Technical Bulletin

Peroxide Assay Kit

Catalogue Number MAK639

Product 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 aging, asthma, diabetes, atherosclerosis, cataracts, inflammatory arthritis and neurodegenerative diseases.

The improved peroxide assay kit is designed to measure peroxide concentration in biological samples without any pretreatment. This 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 540-610 nm, is an accurate measure of the peroxide level in the sample. The optimized formulation substantially reduces interference by substances in the raw samples.

This assay has a detection limit of 0.54 µM (18.4 ng/mL) and linear up to 30 µM (1,020 ng/mL) H_2O_2 in 96-well plate assay.

Components

The kit is sufficient for 100 colorimetric assays in 96-well plates.

- Detection Reagent 20 mL
  Catalogue Number MAK639A
- 3% H_2O_2 Standard 100 µL
  Catalogue Number MAK639B

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (e.g., multichannel pipettor)
- Spectrophotometric multiwell plate reader capable of 540-610 nm.
- Clear plates for colorimetric assays (Catalogue number M2936 or equivalent) Cell culture or tissue culture treated plates are not recommended.
- 1.5 mL microcentrifuge tubes

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped at ambient temperature. Store at room temperature upon receipt.

Preparation Instructions

Bring all reagents to room temperature prior to assay.

Sample Preparation

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.

Standard Preparation

Prepare fresh standards on the day of assay.

Pipette 5 µL 3% H_2O_2 and mix well with 495 µL H2O in a 1.5-mL 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 Table 1.

Table 1.
Standard Dilutions

Std #	Premix Standard (µL)	Purified water (µL)	Conc. (µM)
1	100	0	30
2	60	40	18
3	30	70	9
4	0	100	0

If organic peroxides, e.g. t-butyl hydroperoxide (*t*-Bu-OOH); cumene hydroperoxide (Cu-OOH), are being measured, results will be in H_2O_2 equivalents. Alternatively, substitute H_2O_2 in the standard curve with the peroxide of interest.

Procedure

Assay Reaction

1. Transfer 40 µL diluted standards and each sample into separate wells of a clear flat-bottom 96-well plate.
2. 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 a 1.5-mL tube and centrifuge 2 minutes 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.

Results

Calculations

Subtract blank OD (water, #4) 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.

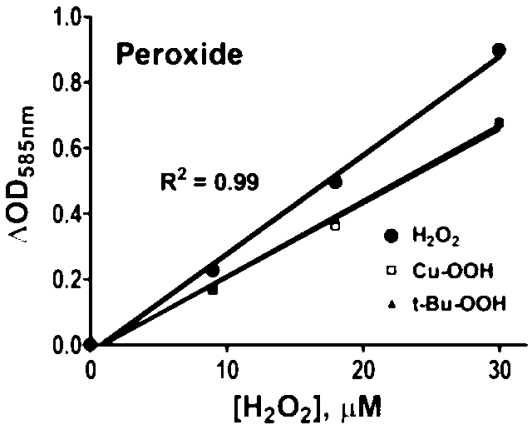

Figure 1.
Exemplary standard curve

References

1. Zhang N et al (2021) Analytical methods for determining the peroxide value of edible oils: A mini-review. Food Chem. 358:129834.
2. Dringen R, Pawlowski PG, Hirrlinger J (2005). Peroxide detoxification by brain cells. J Neurosci Res. 79(1-2):157-65.
3. Torres-García I (2021). Marine Terpenic Endoperoxides. Mar Drugs. 19(12): 661.

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