Intracellular Fluorimetric Hydrogen Peroxide Assay Kit (Green)

Cat. No. Kit-0986 **Lot. No.** (See product label)

SPECIFICATION

Product Overview	This Cell Intracellular Fluorimetric Hydrogen Peroxide Assay Kit uses our unique Green Peroxide Sensor to quantify hydrogen peroxide in live cells. Green peroxide sensor is cellpermeable, and generates the green fluorescence when it reacts with hydrogen peroxide. This kit provides a sensitive tool to monitor hydrogen peroxide level in living cells. The kit is also optimized with "mix and read" assay format for solution based assay. It provides a sensitive, one-step fluorimetric assay to detect as little as 0.3 nanomoles of H2O2 in a 100 µ assay volume (3 µM). The assay can be performed in a convenient 96-well or 384-well microtiterplate format. Its signal can be easily read by either a fluorescence microplate reader at Ex/Em = 490/520 nm for H2O2 detection in solution or a fluorescence microscopy and a flow cytometry for live cell H2O2 detection.
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	200 assays
Kit Components	Component A: Green Peroxide Sensor 1 vial

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

	Component B: H2O2 1 vial (3% stabilized solution, 200 µL) Component C: Assay Buffer 1 bottle (20 mL) Component D: DMSO 1 vial (200 µL)
Features & Benefits	Broad Application: Can be used for quantifying hydrogen peroxide in live cells, in solutions, and in cell extracts. Continuous: Easily adapted to automation without a separation step. Convenient: Formulated to have minimal hands-on time. No wash is required.
Preparation	1. Prepare stock solutions: 1.1 Green Peroxide Sensor stock solution (250X): Add 50 uL of DMSO (Component D) into the vial of Green Peroxide Sensor (Component A). The stock solution should be used promptly. Any remaining solution should be aliquoted and refrozen at -20°C. Note: Avoid repeated freeze-thaw cycles and protect from light. 1.2 20 mM H2O2 stock solution: Add 22.7 uL of 3% H2O2 (0.88 M, Component B) into 977 uL of Assay Buffer (Component C). Note: The diluted H2O2 solution is not stable. The unused portion should be discarded. 2. Prepare 1X Green Peroxide Sensor working solution: Add 20 uL of Green Peroxide Sensor stock solution (250X, from Step 1.1) into 5 mL of Assay Buffer (Component C). 3. Prepare serially diluted H2O2 standards (0 to 1000 µM): 3.1 Add 50 µL of 20 mM H2O2 solution (from Step 1.2) into 950 µL of Assay Buffer (Component C) to get 1000 µM H2O2 solution. 3.2 Take 200 µL of 1000 µM H2O2 solution to perform 1:3 serial dilutions to get 300, 100, 30, 10, 3, 1, 0.3 and 0 µM serially diluted H2O2 stands. 3.3 Add H2O2 standards and H2O2-containing test samples into a solid black 96-well microplate as described in Tables 1 and 2. Table 1 Layout of H2O2 standards and test samples in a solid black 96-well

	microplate BL BL TS TS …. …. HS1 HS1 …. …. …. …. HS2 HS2 HS3 HS3 HS4 HS4 HS5 HS5 HS6 HS6 HS7 HS7 Note: HS= H2O2 Standards; BL=Blank Control; TS=Test Samples Table 2 Reagent composition for each well H2O2 Standard: Serial dilutions*: 50 µL Blank Control: Assay Buffer (Component C): 50 µL Test Sample: 50 µL
Assay Protocol	4. Run H2O2 assay in supernatants reaction: 4.1 Add 50 µL of H2O2 reaction mixture (from Step 2) into each well of H2O2 standard, blank control, and test samples (see Step 3.3) to make the total H2O2 assay volume of 100 µ/well. Note: For a 384-well plate, add 25 µL of sample and 25 µL of 1X Green peroxide Sensor working solution into each well. 4.2 Incubate the reaction at room temperature for 15 to 30 minutes, protected from light. 4.3 Monitor the fluorescence increase at Ex/Em = 490±10/520±10 nm (optimal Ex/Em = 490/520) with a fluorescence plate reader. 5. Run H2O2 assay for cells: Green Peroxide Sensor can be loaded passively into living cells and report the micromolar changes in intracellular H2O2 concentrations. The following is a suggested microscope imaging protocol that can be modified to meet specific

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	research needs. 5.1 Activate the cells as desired. 5.2 Wash the cells with PBS buffer, incubated the cells with 100 µ/well 1X Green Peroxide Sensor working solution (from Step 2) for 5 to 60 minutes or your desired time. Note: For a 384-well plate, add 25 µL/well of 1X Green Peroxide Sensor working solution. 5.3 Monitor the fluorescence increase at excitation 490 nm and emission at 525nm using a fluorescence plate reader with bottom read mode. Or image the fluorescence change with a fluorescence microscope using FITC channel.

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