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

Catalog number: 11505
Unit size: 100 Tests

Component	Storage	Amount
Component A: OxiVision™ Blue 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 states. It is involved in many biological events 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™ Blue peroxide sensor to quantify hydrogen peroxide in live cells. OxiVision™ Blue peroxide sensor is cell-permeable, and generates blue 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™ Blue Peroxide Sensor
3. Treat cells with test compounds
4. Monitor fluorescence intensity with flow cytometer Pacific Blue Channel (Ex/Em = 405/450 nm)

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

KEY PARAMETERS

Instrument:	Flow cytometer
Excitation:	405 nm laser
Emission:	450/40 nm filter
Instrument specification(s):	Pacific Blue 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™ Blue Peroxide Sensor stock solution:
Add 100 µL of DMSO (Component B) into the vial of OxiVision™ Blue peroxide sensor (Component A), and mix them well.

Note 1 µL of reconstituted OxiVision™ Blue peroxide sensor stock solution is enough for 0.5 mL cells. The stock solution should be used promptly. Keep 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™ Blue Peroxide Sensor stock solution in full medium or in your desired buffer at 37°C for 20 - 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's 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. Monitor the fluorescence intensity at Pacific Blue channel (Ex/Em=405/450 nm) using a flow cytometer. Gate on the cells of interest, excluding debris.

EXAMPLE DATA ANALYSIS AND FIGURES

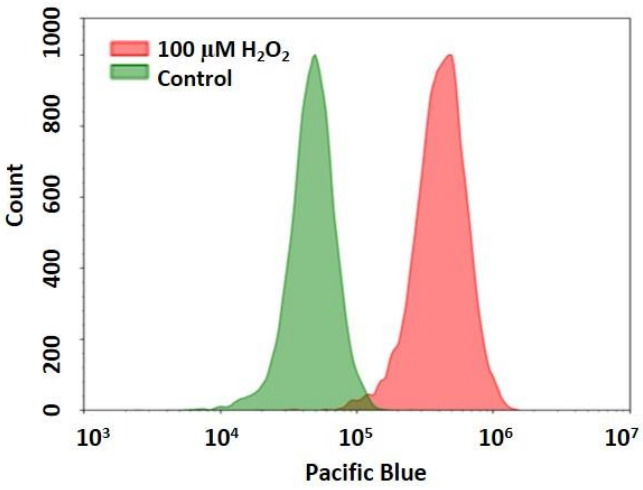

Figure 1. Detection of hydrogen peroxide in Jurkat cells using Cell Meter™ Intracellular Fluorimetric Hydrogen Peroxide Assay Kit (Cat#: 11505). Jurkat cells were stained with OxiVision™ Blue peroxide sensor for 30 minutes and treated with 100 µM hydrogen peroxide at 37 °C for 90 minutes. Cells stained with OxiVision™ Blue peroxide sensor but without hydrogen peroxide treatment were used as control.

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