CREATIVE BIOMART®
Assay Kit

# Intracellular Fluorimetric Hydrogen Peroxide Assay Kit (Blue)

## Product Information

**Cat**

Kit-0987

**Common Name**

H2O2

**Cat.No.** Kit-0987

**Product Overview**

This Intracellular Fluorimetric Hydrogen Peroxide Assay Kit uses our unique Blue peroxide sensor to quantify hydrogen peroxide in live cells. 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 fluorescence microscopy.

**Description**

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 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.

**Storage**

Keep in freezer and avoid exposure to light.

**Size**

100 Tests

**Kit Components**

Component A: Blue peroxide sensor 1 vial
Component B: Assay Buffer 1 bottle (20 mL)
Component C: DMSO 1 vial (100 μL)

**Preparation**

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1. Prepare cells:
1.1. For adherent cells: Plate cells overnight in growth medium at 10,000 to 40,000 cells/well/90 μL for a 96- well plate or 2,500 to 10,000 cells/well/22.5 μL for a 384-well plate.
1.2. For non-adherent cells: Centrifuge the cells from the culture medium and suspend the cell pellets in culture medium at 50,000-100,000 cells/well/90 µL for a 96-well poly-D lysine plate or 10,000-25,000 cells/well/22.5 µL for a 384-well poly-D lysine plate. Centrifuge the plate at 800 rpm for 2 minutes with brake off prior to your experiment.
Note: Each cell line should be evaluated on an individual basis to determine the optimal cell density.
2. Prepare Blue peroxide sensor stock solution:
2.1 Prepare Blue peroxide sensor stock solution (500X): Add 40 µL of DMSO (Component C) into the vial of Blue peroxide sensor (Component A), and mix them well.
Note: 20 μL of reconstituted of reconstituted Blue peroxide sensor stock solution is enough for 1 plate. The stock solution should be used promptly. Any remaining solution should be aliquoted and frozen at -20°C. Avoid repeated freeze-thaw cycles and protect from light.
2.2 Prepare Blue peroxide sensor working solution (10X): Add 10 μL of 500X DMSO reconstituted Blue peroxide sensor stock solution (from Step 2.1) into 500 μL of Assay Buffer (Component B), and mix them well.
Note: The working solution is not stable; prepare it as needed before use.

## Assay Protocol

3. Run the hydrogen peroxide assay:
3.1 Add 10 µL/well (96-well plate) of Blue peroxide sensor working solution (from Step 2.2) in 90 µL cell culture per well in the cell plate.
Note 1: It is not necessary to wash cells before staining. It's recommended to stain the cells in full medium.
Note 2: For a 384-well plate, add 2.5 μL/well of 1X Blue peroxide sensor working solution.
3.2 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 1: 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. Add 1X

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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.
Note 2: We treated Jurkat cells with 100 µM hydrogen peroxide in full medium at 37°C for 90 minutes to induce hydrogen peroxide.
3.3 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). Take images using fluorescence microscope with a DAPI filter.

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