Intracellular Fluorimetric Hydrogen Peroxide Assay Kit (Green, Optimized for Flow Cytometry)

Cat. No. Kit-0989 **Lot. No.** (See product label)

SPECIFICATION

Product Overview	This Intracellular Fluorimetric Hydrogen Peroxide Assay Kit uses our unique Green peroxide sensor to quantify hydrogen peroxide in live cells. Green 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.
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: Green peroxide sensor 1 vial Component B: DMSO 1 vial (200 µL)
Preparation	1. Prepare cells: For each sample, prepare cells in 0.5 mL growth medium or buffer of your choice at a density of 5×10^5 to 1×10^6 cells/mL.

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	Note: Each cell line should be evaluated on an individual basis to determine the optimal cell density for hydrogen peroxide induction. 2. Prepare Green peroxide sensor stock solution: Add 100 µ of DMSO (Component B) into the vial of Green peroxide sensor (Component A), and mix them well. Note: 1 µL of reconstituted Green peroxide sensor stock solution is for 0.5 mL cells. The stock solution should be used promptly. Any remaining solution should be aliquoted and refrozen at -20°C. Avoid repeated freeze-thaw cycles and protect from light.
Assay Protocol	3. Run the hydrogen peroxide assay: 3.1 Stain cells with Green peroxide sensor in full medium or in your desired buffer at 37ºC for 30 minutes, protected from light. 3.2 Treat cells with test compounds in full medium or in your desired buffer at 37ºC 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. 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 2: We treated Jurkat cells with 100 µM hydrogen peroxide in full medium at 37ºC for 90 minutes to induce hydrogen peroxide. 3.3 Alternatively, treat cells with tested compounds at 37 ºC for desired period of time (as in Step 3.2). Remove the treatment solution, then stain cells with Green peroxide sensor in full medium or in your desired buffer at 37ºC for desired period of time. 3.4 Monitor the fluorescence intensity at FITC channel (Ex/Em = 490/530 nm) using a flow cytometer. Gate on the cells of interest, excluding debris.

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