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User's Manual and Instructions

Peroxide Assay Kit (Z5030034)

Quantitative Colorimetric Peroxide Determination at 585nm

DESCRIPTION

Peroxide (e.g. hydrogen peroxide H_2O_2) is one of the key reactive oxygen species formed under oxidative stress conditions. High levels of peroxide formation have been linked to pathological conditions such as ageing, asthma, diabetes, atherosclerosis, cataract, inflammatory arthritis and neurodegenerative diseases.

Simple, direct and automation-ready procedures for quantitative determination of peroxide find wide applications in research and drug discovery. Biochain's peroxide assay kit is designed to measure peroxide concentration in biological samples without any pretreatment. The improved method utilizes the chromogenic Fe^{3+}-xylenol orange reaction, in which a purple complex is formed when Fe^{2+} provided in the reagent is oxidized to Fe^{3+} by peroxides present in the sample. The intensity of the color, measured at 540-610nm, is an accurate measure of the peroxide level in the sample. The optimized formulation substantially reduces interference by substances in the raw samples.

KEY FEATURES

Sensitive and accurate. Enhanced color intensity using sorbitol. Detection range 0.2 μM (7 ng/mL) to 30 μM (1,020 ng/mL) H_2O_2 in 96-well plate assay.
Simple and high-throughput. The procedure involves addition of a single detection reagent and incubation for 30 min. Can be readily automated as a high-throughput assay in 96-well plates for thousands of samples per day.

APPLICATIONS:

Direct Assays: H_2O_2 in biological samples (e.g. serum, citrate-plasma, urine, cell lysate, culture medium).
Pharmacology: effects of drugs on peroxide metabolism.

KIT CONTENTS (250 tests in 96-well plates)

Reagent A: 1 mL
Reagent B: 50 mL
Standard: 100 μL 3% stabilized H_2O_2.

Kit shipped at room temperature.
Storage conditions. The kit is shipped at room temperature. Store all reagents at 4 °C. Shelf life of at least 6 months.

Precautions: reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

PROCEDURES

Sample Treatment: several chemicals are known to interfere and should be avoided in sample preparation. These include ascorbic acid, EDTA, heparin, DMSO (>0.02%), NP-40 (>0.6%), SDS (>0.12%), Tris (>8mM) and ethanol (>0.4%).

Samples can be analyzed immediately after collection, or stored in aliquots at –20 °C. Avoid repeated freeze-thaw cycles.

Reagent Preparation: Equilibrate to room temperature before assay. Prepare enough Detection Reagent by mixing 1 volume of Reagent A with 100 volumes Reagent B.

Procedure using 96-well plate:

1. *Standards*. Prepare fresh standards on the day of assay. Pipette 5 μL 3% H_2O_2 and mix well with 495 μL H_2O in a 1.5-mL Eppendorf tube. Mix 5 μL of this solution with 1465 μL H_2O. The final H_2O_2 concentration is 30 μM (labeled "Premix"). Dilute standard as shown in the Table.

2. Transfer 40 μL diluted standards and each sample into separate wells of a clear flat-bottom 96-well plate. Add 200 μL Detection Reagent to all standards and samples.

No	Premix + H_2O	Vol (μL)	H_2O_2 (μM)
1	100μL + 0μL	100	30
2	80μL + 20μL	100	24
3	60μL + 40μL	100	18
4	40μL + 60μL	100	12
5	30μL + 70μL	100	9
6	20μL + 80μL	100	6
7	10μL + 90μL	100	3
8	0μL + 100μL	100	0

3. Incubate 30 min at room temperature and read optical density at 540-610nm (peak absorbance at 585nm).

   Note: if in rare cases, precipitation occurs after adding the Detection Reagent to a sample, transfer the whole reaction mixture of this sample well into a 1.5-mL Eppendorf tube and centrifuge 2 min at 14,000 rpm. Carefully remove 200 μL supernatant into a clean well and read OD. *Multiply the OD reading by 1.2 to account for the volume change*.

CALCULATION

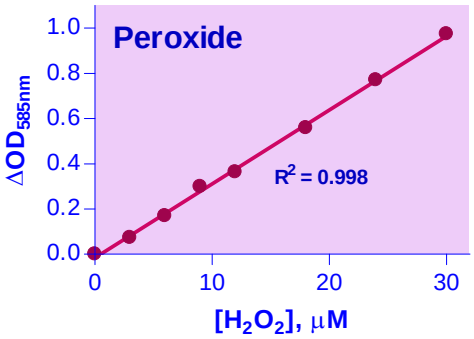
Subtract blank OD (water, #8) from the standard OD values and plot the OD against H_2O_2 concentrations. Subtract blank OD from Sample OD. Determine the sample peroxide content from the standard curve.
Conversions: 1 μM H_2O_2 equals 34 ng/mL or 34 ppb.

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipetting devices and accessories, 96-well plates and plate reader.

EXAMPLES:

Duplicate assays for goat serum, human serum, 293 cell culture medium and fresh human urine gave peroxide content of 8.7± 2.8, 14.2 ± 2.7, 2.4 ± 0.0 and 1.4 ± 0.6 μM (n = 2).



Standard Curve in 96-well plate assay

PUBLICATIONS

[1]. Chi Li Yu et al (2008). A novel caffeine dehydrogenase in pseudomonas sp. strain CBB1 oxidizes caffeine to trimethyluric acid. J. Bacteriol. 190(2):772–776.

[2]. Deshmane, S.L. et al (2009). Activation of the oxidative stress pathway by HIV-1 Vpr leads to induction of hypoxia-inducible factor 1alpha expression. J Biol Chem. 284(17):11364-11373.

[3]. Giao, N.N. et al (2007). Water deficit induced pollen Sterility associated with a programmed cell death and oxidative stress in rice anthers. Proceedings the 2nd International Rice for the Future pp202-209.