***Oxis*Research™**
A Division of AOXRE LLC

BIOXYTECH® H_2O_2-560 ™
Quantitative Hydrogen Peroxide Assay

For Research Use Only. Not For Use In Diagnostic Procedures.
Store at 2 to 8°C
Catalog Number: 21024

INTRODUCTION

INTENDED USE

The H_2O_2-560 Assay is intended for the colorimetric quantitative determination of hydrogen peroxide (total hydroperoxides) in aqueous samples.

INTRODUCTION

The study of hydrogen peroxide in biological systems prompts the need for rapid and quantitative measurement of hydrogen peroxide. The researcher also requires a method of measuring hydrogen peroxide in laboratory reagents and detergents to prevent the unintended contribution of peroxidation by reagent use. The H_2O_2-560 Assay allows for simple colorimetric measurement of hydrogen peroxide.

PRINCIPLES OF THE PROCEDURE

The H_2O_2-560 Assay is based on the oxidation of ferrous ions (Fe^{2+}) to ferric ions (Fe^{3+}) by hydrogen peroxide under acidic conditions, equation (1). The ferric ion binds with the indicator dye xylenol orange (3,3'-bis[N,N-di(Carboxymethyl)-aminomethyl]-o-cresolsulfone-phthalein, sodium salt) to form a stable colored complex which can be measured at 560 nm, equation (2).

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + HO^{\bullet} + OH^{-} \qquad (1)$$

$$Fe^{3+} + XO \rightarrow Fe^{3+}\text{—}XO \qquad (2)$$

The Fe^{3+}-xylenol orange complex has an extinction coefficient of 1.5 X 10^4 $M^{-1}\bullet cm^{-1}$ at 560 nm when ferric ions are added in the absence of hydrogen peroxide. However, in the presence of sorbitol there is substantial chain oxidation of ferrous ion, increasing the color yield. This results in an apparent extinction coefficient of 2.67 X 10^5 $M^{-1}\bullet cm^{-1}$, indicating that approximately 18 moles of Fe^{2+} are oxidized to Fe^{3+} for every mole of hydrogen peroxide present, increasing the sensitivity of the assay.

REAGENTS

Materials Provided

R1: Ammonium iron(II) sulfate, H_2SO_4, 1 mL.
R2: Sorbitol, xylenol orange in water, 2 X 50 mL

Warnings and Precautions

- **For Research Use Only. Not For Use In Diagnostic Procedures.**
- For *In Vitro* Use Only.
- Wear appropriate laboratory attire, including eye protection and disposable gloves.
- In case of accidental exposure to skin, eyes or mucous membranes, thoroughly rinse affected area with water.

Preparation

Prepare working reagent by mixing 1 volume of R1 with 100 volumes of R2. Prepare only the volume needed for the day's assays.

Reagent Storage

Store the reagents at 2-8°C. Reagents are stable until the expiration date on the label. The prepared working reagent is stable for 12 hours when stored at 2-8°C. Discard unused working reagent at the end of the day.

PROCEDURE

Materials Required But Not Provided

- 30% H_2O_2
- Deionized water
- Adjustable pipets with disposable tips
- Test tubes and spectrophotometric cuvettes or microplates
- Spectrophotometer or microplate reader capable of an absorbance reading in the 520-600 nm range
- 2.5 M H_2SO_4 (for optional sample blank)

Standard Preparation

1. Prepare fresh standards for each use.
2. Prepare a 25 mM standardized H_2O_2 stock solution by diluting 285 µL 30% H_2O_2 to 100 mL with DI H_2O.
3. Standardize using 43.6 $M^{-1} \bullet cm^{-1}$ as the molar extinction coefficient for H_2O_2 at 240 nm, *i.e.*, in a 1 cm cuvette, a 25 mM H_2O_2 solution will have an absorbance of 1.09 at 240 nm.
4. Dilute 25 mM H_2O_2 1/250 with DI H_2O to give a 100 µM standard (highest standard).
5. Perform dilutions of the 100 µM standard to obtain a range of standards with appropriate concentrations for the samples to be assayed.

Assay Method

1. Add 1 volume of sample or standard to 10 volumes of working reagent.
2. Mix well and incubate at room temperature for 25-30 minutes.
3. Zero the spectrophotometer.
4. Measure the absorbance at 560 nm.

Stability of Final Reaction Material

The incubation step is necessary for the reaction to reach an endpoint. Once formed, the complex is stable for up to 12 hours. The absorbance should be measured the same day.

Assay Notes

- Excessive H_2O_2 can bleach color from the dye, resulting in lower measured absorbance. If the target concentration of the sample is unknown, perform a 1:100 dilution of the sample and repeat the assay. Absorbance values for the diluted sample that are the same or higher than the original reading indicate excessive H_2O_2. Repeat the process of performing 1:100 dilutions and reassaying the sample until the dilution-corrected concentration is consistent.
- If endogenous iron is suspected, such as in biological samples, a sample blank should be assayed by replacing the working reagent with “blanking” reagent (1 volume 2.5 M H_2SO_4 mixed with 100 volumes R2). The measured absorbance should be subtracted from the sample absorbance.

RESULTS

Standard Curve

- Use a range of standards appropriate for the samples to measure.
- The maximum standard concentration to use is 100 µM. At concentrations above 100 µM the standard curve reaches a plateau.
- Plot absorbance *versus* standard concentration.
- The standard curve can be *approximated* by linear regression.
- Other curve fitting or graphical interpolation may be used.

Sample Values

- Subtract the blank absorbance values from sample absorbance values to obtain the net absorbance.
- If using linear regression or other mathematical curve fit, obtain concentration from the net absorbance using the equation of the standard curve.
- If using interpolation, obtain the concentration graphically from the plot.

LIMITATIONS OF THE PROCEDURE

- Endogenous iron and other transition metals may increase color development. Use the sample blanking procedure if needed.
- High concentrations of fructose, sorbitol, sucrose, glucose and formic acid in samples result in increased color yield.

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