# Lipid Peroxidation (LPO) Assay

## (Cat. # BAQ067)

## INTRODUCTION

Lipid peroxidation is a well-known example of oxidative damage in cell membranes, lipoproteins, and other lipid-containing structures. Peroxidative modification of unsaturated phospholipids, glycolipids, and cholesterol can occur in reactions triggered by i) free radical species such as oxyl radicals, peroxyl radicals, and hydroxyl radicals derived from iron-mediated reduction of hydrogen peroxide or ii) non-radical species such as singlet oxygen, ozone, and peroxynitrite generated by the reaction of superoxide with nitric oxide.

Malondialdehyde (MDA) and 4-hydroxyalkenals are important toxic byproducts of lipid peroxidation. The measurement of the amounts of such aldehydes has been widely used as an index of lipid peroxidation in vitro and in vivo.

4-Hydroxynonenal (4-HNE) is produced as a major product of the peroxidative decomposition of ω-6 polyunsaturated fatty acids (PUFA) and possesses cytotoxic, hepatotoxic, mutagenic, and genotoxic properties. Increased levels of HNE were found in plasma and various organs under oxidative stress conditions.

MDA is in many instances the most abundant individual aldehyde resulting from lipid peroxidation. In vitro MDA can alter proteins, DNA, RNA, and many other biomolecules.

G-Biosciences' LPO assay kit measures MDA and HNE concentrations as an index of lipid peroxidation. Reactions between indoles and aldehydes (MDA and HNE) are initiated by acid-catalyzed attack at the 3-position of the indole ring to give a diindolylalkane (chromophore) with maximum absorbance in the region of 580-620 nm.

In our assay an indol (Reagent A) reacts quickly with MDA and HNE in acidic medium, yielding a chromophore (C) with a high molar extinction coefficient at its maximal absorption wavelength of 586 nm.

$$\text{Reagent A} + \text{MDA} \rightarrow \text{C} \ (\lambda_{max} = 586 \text{ nm})$$
$$\text{Reagent A} + \text{HNE} \rightarrow \text{C} \ (\lambda_{max} = 586 \text{ nm})$$

## ITEM(S) SUPPLIED

| Description | 200 tests (96 well plate) |
|---|---|
| LPO Solvent | 1 vial |
| LPO Reagent A | 3 vials (powder) |
| LPO Reagent B | 5 vials |
| LPO Standard | 1 vial |

## STORAGE CONDITIONS

This kit is shipped at ambient temperature. Store the LPO standard at 4°C upon arrival. All other reagents can be stored at room temperature. If stored and used as directed this kit is stable for 12 months.

## ADDITIONAL ITEMS REQUIRED

- Spectrophotometer microplate reader that can measure at 580-620 nm
- 96 well microtiter plate for microplate assay.
- 1.5ml Tubes
- 20mM Tris buffer, pH 7.4

## SAMPLE PREPARATION

***Tissues and cells***

1. Homogenize at 4°C in 20 mM Tris buffer (pH 7.4).
2. Centrifuge for 10 min at 2,000 x g and 4°C.

***Plasma samples***

Plasma sample does not need prior sample preparation. Use directly in the assay.

## PREPARATION BEFORE USE

***LPO Reagent A Solution***

Add 25 mL of LPO Solvent to each vial of LPO Reagent A and mix well. Once prepared, the mixture must be used immediately.

***Standard preparation***

Prepare calibration curve in 1.5 mL tubes as shown below.

| Sample | Standard [µL] | $H_2O$ Diluent [µL] | Standard [µM] |
|---|---|---|---|
| S1 (Blank) | 0 | 1000 | 0 |
| S2 | 5 | 995 | 5 |
| S3 | 10 | 990 | 10 |
| S4 | 20 | 980 | 20 |
| S5 | 30 | 970 | 30 |
| S6 | 40 | 960 | 40 |
| S7 | 50 | 950 | 50 |
| S8 | 60 | 940 | 60 |

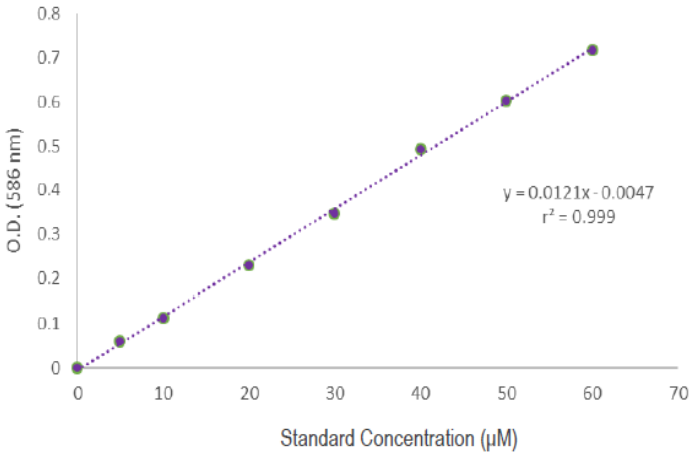
## PROTOCOL

1. Add 100 µL of samples or standard to 325 µL of previously prepared LPO Reagent A solution
2. Add 75 µL of LPO Reagent B to the mixture and mix thoroughly.
3. Incubate the mixture for 40 minutes at 40°C.
4. If the mixture is cloudy, centrifuge it at 5,000 x g, for five minutes at room temperature.
5. Transfer 200 µL of the supernatant from each tube into a 96-well plate.
6. Measure the absorbance at 586 nm using a 96-well plate reader.

## DATA ANALYSIS

Subtract the average absorbance of the Standard 1 (Blank) from itself and all other standards and samples to obtain the corrected absorbance.

Plot the corrected absorbance of standards as a function of their final concentrations.



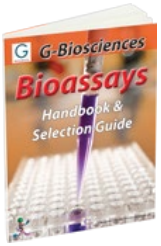

Typical standard curve for LPO assay

Calculate the aldehydes values (MDA + HNE) of the samples using the equation obtained from the linear regression of the standard curve replacing the corrected absorbance values for each sample.

MDA + HNE (µM) = (sample absorbance-y) / slope

## RELATED PRODUCTS

Download our Bioassays Handbook.




http://info2.gbiosciences.com/complete-bioassay-handbook

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