PEROXsay™

A quantitative peroxide assay

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**
PEROXsay™ Component 1	50ml
PEROXsay™ Component 2	0.5ml

STORAGE CONDITIONS

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

ITEMS NEEDED BUT NOT SUPPLIED WITH KIT

30% Hydrogen peroxide solution (8.8M)

PREPARATION BEFORE USE:

Assay Solution:

For microtiter plate assays, you require 200µl Assay Solution for each sample and for cuvettes you will require 1ml Assay Solution.

Add 1 volume of PEROXsay™ Component 2 to 100 volumes PEROXsay™ Component 1 and mix.
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:

A. *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.*

B. *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.
   For a microtiter plate, add 200µl Assay Solution to each well containing 20µl sample.

2. Mix and then incubate at room temperature for 30 minutes.

3. After incubation, measure the absorbance at 560nm.
   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)

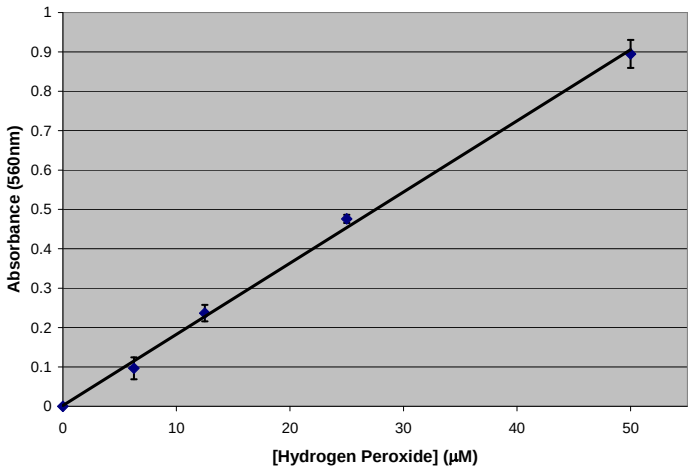

Figure 1: PEROXsay™ Linear Range of Standard Curve. A 1mM hydrogen peroxide solution was serially diluted and 50µl was used in an assay with 500µl PEROXsay™ Assay Solution. Absorbances were measured at 560nm. The error bars show the standard deviation of 10 individual experiments.

RELATED PRODUCTS

1. ***Proteomic Grade Detergents:*** A selection of non-ionic detergents are available that have ultra low levels of protein damaging peroxides and aldehydes. Visit www.GBiosciences.com/proteomic-detergents-products

NOTE: *For other related products, visit our web site at www.GBiosciences.com or contact us.*