CREATIVE BIOMART®
Assay Kit

# Red hydrogen peroxide assay Kit for microplates

## Product Information

**Common Name**

Peroxide

**Cat.No.** Kit-0677

**Product Overview**

This Fluorimetric Hydrogen Peroxide Assay Kit uses our non-fluorescent Red peroxidase substrate to quantify hydrogen peroxide in solutions and cell extracts. 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. It provides a sensitive, one-step fluorometric assay to detect as little as 3 picomoles of H2O2 in a 100 µL assay volume (30 nM). The assay can be performed in a convenient 96-well or 384-well microtiter-plate format and readily adapted to automation. Its signal can be easily read by either a fluorescence microplate reader at Ex/Em = ~540/590 nm or an absorbance microplate reader at ~570 nm.

**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 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 hydrogen peroxide 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 a variety of intracellular pathways.

**Applications**

Fluorescence microscopy, HTS

**Stability**

Stable for at least one year after receipt when stored as recommended.

**Storage**

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Keep in freezer. Avoid exposure to light

**Size**

500 assays

**Kit Components**

A. Red Peroxidase Substrate (1 vial)
B. Hydrogen Peroxide (H2O2) (1 vial (3% stabilized solution, 200 μL))
C. Assay Buffer (100 ml)
D. Horseradish Peroxidase (HRP) (20 units)
E. DMSO (1 ml)

**Features & Benefits**

Broad Application: Can be used for quantifying hydrogen peroxide in solutions, in cell extracts and in live cells; and can also be used for detecting a variety of oxidase activities through enzyme-coupled reactions.
Sensitive: Detect as low as 10 picomoles of H2O2 in solution.
Continuous: Easily adapted to automation without a separation step.
Convenient: Formulated to have minimal hands-on time. No wash is required.
Non-Radioactive: No special requirements for waste treatment.

**Preparation**

1. Prepare stock solutions:
1.1 100X Red peroxidase substrate stock solution: Add 250 uL of DMSO (Component E) into the vial of Red 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).

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Note: The diluted H2O2 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)
Components Volume
Red Peroxidase Substrate Stock Solution (100X, from Step 1.1) 50 uL
20 U/ml Peroxidase Stock Solution (from Step 1.2) 200 uL
Assay Buffer (Component C) 4.75 mL
Total volume 5 mL
3. Prepare serial dilutuions of H2O2 standard (0 to 10 μM):
3.1 Add 1 μL of 20 mM H2O2 solution (from Step 1.3) into 1999 μL of Assay Buffer (Component C) to get a 10 μM H2O2 standard.
3.2 Take 200 μL of 10 μM H2O2 standard to perform 1:3 serial dilutions to get 3, 1, 0.3, 0.1, 0.03, 0.01 and 0 μM serial dilutions of H2O2 standard.
3.3 Add serial dilutions of H2O2 standard and H2O2-containing test samples into a solid black 96-well microplate as described in Tables 2 and 3
Table 2 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 3 Reagent composition for each well
H2O2 Standard: Serial dilutions* 50 μL
Blank Control: Assay Buffer (Component C) 50 μL
Test Sample: 50 μL

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*Note: Add the serially diluted H2O2 standards from 0.01 μM to10 μM into wells from HS1 to HS7 in duplicate. High concentration of H2O2 (e.g., > 100 μM, final concentration) may cause reduced fluorescence signal due to the overoxidation of Red (to a non-fluorescent product).

**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 µL/well.
Note: For a 384-well plate, add 25 μL of sample and 25 μL of H2O2 reaction mixture in each well.
4.2 Incubate the reaction at room temperature for 15 to 30 minutes, protected from light.
4.3 Monitor the fluorescence increase with a fluorescence plate reader at Ex/Em = 540 ± 10 /590 ± 10 nm (optimal Ex/Em = 540/590 nm).
Note: The contents of the plate can also be transferred to a white clear bottom plate and read by an absorbance microplate reader at the wavelength of 576 ± 5 nm. The absorption detection has lower sensitivity compared to fluorescence reading.
5. Run H2O2 assay for cells:
The Fluorimetric 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 that is 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 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.

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5.4 Incubate the reaction at room temperature for 15 to 30 minutes, protected from light.
5.5 Monitor the fluorescence increase with a fluorescence plate reader at Ex/Em= 540 ± 10/590 ± 10 nm (optimal Ex/Em = 540/590 nm).

**Analysis**

The fluorescence in blank wells (with the assay buffer only) is used as a control, and is subtracted from the values for those wells with the H2O2 reactions.
Note: The fluorescence background increases with time, thus it is important to subtract the fluorescence intensity value of the blank wells for each data point.

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