CREATIVE BIOMART®
Assay Kit

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

Product Information

Cat

Kit-0988

Common Name

H2O2

Cat.No. Kit-0988

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 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: Blue peroxide sensor 1 vial
Component B: DMSO 1 vial (200 μL)

Preparation

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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.

Note: Each cell line should be evaluated on an individual basis to determine the optimal cell density for hydrogen peroxide induction.

2. Prepare Blue peroxide sensor stock solution:

Add 100 µL of DMSO (Component B) into the vial of Blue peroxide sensor (Component A), and mix them well.

Note: 1 μL of reconstituted Blue 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 Blue peroxide sensor in full medium or in your desired buffer at 37°C for 20- 30 minutes, protected from light.

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. 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 37°C for 90 minutes to induce hydrogen peroxide.

3.3 Monitor the fluorescence intensity at Pacific Blue channel (Ex/Em=405/450 nm) using a flow cytometer. Gate on the cells of interest, excluding debris.

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