CREATIVE BIOMART®
Assay Kit

Colorimetric Hydrogen Peroxide Assay Kit

Product Information

Cat

Kit-0990

Common Name

H2O2

Cat.No.

Kit-0990

Product Overview

This Colorimetric Hydrogen Peroxide Assay Kit uses our unique IR peroxidase substrate to quantify hydrogen peroxide in solutions and cell extracts. Upon hydrogen peroxide oxidation the colorless IR generates an intense blue color product that is pH-independent from pH 4 to 10. The existing colorimetric hydrogen peroxide assays (from other vendors) often have severe sample interferences caused by the inherent absorption of biological samples. The near infrared absorption of IR product minimizes the assay background that since the biological samples rarely absorb light beyond 600 nm. It can also be used to detect a variety of oxidase activities through enzyme-coupled reactions. The kit is an optimized 'mix and read' assay that is compatible with HTS liquid handling instruments.

Description

Hydrogen peroxide (H2O2) is a reactive oxygen metabolic by-product that serves as a key regulator for numerous oxidative stress-related states. It is involved in a number of biological events that have been linked to asthma, atherosclerosis, diabetic vasculopathy, osteoporosis, a number of neurodegenerative diseases and Down's syndrome. Perhaps the most intriguing aspect of H2O2 biology is the recent report that antibodies have the capacity to convert molecular oxygen into H2O2 to contribute to the normal recognition and destruction processes of the immune system. Measurement of this reactive species will help to determine how oxidative stress modulates varied intracellular pathways.

Storage

Keep in freezer and avoid exposure to light.

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Size

500 Tests

Kit Components

Component A: IR Peroxidase Substrate 1 vial
Component B: H2O2 1 vial (3% stabilized solution, 200 μL)
Component C: Assay Buffer 1 bottle (100 mL)
Component D: Horseradish Peroxidase 1 vial (20 units)
Component E: DMSO 1 vial (0.5 mL)

Preparation

1. Prepare stock solutions:
1.1 100X IR peroxidase substrate stock solution: Add 250 uL of DMSO (Component E) into the vial of IR Peroxidase Substrate (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 U/mL peroxidase stock solution: Add 1 mL of Assay Buffer (Component C) into the vial of Horseradish Peroxidase (Component D).
Note: The unused HRP solution should be divided into single use aliquots and stored at -20°C.
1.3 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 stock solution is not stable. The unused portion should be discarded.
2. Prepare H2O2 reaction mixture: Prepare the H2O2 reaction mixture according to the following table and keep from light.
Table 1. H2O2 Reaction mixture for one 96-well plate (2X)
100X IR Peroxidase Substrate Stock Solution (from Step 1.1) 50 μL
20 U/mL Peroxidase Stock Solution (from Step 1.2) 200 μL
Assay Buffer (Component C) 4.75 mL
Total volume 5 mL
3. Prepare serial dilutions of H2O2 standard (0 to 50 μM):
Warning 1: IR Peroxidase Substrate (Component A) is unstable in the presence of thiols such as DTT

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and β-mercaptoethanol. If the final concentration of the thiols is higher than 10 uM, it would significantly decrease the assay dynamic range.
Warning 2: NADH and glutathione (reduced form of GSH) may interfere with the assay.
3.1 Add 5 μL of 20 mM H2O2 stock solution (from Step 1.3) into 995 μL of Assay Buffer (Component C) to get 100 μM H2O2 standard.
3.2 Take 200 μL of 100 μM H2O2 standard to perform 1:2 serial dilutions to get 50, 25, 12.5, 6.25, 3.125,1.1.56 and 0 μM serial dilutions of H2O2 standard.
3.3 Add serial dilutions of H2O2 standard and H2O2-containing test samples into a white wall/clear bottom 96-well microplate as described in Tables 2 and 3.
Table 2 Layout of H2O2 standards and test samples in a white wall/clear bottom 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 3 Reagent composition for each well
H2O2 Standard: Serial Dilutions*: 50 μL
Blank Control: Assay Buffer (Component C): 50 μL
Test Sample: 50 μL
*Note: Add the serial diluted of H2O2 standard from 1.56 μM to50 μM into wells from HS1 to HS7 in duplicate.

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 volume of 100 µL/well.
Note: For a 384-well plate, add 25 μL of sample and 25 μL of H2O2 reaction mixture into

Colorimetric Hydrogen Peroxide Assay Kit

each well.
4.2 Incubate the reaction at room temperature for 10 to 30 minutes, protected from light.
4.3 Monitor the absorbance with an absorbance plate reader at 650 nm.
5. Run H2O2 assay for cells:
The Colorimetric Hydrogen Peroxide Assay Kit can be used to measure the release of H2O2 from cells. The following is a suggested protocol that can be modified to meet the specific research needs.
5.1 The H2O2 reaction mixture should be prepared as Step 2 except that the Assay Buffer (Component C) should be replaced with the media used in your cell culture system. Suggested media including (a) Krebs Ringers Phosphate Buffer (KRPB); (b). Hanks Balanced Salt Solution (HBSS); or (c) Serum-free media.
5.2 Prepare cells in a 96-well plate (50 - 100 μL/well), and activate the cells as desired.
Note: The negative controls (media alone and non-activated cells) are included for measuring the background fluorescence.
5.3 Add 50 μL of H2O2 reaction mixture (from Step 5.1) into each well of cells, and H2O2 standards (from Step 3.3).
Note: For a 384-well plate, add 25 μL of cells and 25 μL of H2O2 reaction mixture into each well.
5.4 Incubate the reaction at room temperature for 10 to 60 minutes, protected from light.
5.5 Monitor the absorbance with an absorbance plate reader at 650 nm.

Analysis

The absorbance in blank wells (with the assay buffer only) is used as a control, and is subtracted from the values for those wells with the HRP reactions.

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