EZ-Lipid peroxidation(TBARS) Assay Kit

Oxidative Stress Assay Kit

Cat. No. DG-TBA200

FOR RESEARCH USE ONLY.

NOT FOR USE IN DIAGNOSTIC PROCEDURES.

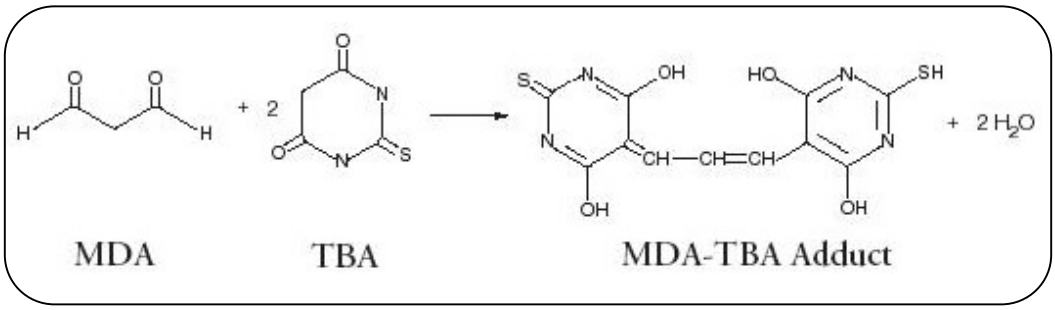
DoGenBio Co., Ltd.

▪ Product Description

Lipid peroxidation is a well-known mechanism of cellular damage in both animals and plants, and is used as an indicator of oxidative stress in cells and tissues. During lipid peroxidation, membrane lipids are degraded, and unstable hydroperoxides are formed. These hydroperoxides are highly unstable and break down into complex compounds such as reactive carbonyl compounds.

Subsequently, peroxidized polyunsaturated fatty acids are degraded into substances like Malondialdehyde (MDA), which is used as a representative marker for lipid peroxidation. The measurement of Thiobarbituric Acid Reactive Substances (TBARS) is a widely known screening method for lipid peroxidation. This method measures the MDA-TBA adduct formed when MDA reacts with Thiobarbituric Acid (TBA), thus providing an indication of the extent of lipid peroxidation.

This assay is based on the reaction between MDA and TBA at 25°C, under acidic conditions, where the MDA-TBA adduct formed is measured colorimetrically by absorbance at 540 nm (ranging from 530 to 540 nm). (The detection limit of this method is 1.0 μM.)



Formation of MDA-TBA Adduct from the Reaction of MDA and TBA

▪ Kit Contents and Storage Conditions

Component	200 assay	Storage
Indicator	2 vial	4 ℃, 2 months
Acid reagent	20 ㎖ x 2	4 ℃, 2 months
100X BHT	100 ㎕	4 ℃, 2 months
MDA standard (10 mM)	100 ㎕	4 ℃, 2 months

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* This product is for research use only and is not intended for human or diagnostic use.

* In terms of the number of tests that can be performed with this product, 100 assays means that it provides reagents that can process 100 wells based on 1 well of a 96 well plate. Among these, considering standard, blank, duplication processing per sample, etc., the actual number of samples that can be tested is in the range of 20 to 40 samples. Review the product instructions carefully and determine the number of kits required considering the characteristics of the sample you wish to test.

▪ Preparation of Reagent

Solution	Preparation	Storage and Stability
Indicator solution	Mix 10 mL of Acid Reagent with 1 vial of Indicator and stir while dissolving on a hot plate (~80°C). Once the Indicator has fully dissolved, allow the solution to cool down to room temperature before use.	The dissolved Indicator Solution can be stored at 4°C for up to 7 days. If precipitation occurs, dissolve it again using a water bath, then allow it to cool to room temperature before use.

* Do not leave the Acid Reagent at room temperature for more than 1 hour.
* After preparing the indicator solution, precipitation may occur at room temperature if the surrounding temperature is low.
* The MDA Standard stock should be prepared immediately before use and used within 24 hours.

▪ General Protocol

1. Standard preparation

Mix 20 µL of 10 mM MDA Standard with 9.98 mL of dH_2O to prepare a 20 µM MDA Standard Stock. Prepare the MDA standard by adding the solution to the microtubes as shown below.

Standard No.	Volume of 20 µM MDA Standard	Volume of dH_2O	Final standard volume in tube	Final standard MDA Conc. (µM)
0	0 ㎕	200 ㎕	200 ㎕	0
1	5 ㎕	195 ㎕	200 ㎕	0.5
2	10 ㎕	190 ㎕	200 ㎕	1.0
3	25 ㎕	175 ㎕	200 ㎕	2.5
4	50 ㎕	150 ㎕	200 ㎕	5
5	100 ㎕	100 ㎕	200 ㎕	10
6	150 ㎕	50 ㎕	200 ㎕	15
7	200㎕	0 ㎕	200 ㎕	20

* It is recommended to measure the standard each time you perform the experiment.

2. Sample preparation

- Keep the temperature of the samples low to ensure that all lipid peroxides remain stable throughout the experimental process.
- Do not refreeze the samples and store them in the dark to prevent photooxidation.
- Urine samples can be used immediately, but centrifuge to remove insoluble particles and use only the supernatant. Perform the analysis as soon as possible after collection. Reliable data can still be obtained within 24 hours. If analysis is to be performed on a later day, store the samples at -70°C.
- Plasma or serum can be analyzed directly without dilution. If analysis is to be performed after deproteinization, follow the steps below or use a 10KD spin column.

① Add 100 µL of saturated ammonium sulfate to 0.5 mL of plasma or serum sample.
② Add 35 mg of TCA to each sample, then vortex. This will result in a cloudy precipitate.
③ Centrifuge and proceed with analysis using only the supernatant.

- Tissue or Cell

① Since hemoglobin may interfere with the experiment, perform perfusion using PBS containing heparin.
② Add PBS containing 1X BHT
  - Tissue : 50 to 100 mg/ml in PBS containing 1X BHT
  - Cell : 1-2 x 10^7 cells/ml in PBS containing 1X BHT
③ Homogenize the tissue or cell sample on ice..
④ Centrifuge and proceed with analysis using only the supernatant.

Protocol for Measuring Free MDA

1. Prepare the microtubes as follows.

   1) Standards : Add 200 µL of Indicator Solution to 200 µL of standard.
   2) Samples : Add 200 µL of Indicator Solution to 200 µL of sample.
   3) Blank **(Important, do not skip this step)**
      : Add 200 µL of Acid Reagent to 200 µL of sample.

2. Incubate at room temperature for 45 minutes.
3. Add 150 µL into the microplate, then measure the absorbance at 540 nm using a plate reader.

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Protocol for Total MDA Measurement

1. Prepare the microtubes as follows.

   1) **Standards** : Add 200 ㎕ of Indicator Solution to 200 ㎕ of standard.
   2) **Samples** : Add 200 ㎕ of Indicator Solution to 200 ㎕ of sample.
   3) **Blank (Important, do not skip this step)**
      : Add 200 ㎕ of Acid Reagent to 200 ㎕ of sample.

2. Incubate at 65°C for 45 minutes.

3. Add 150 ㎕ per well into the microplate, then measure the absorbance at 540 nm using a plate reader.

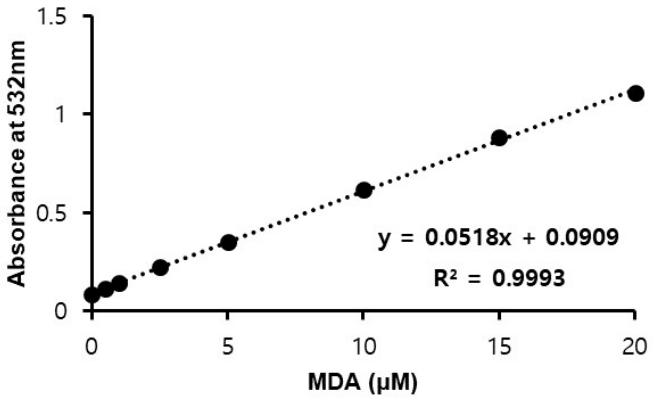
▪ Calculation

1. Subtract the OD value of the blank from all measurement values.

2. Average the duplicate measurements for each standard well, sample well, and blank well.

3. Plot the standard curve using the absorbance of the MDA standard.
   (MDA standard concentration vs OD at 540 nm)

4. Calculate the concentration of MDA for each sample using the linear equation shown by the standard curve. If the sample was diluted, the final result must be multiplied by the sample dilution factor. For serum or plasma samples, if protein removal was performed as above, an additional dilution factor of 1.2 is applied.



MDA standard curve. Assay was performed following the kit protocol.

▪ Related Product

	Products	Catalog No.	Assay
Oxidative Stress Assay Kit	EZ-Superoxide Dismutase (SOD) Assay Kit (Colorimetric)	DG-SOD400	400 Assay
	EZ-Glutathione Assay Kit (Colorimetric)	DG-GLU200	200 Assay
	EZ-Catalase Assay Kit (Fluorometric/Colorimetric)	DG-CAT400	400 Assay
	EZ-Hydrogen peroxide/Peroxidase Assay Kit (Fluorometric/Colorimetric)	DG-PER500	500 Assay
	EZ-Lipid Peroxidation (TBARS) Assay Kit (Colorimetric)	DG-TBA200	200 Assay
	EZ-Total Antioxidant Capacity (TAC) Assay Kit (Colorimetric)	DG-TAC200	200 Assay
	EZ-DPPH Antioxidant Assay Kit (Colorimetric)	DG-DPH400	400 Assay
	EZ-ABTS Antioxidant Assay Kit (Colorimetric)	DG-ABT400	400 Assay
	EZ-Glutathione Peroxidase Assay Kit (Colorimetric)	DG-GPX100	100 Assay
Metabolism Assay Kit	EZ-Lactate Assay Kit (Colorimetric)	DG-LAC100	100 Assay
	EZ-Acetylcholinesterase Assay Kit (Colorimetric)	DG-ACE100	100 Assay
	EZ-Ascorbic Acid Assay Kit (Colorimetric)	DG-ASC100	100 Assay
	EZ-ATP Assay Kit (Fluorometric/Colorimetric)	DG-ATP100	100 Assay
	EZ-Free Fatty Acid Assay Kit (Fluorometric/Colorimetric)	DG-FFA100	100 Assay
	EZ-Free Glycerol Assay Kit (Fluorometric/Colorimetric)	DG-FGC100	100 Assay
	EZ-Glucose Assay Kit (Fluorometric/Colorimetric)	DG-GCS100	100 Assay
	EZ-HDL, LDL/VDL Assay Kit (Fluorometric/Colorimetric)	DG-CHO100	100 Assay
	EZ-Total Cholesterol Assay Kit (Fluorometric/Colorimetric)	DG-TSC100	100 Assay
	EZ-Triglyceride Quantification Assay Kit (Fluorometric/Colorimetric)	DG-TGC100	100 Assay
	EZ-Nitric Oxide Assay kit (Colorimetric)	DG-NO500	500 Assay
	EZ-Total Collagen Assay Kit (Colorimetric)	DG-COL100	100 Assay
	EZ-Ethanol Assay Kit (Colorimetric)	DG-ETH100	100 Assay