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Product Information

Peroxide Assay Kit

Catalog Number **MAK311**
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Product Description
Hydrogen peroxide (H_2O_2), a reactive oxygen species (ROS) produced through the metabolism of molecular oxygen, serves as both an intracellular signaling messenger and a source of oxidative stress. Hydrogen peroxide is generated in cells via multiple mechanisms such as the NOX-mediated ROS production by neutrophils and macrophages (respiratory burst) or by the dismutase of superoxide anions produced as a result of electron leak during mitochondrial respiration. Abnormal hydrogen peroxide production contributes to oxidative cell damage and the progression of diseases such as asthma, atherosclerosis, osteoporosis, and neurodegeneration.

The Peroxide Assay Kit provides a simple and high-throughput adaptable assay for quantitative determination of peroxide concentration without any pretreatment in a variety of biological samples, such as serum, citrate-plasma, urine, cell lysates, and culture medium. This assay utilizes the chromogenic Fe^{3+}-xylenol orange reaction, in which a purple complex is formed when Fe^{2+} is oxidized to Fe^{3+} by peroxides present in the sample, generating a colorimetric (585 nm) result, proportional to the level of peroxide present. The optimized formulation reduces interference by substances in the raw samples.

A concentration of 1 μM H_2O_2 equals 34 ng/mL or 34 ppb. The kit has a detection range of 0.2–30 μM (7–1,020 ng/mL) H_2O_2 in a 96 well format.

Components
The kit is sufficient for 250 assays in 96 well plates.

Component	Amount
Reagent A Catalog Number MAK311A	1 mL
Reagent B Catalog Number MAK311B	50 mL
3% stabilized H_2O_2 Standard Catalog Number MAK311C	100 μL

Reagents and Equipment Required but Not Provided.
- 96 well flat-bottom plate – It is recommended to use clear plates for colorimetric assays.
- Spectrophotometric multiwell plate reader

Precautions and Disclaimer
This product is 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.

Preparation Instructions
Briefly centrifuge vials before opening. Use ultrapure water for the preparation of reagents. To maintain reagent integrity, avoid repeated freeze/thaw cycles.

Detection Reagent – Allow Reagents A and B to come to room temperature before use. Prepare enough Detection Reagent by mixing 1 volume of Reagent A with 100 volumes of Reagent B.

3% H_2O_2 Standard – Prepare fresh standards on the day of assay. Dilute 5 μL of 3% H_2O_2 standard with 495 μL of ultrapure water. Mix well and dilute further by adding 5 μL of the solution with 1,465 μL of ultrapure water. The final H_2O_2 concentration is 30 μM.

Storage/Stability
The kit is shipped at room temperature. Storage at 2–8 °C is recommended.

Procedure

All samples and standards should be run in duplicate. Use ultrapure water for the preparation of standards and samples.

H_2O_2 Standards for Colorimetric Detection

Add 0, 10, 20, 30, 40, 60, 80, and 100 μL of the 30 μM Standard Working Solution into wells of a 96 well plate. Add water to each well to bring the volume to 100 μL, generating 0 (blank), 3, 6, 9, 12, 18, 24, and 30 μM standards.

Sample Preparation

Samples can be assayed immediately after collection. All samples should be aliquoted and stored at –20 °C. Avoid repeated freeze-thaw cycles.

Note: Several chemicals used in sample preparation are known to interfere with this assay, thus should be avoided. These include ascorbic acid, EDTA, heparin, DMSO (>0.02%), NP-40 (>0.6%), SDS (>0.12%), Tris (>8 mM), and ethanol (>0.4%).

Assay Reaction

1. Add 40 μL of each standard into wells of a 96 well plate.
2. Add 40 μL of each sample into separate wells.
3. Add 200 μL of prepared Detection Reagent to each sample and standard well.
4. Incubate the reaction for 30 minutes at room temperature.
5. Measure the absorbance at 585 nm (A_{585}). (Optical density at 540–610 nm)

Note: If precipitation occurs after adding the Detection Reagent to a sample, transfer the reaction mixture to a 1.5 mL tube and centrifuge for 2 minutes at 14,000 rpm. Carefully remove 200 μL of the supernatant and add to a new well. Read absorbance and multiply the OD reading by 1.2 to account for the volume change.

Results

Calculations

Note: A new standard curve must be set up each time the assay is run.

The background is the value obtained for the 0 (assay blank) 3% H_2O_2 Standard. Correct for the background by subtracting the 0 (assay blank) value from all readings. Background values can be significant and must be subtracted from all readings. Use the values obtained from the appropriate 3% H_2O_2 standards to plot a standard curve. Determine the peroxide content of the samples from the standard curve.

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay not working	Cold assay buffer	Assay Buffer must be at room temperature
	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	For colorimetric assays, use clear plates
Samples with erratic readings	Samples prepared in different buffer	Use the Assay Buffer provided or refer to Technical Bulletin for instructions
	Cell/Tissue culture samples were incompletely homogenized	Repeat the sample homogenization, increasing the length and extent of homogenization step.
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if samples will be used multiple times
	Presence of interfering substance in the sample	If possible, dilute sample further
	Use of old or inappropriately stored samples	Use fresh samples and store correctly until use
Lower/higher readings in samples and standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Use of expired kit or improperly stored reagents	Check the expiration date and store the components appropriately
	Allowing the reagents to sit for extended times on ice	Prepare fresh Reaction Mix before use
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Non-linear standard curve	Use of partially thawed components	Thaw and resuspend all components before preparing the reaction mix
	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Pipetting errors in the Reaction Mix	Prepare a Reaction Mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the plate well
	Standard stock is at incorrect concentration	Refer to the standard dilution instructions in the Technical Bulletin
	Calculation errors	Recheck calculations after referring to Technical Bulletin
	Substituting reagents from older kits/lots	Use fresh components from the same kit
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further
	Sample readings above/below the linear range	Concentrate or dilute samples so readings are in the linear range

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