Lipid Peroxidation Colorimetric Assay Kit

03/19

(Catalog # K454-100; 100 assays; Store at 4°C)

I. Introduction:

Lipid peroxidation is a type of oxidative damage that affects cellular membranes, lipoproteins, and other lipid-containing molecules. This series of chain reactions occur between highly reactive oxygen species and unsaturated fatty acids in cell membranes, which leads to cell damage. Lipid peroxides, derived from polyunsaturated fatty acids are unstable and decompose forming malondialdehyde (MDA) and 4-hydroxyalkenals. The measurement of MDA is widely used and is considered as an indicator of lipid peroxidation. Traditionally, 2-Thiobarbituric acid (TBA) method has been widely used for the colorimetric detection and measurement of MDA in samples. However, this reaction is relatively nonspecific since both free and protein-bound MDA react with TBA. BioVision's Lipid Peroxidation Assay Kit uses a proprietary set of reagents that minimizes the interference from other lipid peroxidation products including 4-hydroxyalkenals. The principle is based on the reaction between a specific chromogenic reagent and MDA at 45 °C. The assay is simple to perform, and yields a strong, yet stable colorimetric signal (OD 586 nm) that is proportional to the amount of MDA in samples. Our assay can detect as low as 3 nmol MDA in samples.

II. Applications:

- Measurement of lipid peroxidation in tissues and serum samples

III. Sample Type:

- Serum
- Plasma
- Tissues (*e.g.* liver)

IV. Kit Contents:

Components	K454-100	Cap Code	Part Number
Chromogenic Reagent	6 ml	NM	K454-100-1
Diluent A	5 ml	Red NM	K454-100-2
Diluent B	5 ml	Blue NM	K454-100-3
BHT	1 ml	Purple	K454-100-4
MDA Standard	200 µl	Yellow	K454-100-5

V. User Supplied Reagents and Equipment:

- 96-well clear plate with flat bottom
- Multi-well spectrophotometer
- Concentrated HCl (12 N)
- Acetonitrile
- Isopropyl alcohol

VI. Storage Conditions and Reagent Preparation:

Upon arrival, store kit at 4°C, protected from light. Briefly centrifuge small vials prior to opening. Read the entire protocol before performing the assay.

- **Chromogenic Reagent:** Prepare working solution (500 µl/assay) immediately before use. Dilute 10-fold with acetonitrile, (i.e. 1 ml Chromogenic Reagent + 9 ml Acetonitrile). Dilute enough reagents for the number of assays to be performed. Discard unused diluted reagents
- **Diluent A:** Prepare working solution (~200 µl/assay) immediately before use. Dilute Diluent A 62.5-fold with **isopropanol** (i.e. 64 µl Diluent A + 3936 µl Isopropyl alcohol). Dilute enough reagents for the number of assays to be performed. Discard unused diluted reagents.
- **Diluent B:** Prepare working solution (500 µl/100 mg of tissue). Dilute Diluent B 10-fold with ddH_2O (i.e. 500 µl Diluent B + 4.5 ml of ddH_2O). Diluted solution can be stored at 4 ºC and should be used within 2 months.
- **Developer Mix:** Prepare Developer Mix (650 µl/assay) as follows: Mix diluted Chromogenic Reagent and diluted Diluent A at a ratio of 3:1 (i.e. 495 µl diluted Chromogenic Reagent + 165 µl diluted Diluent A). Discard unused diluted reagents.
- **MDA Standard:** Ready-to-use as supplied.

VII. Lipid Peroxidation Assay Protocol:

1. Sample Preparation:

For tissues: Prepare homogenizing buffer (1 ml of diluted Diluent B + 3 µl BHT). Rapidly homogenize Samples (~200 mg) in 1 ml ice-cold homogenizing buffer. Keep on ice for 10 min. Centrifuge at 3000 x *g* and 4°C for 10 min. Transfer supernatant to a fresh tube. Bring pH of the tissue supernatant to 1.5 using concentrated HCl.

For serum: Add 3 µl BHT to 1 ml of Serum Sample and adjust pH to 1.5.

Sample Hydrolysis: For all Samples: Aliquot 200 µl of acidified supernatants (pH 1.5), and hydrolyze at 60 ºC for 80 minutes.

Notes: a) Approximately 10 µl of concentrated HCl is required to bring 1 ml of Serum Sample to pH 1.5. Users need to verify the amount of HCl needed for their respective Samples.

b) Make sure to bring the heat block to 60°C before starting the hydrolysis step.

2. **MDA Standard Curve:** Prepare 100 mM solution by diluting 25 µl of MDA Standard (6 M) with 1475 µl diluted Diluent B. Further dilute to 10 mM by adding 100 µl of 100 mM MDA solution to 900 µl ddH_2O. Prepare the working solution (200 µM) by adding 20 µl of 10 mM MDA solution to 980 µl of ddH_2O. Add 0, 10, 20, 40, 60, 80, 100 µl of 200 µM MDA Standard to individual fresh centrifuge vials. Bring the volume of each Standard to 200 µl with ddH_2O to generate 0, 2, 4, 8, 10, 16, 20 nmol/vial (0, 0.4, 0.8, 1.6, 2.4, 3.2, 4 nmol/well of MDA Standard respectively – see step 3. Development).
3. **Development:** Add 650 µl Developer Mix (see Section VI) and 150 µl of concentrated HCl to each vial containing 200 µl Standard and 200 µl hydrolyzed Sample(s). Incubate at 45 ºC for 60 minutes on a pre-heated heat block. At the end of the incubation time, centrifuge Samples at 9000 x *g* and RT for 10 min. collect the clear blue supernatant. Transfer 200 µl of each (Standards and Samples) into a 96-well clear plate.
   Note: For unknown Samples, we suggest testing several doses to ensure the readings are within the Standard Curve range.
4. **Measurement:** Measure absorbance at OD 586 nm at RT in end-point mode.

   Note: The color is stable for at least an hour at room temperature, when stored in dark. In order to confirm that there is no interference in the Sample, it is important to perform a wavelength scan from 400 nm to 700 nm using Sample Reaction Mixture and compare it with the Standard. The lack of peak at 586 nm suggests interference.
5. **Calculation:** Subtract 0 Standard reading from all readings including Standard and Sample(s) to get corrected O.D. Plot the MDA Standard Curve. Apply the corrected Sample OD to the MDA Standard Curve to get B nmol of MDA present in the Sample.

Sample Lipid peroxidation = ([B/(V/5)]/P)*D = nmol/ml

Where: **B** = MDA amount from Standard Curve (nmol)
P = Amount of tissue per well (mg). (P= 1 for serum samples)
V = Sample volume added into the reaction well (ml) (= 0.2 ml)
5 = Correction for using 200 µl (into each well of the 96-well plate) from the 1000 µl Reaction Mix
D= Pre-hydrolysis Sample dilution factor (D= 1 for undiluted Samples)

Note: The dilution factor D is only needed if the sample is diluted before the hydrolysis step.

a)

b)

Figures: (A) MDA Standard Curve. (B) MDA concentration in human serum (200 µl) and rat liver lysate (200 mg). Assay was performed following the kit protocol. Serum: 3.7 ± 0.15; Rat Liver Lysate: 3.14 ± 0.031.

VIII. RELATED PRODUCTS:

Malate Colorimetric Assay Kit (K637)
Pyruvate Colorimetric /Fluorometric Assay Kit (K609)
Citrate Colorimetric/Fluorometric Assay Kit (K655)
Citrate Synthase Activity Colorimetric Assay Kit (K318)
Succinate (Succinic Acid) Colorimetric Assay Kit (K649)
α-Ketoglutarate Colorimetric Assay Kit (K677)
Lipid Peroxidation (MDA) Colorimetric/Fluorometric Assay Kit (K739)
Fumarate Colorimetric Assay Kit (K633)
PicoProbe™ Acetyl-CoA Fluorometric Assay Kit (K317)
Oxaloacetate Colorimetric/Fluorometric Assay kit (K659)
Isocitrate Colorimetric Assay Kit (K656)
Isocitrate Dehydrogenase Activity Assay Kit (K756)
Aconitase Activity Colorimetric Assay Kit (K716)

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