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

Catalog number: 11504
Unit size: 100 Tests

Component	Storage	Amount
Component A: OxiVision™ Blue Peroxide Sensor	Freeze (<-15 °C), Minimize light exposure	1 vial
Component B: Assay Buffer	Freeze (<-15 °C)	1 bottle (20 mL)
Component C: DMSO	Freeze (<-15 °C)	1 vial (100 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 for fluorescence microscopy.

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 DAPI filter or Ex/Em = 405/450 nm

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

KEY PARAMETERS

Instrument:	Fluorescence microscope
Excitation:	DAPI filter
Emission:	DAPI filter
Recommended plate:	Black wall/clear bottom

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 (500X):
Add 40 µL of DMSO (Component C) into the vial of OxiVision™ Blue Peroxide Sensor (Component A) and mix well to make 500X OxiVision™ Blue Peroxide Sensor stock solution.

Note 20 µL of reconstituted OxiVision™ Blue peroxide sensor stock solution is enough for 1 plate. The stock solution should be used promptly. Protect from light.

PREPARATION OF WORKING SOLUTION

Add 10 µL of 500X OxiVision™ Blue Peroxide Sensor stock solution into 500 µL of Assay Buffer (Component B) and mix well to make OxiVision™ Blue Peroxide Sensor working solution.

Note This OxiVision™ Blue Peroxide Sensor working solution is not stable; prepare it as needed before use.

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. Add 10 µL/well (96-well plate) or 2.5 µL/well (384-well plate) of OxiVision™ Blue Peroxide Sensor working solution in 90 µL cell culture per well in a 96-well cell plate or 22.5 µL cell culture per well in a 384-well cell plate.

   Note It is not necessary to wash cells before staining. It's recommended to stain the cells in full medium.

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. Add 1X Hank's salt solution and 20 mM Hepes buffer (HHBS) or the buffer of your choice into the cells after aspiration. Alternatively, cells can be treated in serum-free media. 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. Wash cells with DPBS 1 - 2 times, and replace with 100 uL/well (for 96-well plate) or 25 uL/well (for 384- well plate) Assay Buffer (Component C).

4. Take images using fluorescence microscope with a DAPI filter.

EXAMPLE DATA ANALYSIS AND FIGURES

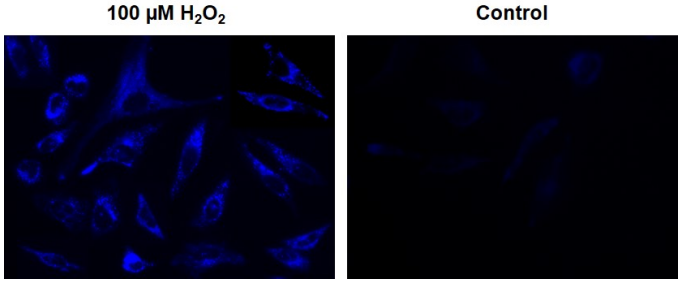

Figure 1. Fluorescence images of intracellular hydrogen peroxide in HeLa cells using Cell Meter™ Intracellular Fluorimetric Hydrogen Peroxide Assay Kit (Cat# 11504). HeLa cells at 10,000 cells/well/100 µL were seeded overnight in a Costar black wall/clear bottom 96-well plate. 100 µM H_2O_2: HeLa cells were stained with OxiVision™ Blue peroxide sensor for 30 minutes and treated with 100 µM hydrogen peroxide at 37 °C for 90 minutes. Control: Cells were stained with OxiVision™ Blue peroxide sensor but without hydrogen peroxide treatment. The

fluorescence signals were measured using fluorescence microscope with a DAPI filter.

DISCLAIMER