Lipid Peroxidation (LPO) Assay Kit

Catalog No.: abx096010

Size: 100 Assays

Storage: Store all kit components at 4°C in the dark.

Application: For quantitative detection of Lipid Peroxide (LPO) content in serum, plasma, tissue homogenates, and urine.

Detection Range: 0.7 µmol/L – 80 µmol/L

Sensitivity: 0.7 µmol/L

Introduction: Reactive oxygen species degrade polyunsaturated lipids, forming malondialdehyde. This compound is a reactive aldehyde and is one of the many reactive electrophile species that cause toxic stress in cells and form covalent protein adducts referred to as advanced lipoxidation end-products (ALE), in analogy to advanced glycation end-products (AGE). The production of this aldehyde is used as a biomarker to measure the level of oxidative stress in an organism. Malondialdehyde reacts with deoxyadenosine and deoxyguanosine in DNA, forming DNA adducts, the primary one being M1G, which is mutagenic. The guanidine group of arginine residues condense with malondialdehyde to give 2-aminopyrimidines. Human ALDH1A1 aldehyde dehydrogenase is capable of oxidizing malondialdehyde.

Principle of the assay: Quantification of lipid peroxidation is essential to assess oxidative stress in pathophysiological processes. Lipid peroxidation forms Lipid peroxides (LPO) as natural by-products. Measuring the end products of lipid peroxidation is one of the most widely accepted assays for oxidative damage. Abbexa's LPO Assay Kit provides a convenient tool for sensitive detection of the LPO in a variety of samples. The LPO in the sample reacts with the Substrate to produce a colored compound, which can be measured spectrophotometrically at 586 nm.

Kit components

1. 96 well microplate
2. Substrate: 60 ml
3. Substrate Diluent: 20 ml
4. Acid reagent: 20 ml
5. Standard (100 µmol/L): 6 ml
6. Plate sealer: 2

Materials Required But Not Provided

1. Microplate reader (586 nm)
2. Incubator and water bath
3. Microcentrifuge tubes
4. High-precision pipette and sterile pipette tips
5. Double distilled water
6. Homogenization Medium (0.01 M, pH 7.4 PBS; or 20 mM, pH 7.4 Tris-HCl)
7. Absolute ethanol
8. Mortar
9. Timer
10. Ice
11. Sonicator

Protocol

A. Preparation of Sample and Reagents

1. Reagents

- **Substrate Working Solution**
  Dilute the Substrate with the Substrate Diluent 4-fold (for example. Add 20 ml of Substrate Diluent to 60 ml of Substrate) and mix thoroughly. Prepare immediately before use.

2. Samples

- **Tissue samples**
  Weigh the sample accurately. For every 1 g of sample, homogenize with 9 ml of Homogenization medium (0.01 M, pH 7.4 PBS; or 20 mM, pH 7.4 Tris-HCl). Centrifuge at 8000 × g at 4°C for 10 minutes. Transfer the supernatant to a new tube, keep on ice and analyze immediately. The protein concentration of the supernatant should be determined separately.

- **Serum samples**
  Samples should be collected into a serum separator tube. Coagulate the serum by leaving the tube undisturbed in a vertical position overnight at 4°C or at room temperature for up to 1 hr. Centrifuge at approximately 2000 × g for 15 mins at 4°C. If a precipitate appears, centrifuge again. Take the supernatant, keep on ice and assay immediately, or aliquot and store at -80°C for up to 1 month.

- **Plasma samples**
  Collect plasma using EDTA or heparin as the anticoagulant. Centrifuge for 10 mins at 700-1000 × g at 4°C, within 30 mins of collection. If precipitate appears, centrifuge again. Avoid hemolytic samples. Take the supernatant (avoid taking the middle layer containing white blood cells and platelets), keep on ice and assay immediately, or aliquot and store at -80°C for up to 1 month.

- **Urine samples**
  Collect fresh urine and centrifuge at 10,000 × g for 15 min at 4°C. Take the supernatant, place on ice and assay immediately.

  Where sample dilutions are required, dilute with PBS (0.01 M, pH 7.4).

B. Assay Procedure

Bring all reagents to room temperature prior to use.

1. Set the sample, standard and blank tubes. We recommend setting up each standard and sample in duplicate.
2. Label 7 tubes with 5 µmol/L, 10 µmol/L, 20 µmol/L, 30 µmol/L, 40 µmol/L, 50 µmol/L and 80 µmol/L. Absolute ethanol serves as the 0 µmol/L blank. Prepare the standard curve according to the following table.

Volume of 100 µmol/L Standard (µl)	Volume of Absolute Ethanol (µl)	Concentration (µmol/L)
0	1000	0
50	950	5
100	900	10
200	800	20
300	700	30
400	600	40
500	500	50
800	200	80

3. Add 200 µl of standard solution to each standard tube.
4. Add 200 µl of prepared sample solution to each sample tube.
5. Add 650 µl of Substrate working solution to all tubes, close the caps and mix fully.
6. Add 150 µl of Acid Reagent to all tubes, close the caps and mix fully.
7. Incubate at 45°C for 60 minutes. Cool to room temperature with running water.
8. Set the sample, standard and blank wells on the 96 well microplate and record their positions.
9. Centrifuge all tubes at 1100 × g at room temperature for 10 minutes. Take 200 µl of the supernatant to the respective microplate wells.
10. Read and record absorbance at 586 nm.

C. Calculations

The standard curve can be plotted as the absolute absorbance of each standard solution (y) vs. the respective concentration (y). A linear fit is recommended for the standard curve (y = ax + b).

Lipid Peroxide (LPO) per L of sample:

$$\mathbf{LPO\ (\mu mol/L)} = \frac{(\mathbf{OD_{Sample}} - \mathbf{OD_{Blank}}) - \mathbf{b}}{\mathbf{a}} \times \boldsymbol{f}$$

Lipid Peroxide (LPO) per g of protein:

$$\mathbf{LPO\ (\mu mol/g)} = \frac{(\mathbf{OD_{Sample}} - \mathbf{OD_{Blank}}) - \mathbf{b}}{\mathbf{a}} \times \frac{\boldsymbol{f}}{\mathbf{C}_{\boldsymbol{P}}}$$

Lipid Peroxide (LPO) per kg of sample wet weight:

$$\mathbf{LPO\ (\mu mol/kg)} = \frac{(\mathbf{OD_{Sample}} - \mathbf{OD_{Blank}}) - \mathbf{b}}{\mathbf{a}} \times \frac{\boldsymbol{f} \times \mathbf{V}}{\boldsymbol{m}}$$

Where:

$\mathbf{OD_{Sample}}$	Absorbance of the sample
$\mathbf{OD_{Blank}}$	Absorbance of the blank well
$\mathbf{b}$	Intercept of the standard curve (y = ax + b)
$\mathbf{a}$	Gradient of the standard curve (y = ax + b)
$\boldsymbol{f}$	Dilution factor of the sample prior to assay
$\boldsymbol{m}$	Wet weight of tissue (g)
$\mathbf{V}$	Volume of sample in the assay (ml)
$\mathbf{C}_{\boldsymbol{P}}$	Concentration of protein in the sample (g/L)