Lipid Peroxidation (LPO) Assay Kit

KB-03-002

200 test (96 well plate)

BQCkit

A brand of Bio QuChem

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All chemicals should be handled with care

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- This kit is for R&D use only

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.

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.

Malondialdehyde (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.

Materials

BQCkit Lipid Hidroperoxide (LPO) Assay kit *KB03002-200 tests* contains:

Product	Quantity	Storage
LPO Solvent	1 vial	RT
LPO Reagent A	3 vials (powder)	RT
LPO Reagent B	4 vials	RT
LPO Standard*	1 vial	4 ºC

* This reagent is stable during 10 days at Room Temperature and is shipped in these conditions. Once received is recommended to keep it at 4ºC.

Bioquochem LPO Kit does NOT contain:

- Tris buffer (pH=7.4)

Assay Principle

Bioquochem 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.

$$\text{Reagent A} + \text{MDA} \rightarrow \text{C} \ (\lambda\text{máx} = 586 \text{ nm})$$

$$\text{Reagent A} + \text{HNE} \rightarrow \text{C} \ (\lambda\text{máx} = 586 \text{ nm})$$

Scheme 1. *Reactions between aldehydes and indoles*

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 it directly in the assay.

Assay Protocol

Reagents preparation

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

Plate set up

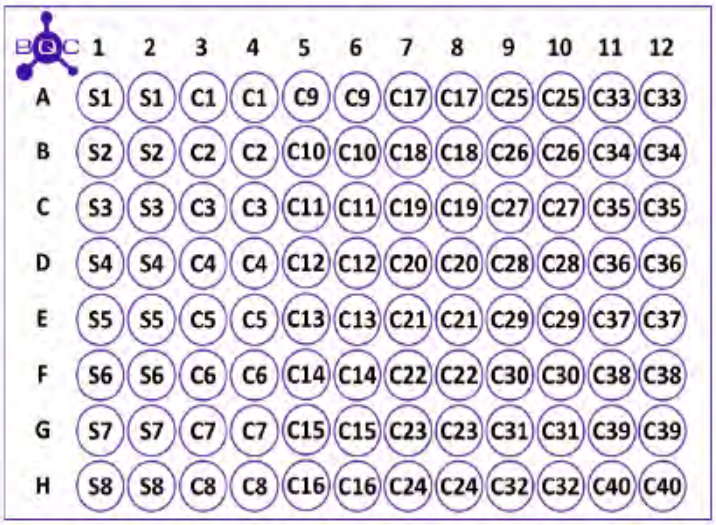

Figure 1. *96-well plate filling format*

S1-S8 = Standards
C1-C40 = Samples

Attention

- If the concentration of MDA and HNE in the samples is not unknown or if it is expected to be beyond the range of the standard curve, we recommend assaying the samples at several dilution levels.

- For optimal results, we recommend running the standards and samples for duplicate, but it is the user´s discretion to do so.
- The blank sample absorbance (A0) must be ≤ 0.01

Standard Preparation

Prepare calibration curve in 1.5 mL tubes as shown below in Table 1.

Table 1. *Reagent volumes needed to carry out the standard curve*

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

Performing the assay

1. For this purpose, use 1.5 mL tubes:
1. Add 100 µL of samples or standard to 325 µL of previously prepared Reagent A solution (see reagents preparation).
2. Add 75 µL of 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 showing the configuration shown in Figure 1.
6. Measure the absorbance at 586 nm using a 96-well plate reader.

Data Analysis

1. Subtract the average absorbance of the Standard 1 (Blank) from itself and all other standards and samples to obtain the corrected absorbance.
2. Plot the corrected absorbance of standards as a function of their final concentrations (Table 1). See Figure 2 for a typical standard curve.

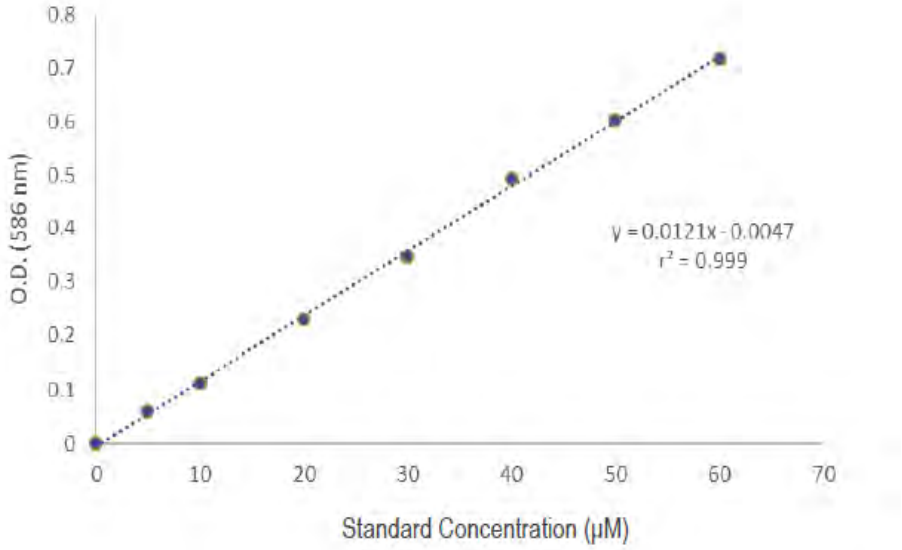

Figure 2. *Typical standard curve for LPO assay*

3. 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

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Said refund or replacement is conditioned on buyer giving written notice to Bioquochem within 30 days after arrival of the material at its destination.

Expiration date: 1 year from the date of delivery

For further details, please refer to our website www.bqckit.com.