen peroxide at 37° C for 90 minutes.

DISCLAIMER

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