Intracellular Fluorimetric Hydrogen Peroxide Assay Kit (Blue)

Cat. No. Kit-0987 **Lot. No.** (See product label)

SPECIFICATION

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	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

Tel: 1-631-559-9269 1-516-512-3133
Email: info@creative-biomart.com Fax: 1-631-938-8127
45-1 Ramsey Road, Shirley, NY 11967, USA

	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 µ for a 96-well poly-D lysine plate or 10,000-25,000 cells/well/22.5 µ 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 µ 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 µ/well (96-well plate) of Blue peroxide sensor working solution (from Step 2.2) in 90 µ 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 ºC

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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 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 37ºC 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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