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A Geno Technology, Inc. (USA) brand name

PEROXsay™

A Quantitative Peroxide Assay

(Cat. # 786-440)

INTRODUCTION

PEROXsay™ is a colorimetric quantitative peroxide assay that measures the oxidation of ferrous (Fe^{2+}) ions to ferric (Fe^{3+}) ions. Basically, the peroxides react with a sugar alcohol converting it to a peroxyl radical that subsequently starts the oxidation of ferrous ions to ferric ions. The acidic pH of the PEROXsay™ Component 2 allows the ferric (Fe^{3+}) ion to complex with xylenol orange, a constituent of PEROXsay™ Component 1, resulting in a change in absorbance that is proportional to the peroxide concentration. The PEROXsay™ is suitable for the following applications; measurement of lipid peroxidation of low density lipoproteins and liposomes, quantifying level of protein damaging peroxides in detergents, and monitoring protein glycation. The PEROXsay™ assay is designed for microtiter plates, but can be scaled up for use with 1ml cuvettes.

ITEM(S) SUPPLIED (Cat. # 786-440)

Description	Size
PEROXsay™ Component 1	50ml
PEROXsay™ Component 2	0.5ml

STORAGE CONDITIONS

The kit is shipped at ambient temperature. Store the kit at 4°C, when stored properly the kit is stable for 1 year.

ADDITIONAL ITEMS REQUIRED

30% Hydrogen peroxide solution (8.8M)

PREPARATION BEFORE USE:

Assay Solution

1. For microtiter plate assays, you require 200µl Assay Solution for each sample and for cuvettes you will require 1ml Assay Solution.
2. Add 1 volume of PEROXsay™ Component 2 to 100 volumes PEROXsay™ Component 1 and mix.
3. The Assay Solution must be made fresh on the day of the assay.

Hydrogen Peroxide Standards

1. Add 5µl 30% Hydrogen Peroxide solution to 440ml deionized (DI) water to give a 100µM concentration.
2. Serially dilute the 100µM hydrogen peroxide solution four times to give hydrogen peroxide standards of 6.25, 12.5, 25 and 50µM.
   NOTE: *To standardize the starting 30% hydrogen peroxide solution, use the molar coefficient of 43.6* $M^{-1}cm^{-1}$ *for hydrogen peroxide at 240nm.*

PROTOCOL

NOTE: *The linear range for this assay is 0-50µM. Dilute samples with higher peroxide concentrations. In addition, samples with >1mM peroxide may cause bleaching and low absorbance reading, to alleviate this issue assay a 1:100 dilution in parallel.*

NOTE: *For samples that may have chelating proteins, transition metals or strong absorbance at or near 560nm, use a blank of* PEROXsay™ *Component 1 without* PEROXsay™ *Component 2. Subtract this blank from the assayed sample to control for the above interferences.*

1. For each volume of sample, add 10 volumes of Assay Solution.
   NOTE: *For a microtiter plate, add 200µl Assay Solution to wells with20µl sample.*
2. Mix and then incubate at room temperature for 30 minutes.
3. After incubation, measure the absorbance at 560nm.
   NOTE: *Absorbances can be read at 560-600nm, for plate readers use 595nm*
4. Plot a standard curve using the absorbances of the hydrogen peroxide samples and calculate the concentration of peroxides in your sample. (See Figure 1)

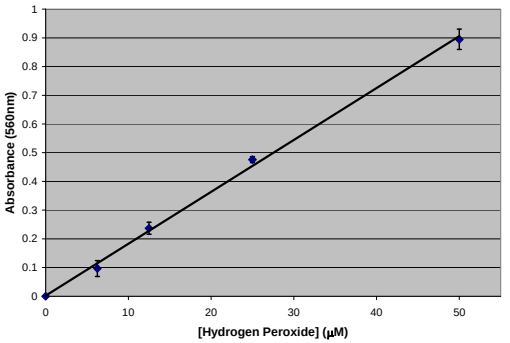
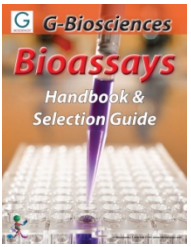


Figure 1: PEROXsay™ Linear Range of Standard Curve. A 1mM hydrogen peroxide solution was serially diluted and 50µl was used in an assay with 500µl PEROXsay™ Assay Solution. Absorbances were measured at 560nm. The error bars show the standard deviation of 10 individual experiments.

RELATED PRODUCTS

Download our Protease & Phosphatase Inhibitors, Enzyme & Assays Handbook.



http://info.gbiosciences.com/complete-bioassay-handbook

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