Datasheet for ABIN1000268

Peroxide Assay Kit

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Overview

Quantity:	250 tests
Target:	Peroxide
Application:	Biochemical Assay (BCA)

Product Details

Sample Type:	Serum, Plasma (citrate), Urine, Cell Lysate, Cell Culture Supernatant
Specificity:	0.4 µM
Characteristics:	Sensitive and accurate. Enhanced color intensity using sorbitol. Detection range 0.2 µM (7 ng/mL) to 30 µM (1,020 ng/mL) H2O2 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.
Components:	Reagent A: 1 mL. Reagent B: 50 mL. Standard: 100 µL 3% stabilized H2O2.
Material not included:	Pipetting devices and accessories, 96-well plates and plate reader.

Target Details

Target:	Peroxide
Background:	Quantitative determination of peroxide by colorimetric (585nm) method. Procedure: 30 min. Peroxide (e.g. hydrogen peroxide H2O2) is one of the key reactive oxygen species formed under oxidative stress conditions. High levels of peroxide formation have been linked to pathological

Target Details

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.

Application Details

Application Notes:	Direct Assays: H2O2 in biological samples (e.g. serum, citrate-plasma, urine, cell lysate, culture medium). Pharmacology: effects of drugs on peroxide metabolism.
Protocol:	Procedure using 96-well plate: 1. Standards. Prepare fresh standards on the day of assay. Pipette 5 µL 3% H2O2 and mix well with 495 µL H2O in a1.5-mL Eppendorf tube. Mix 5 µL of this solution with 1465 µL H2O. The final H2O2 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. 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 a1.5-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.
Reagent Preparation:	Equilibrate to room temperature before assay. Prepare enough Detection Reagent by mixing 1 volume of Reagent A with 100 volumes Reagent B.
Sample Preparation:	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.
Calculation of Results:	Subtract blank OD (water, #8) from the standard OD values and plot the OD against H2O2 concentrations. Subtract blank OD from Sample OD. Determine the sample peroxide content from the standard curve.

Application Details

	Conversions: 1 µM H2O2 equals 34 ng/mL or 34 ppb.
Restrictions:	For Research Use only

Handling

Storage:	4 °C

Publications

Product cited in:

Peng, Ye, Rakheja, Tu, Wang, Du, Zhou, Vaziri, Hu, Mohan, Zhou: "The green tea polyphenol (-)-epigallocatechin-3-gallate ameliorates experimental immune-mediated glomerulonephritis." in: **Kidney international**, Vol. 80, Issue 6, pp. 601-11, (2011) (PubMed).

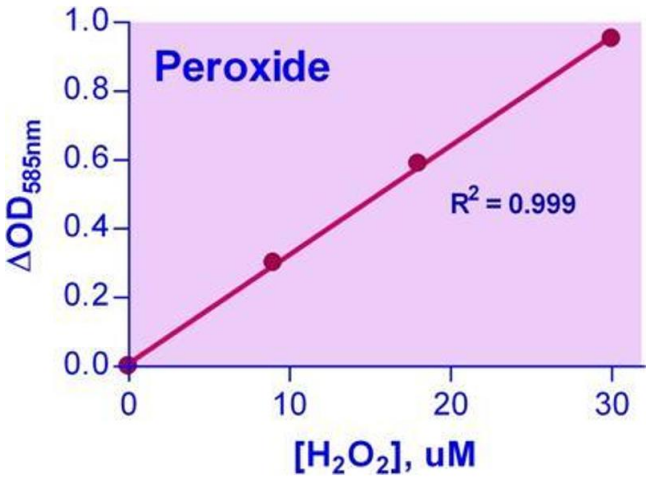
Fedeles, Zhu, Young, Hillier, Proffitt, Essigmann, Croy: "Chemical genetics analysis of an aniline mustard anticancer agent reveals complex I of the electron transport chain as a target." in: **The Journal of biological chemistry**, Vol. 286, Issue 39, pp. 33910-20, (2011) (PubMed).

Jawed, Shah, Jamall, Simjee: "N-(2-hydroxy phenyl) acetamide inhibits inflammation-related cytokines and ROS in adjuvant-induced arthritic (AIA) rats." in: **International immunopharmacology**, Vol. 10, Issue 8, pp. 900-5, (2010) (PubMed).

Fan, Hussien, Brooks: "H2O2-induced mitochondrial fragmentation in C2C12 myocytes." in: **Free radical biology & medicine**, Vol. 49, Issue 11, pp. 1646-54, (2010) (PubMed).

Toschi, Lee, Thompson, Gadir, Yellen, Drain, Ohh, Foster: "Phospholipase D-mTOR requirement for the Warburg effect in human cancer cells." in: **Cancer letters**, Vol. 299, Issue 1, pp. 72-9, (2010) (PubMed).

There are more publications referencing this product on: Product page



Biochemical Assay

Image 1.