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A Geno Technology, Inc. (USA) brand name

PEROXsay™-LIPID

A lipid compatible quantitative peroxide assay

(Cat. #786-441)



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INTRODUCTION

PEROXsay™-LIPID is a colorimetric quantitative peroxide assay that measures the oxidation of ferrous (Fe^{2+}) ions to ferric (Fe^{3+}) ions in the presence of lipids. Basically, the peroxides oxidize ferrous ions to ferric ions. The acidic pH of the PEROXsay™ Component 2 allows the ferric (Fe^{3+}) ion to complex with xylene orange, a constituent of PEROXsay™-LIPID Component 1, resulting in a change in absorbance that is proportional to the peroxide concentration.

The PEROXsay™-LIPID 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. # 786-441)

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

STORAGE CONDITIONS

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

ADDITIONAL ITEM(S) REQUIRED

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™-LIPID 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 $43.6 \text{ M}^{-1}\text{cm}^{-1}$ for hydrogen peroxide at 240nm.

PROTOCOL

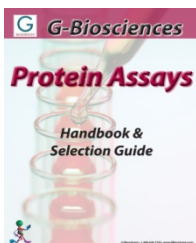
NOTE: The linear range for this assay is 0-50µM. Dilute samples with higher peroxide concentrations. In addition, samples with >1mM peroxide may cause bleaching and low absorbance reading, to alleviate this issue assay a 1:100 dilution in parallel.

For samples that may have chelating proteins, transition metals or strong absorbance at or near 560nm, use a blank of PEROXsay™ -LIPID 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.

RELATED PRODUCTS

Download our Protein Assays Handbook.



<http://info.gbiosciences.com/complete-protein-assay-guide>

For other related products, visit our website at www.GBiosciences.com or contact us.

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