

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 μ M). 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

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	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 µL 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 µL of 3% H₂O₂ (0.88 M, Component B) into 977 µL 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 µL 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

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 μ L/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.