Peroxide Assay Kit (Colorimetric)

LS-K270-250 (250 Tests) • **Store at 4°C**

Introduction

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. This 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.

Components

Component	K270-250
	250 Tests
Reagent A	1 mL
Reagent B	50 mL
Standard (3% stabilized H_2O_2)	100 µL

Materials Not Supplied

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

Storage

The kit is shipped at room temperature. Store all reagents at 4 °C. Shelf life: 12 months after receipt.

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Assay Procedure

Sample Treatment

Several chemicals are known to interfere and should be avoided in sample preparation. These include ascorbic acid, EDTA, heparin, sodium pyruvate (>1mM), 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.

Calculations

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.

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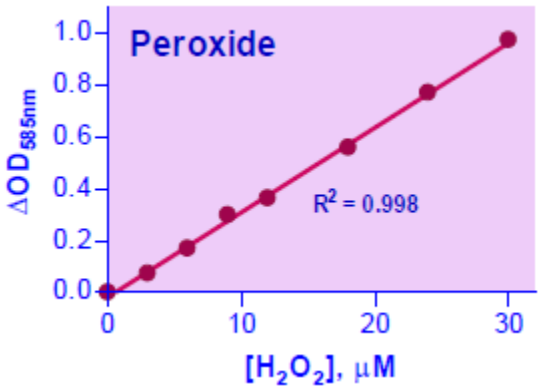
Sample Data

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

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