ab287860 – Lipid Peroxidation Colorimetric Assay Kit

For quantitative measurement of Lipid Peroxidation in tissues and serum samples.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab287860

Storage and Stability

Upon arrival, store kit at 4°C, protected from light.

Materials Supplied

Item	Quantity	Storage Condition
Chromogenic Reagent	6 mL	4°C
Diluent A	5 mL	4°C
Diluent B	5 mL	4°C
BHT	1 mL	4°C
MDA Standard	200 µL	4°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

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

Reagent Preparation

- Read the entire protocol before performing the assay.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.

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 ddH2O (i.e. 500 µL Diluent B + 4.5 mL of ddH2O). Diluted solution can be stored at 4 °C 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.

Assay Protocol

Sample Preparation:

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.

Serum/Plasma (Heparin): Add 3 µL BHT to 1 mL of Serum/Plasma (Heparin) 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 °C for 80 minutes.

Δ 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.*

MDA Standard Curve:

1. Prepare 100 mM solution by diluting 25 µL of MDA Standard (6 M) with 1475 µL diluted Diluent B.
2. Further dilute to 10 mM by adding 100 µL of 100 mM MDA solution to 900 µL ddH2O.
3. Prepare the working solution (200 µM) by adding 20 µL of 10 mM MDA solution to 980 µL of ddH2O.
4. Add 0, 10, 20, 40, 60, 80, 100 µL of 200 µM MDA Standard to individual fresh centrifuge vials.
5. Bring the volume of each Standard to 200 µL with ddH2O 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).

Development:

1. Add 650 µL Developer Mix and 150 µL of concentrated HCl to each vial containing 200 µL Standard and 200 µL hydrolyzed Sample(s). Incubate at 45 °C for 60 minutes on a pre-heated heat block.
2. At the end of the incubation time, centrifuge Samples at 9000 x g and RT for 10 min. collect the clear blue supernatant.
3. Transfer 200 µL of each (Standards and Samples) into a 96-well clear plate.

Δ Note: *For unknown Samples, we suggest testing several doses to ensure the readings are within the Standard Curve range.*

Measurement:

Measure absorbance at OD 586 nm at RT in end-point mode.

Δ 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.*

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)

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

Technical Support

For all technical or commercial enquiries please go to:
www.abcam.com/contactus
www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)