Cell Meter™ Intracellular Fluorimetric Hydrogen Peroxide Assay Kit *Green Fluorescence Optimized for Flow Cytometry*

Catalog number: 11506
Unit size: 100 Tests

Component	Storage	Amount
Component A: OxiVision™ Green peroxide sensor	Freeze (<-15 °C), Minimize light exposure	1 vial
Component B: DMSO	Freeze (<-15 °C)	1 vial (200 µL)

OVERVIEW

Hydrogen peroxide is a reactive oxygen metabolic by-product that serves as a key regulator for a number of oxidative stress-related events. It is involved in many biological processes that are linked to asthma, atherosclerosis, diabetic vasculopathy, osteoporosis, a number of neurodegenerative diseases and Down's syndrome. The measurement of this reactive species is helpful for determining how oxidative stress modulates various intracellular pathways. This Cell Meter™ Intracellular Fluorimetric Hydrogen Peroxide Assay Kit uses our unique OxiVision™ Green peroxide sensor to quantify hydrogen peroxide in live cells. OxiVision™ Green peroxide sensor is cell-permeable, and generates green fluorescence when it reacts with hydrogen peroxide. This kit provides a sensitive tool to monitor hydrogen peroxide level in living cells, and it is optimized to be used in flow cytometry.

AT A GLANCE

Protocol summary

1. Prepare cells in growth medium
2. Stain cells with OxiVision™ Green peroxide sensor
3. Treat cells with test compounds
4. Monitor fluorescence intensity with flow cytometer FITC channel (Ex/Em = 490/530 nm)

Important Thaw all the four components at room temperature before use.

KEY PARAMETERS

Instrument:	Flow cytometer
Excitation:	488 nm laser
Emission:	530/30 nm filter
Instrument specification(s):	FITC channel

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 Peroxide Sensor stock solution:
Add 100 µL of DMSO (Component B) into the vial of OxiVision™ Green Peroxide Sensor (Component A), and mix well.

Note 1 µL of reconstituted OxiVision™ Green Peroxide Sensor stock solution is for 0.5 mL cells. The stock solution should be used promptly. Protect from light.

PREPARATION OF CELL SAMPLES

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

SAMPLE EXPERIMENTAL PROTOCOL

1. Stain cells with OxiVision™ Green Peroxide Sensor stock solution in full medium or in your desired buffer at 37°C for 30 minutes, protected from light.

2. Treat cells with test compounds in full medium or in your desired buffer at 37°C for desired period of time. For control samples (untreated cells), add the corresponding amount of compound buffer.

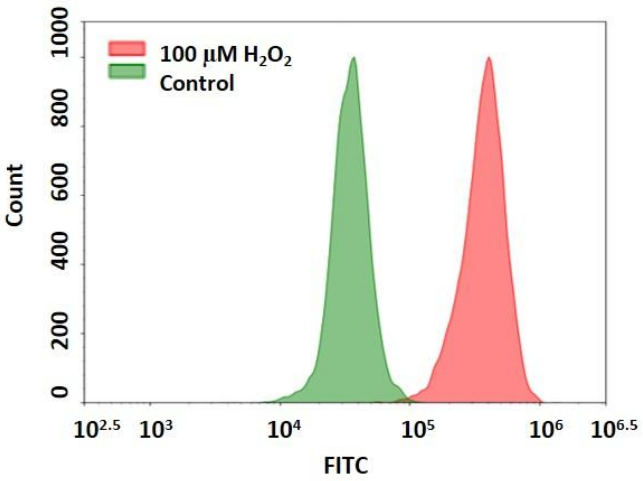
   Note It is recommended to treat cells in full medium. However, if tested compounds are serum sensitive, growth medium and serum factors can be aspirated away before treatment. Resuspend cells in 1X Hank's salt solution and 20 mM Hepes buffer (HHBS) or the buffer of your choice after aspiration. Alternatively, cells can be treated in serum-free media.

   Note We treated Jurkat cells with 100 µM hydrogen peroxide in full medium at 37°C for 90 minutes to induce hydrogen peroxide. See Figure 1 for details.

3. Alternatively, treat cells with tested compounds at 37°C for desired period of time. Remove the treatment solution, then stain cells with OxiVision™ Green Peroxide Sensor stock solution in full medium or in your desired buffer at 37°C for desired period of time.

4. Monitor the fluorescence intensity at FITC channel (Ex/Em = 490/530 nm) using a flow cytometer. Gate on the cells of interest, excluding debris.

EXAMPLE DATA ANALYSIS AND FIGURES



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

Figure 1. Detection of hydrogen peroxide in Jurkat cells using Cell Meter™ Intracellular Fluorimetric Hydrogen Peroxide Assay Kit (Cat#: 11506). Jurkat cells were stained with OxiVision™ Green peroxide sensor for 30 minutes and treated with 100 μM hydrogen peroxide at 37 °C for 90 minutes. Cells stained with OxiVision™ Green peroxide sensor but without hydrogen peroxide treatment were used as control.

DISCLAIMER