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Lipid Peroxidation Assay Kit (CV0017)

Cat. No: CV0017
96 Assays

Introduction

Oxidative stress in the cellular environment results in the formation of highly reactive and unstable lipid hydroperoxides. Decomposition of the unstable peroxides derived from polyunsaturated fatty acids results in the formation of malondialdehyde (MDA), which can be quantified colorimetrically following its controlled reaction with thiobarbituric acid. Thiobarbituric Acid Reactive Substances (TBARS) assay was proposed over 40 years ago and is now the most commonly used of method to screening and monitoring lipid oxidation. TBARS method has been used to evaluate a wide range of samples that include human and animal tissues and fluids, drugs, foods and natural products. The sensitivity of measuring Thiobarbituric Acid Reactive Substances (TBARS) has made this assay the method of choice for screening and monitoring lipid peroxidation, a major indicator of oxidative stress. Even though there remains a controversy cited in literature regarding the specificity of TBARS toward compounds other than MDA, it still remains the most widely employed assay used to determine lipid peroxidation.

This assay is based on the reaction of malondialdehyde (MDA) with thiobarbituric acid (TBA); TBA reacts with MDA to form a pink chromogen, which can be detected spectrophotometrically at 532 nm.

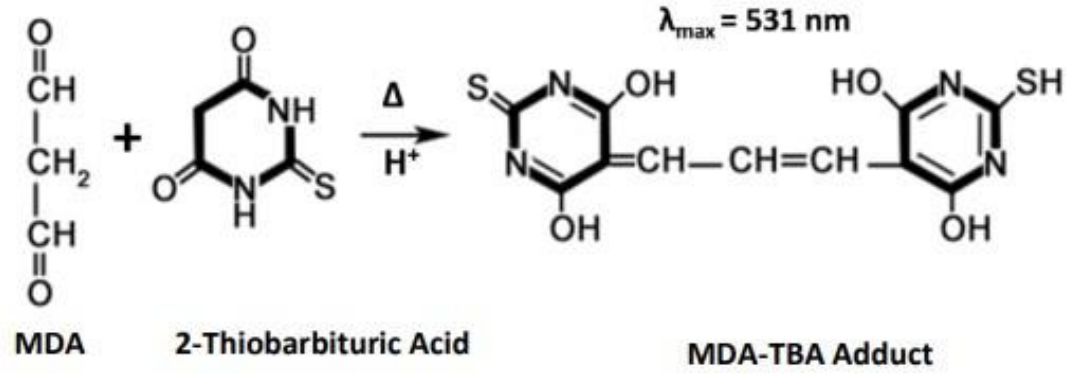
Kit Contents

Thiobarbituric Acid (TBA)	4 x 0.53 g
Diluent (contain acetic acid)	40 ml
Diluent 2 (contain NaOH)	20 ml
SDS Solution	10 ml
MDA Standard (500 µM)	8 ml
96-Well Solid Black plate	1
96-Well Solid Clear plate	1
Adhesive strips	2

Store unopened kit at 2-8 °C.

PRODUCT USE LIMITATION

This product is developed, designed and sold exclusively for research purposes and in vitro use only. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals. Please refer to www.assaygenie.com for Material Safety Data Sheet of the product.



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Reagent Preparation

Thiobarbituric Acid: Each vial contains 530 mg of thiobarbituric acid (TBA). It is ready to use to prepare the Color Reagent.
Diluent 1: The bottle contains 40 mL of concentrated acetic acid. Dilute 40 mL of Diluent 1 with 160 mL of HPLC-grade water. This solution is used in preparing the Color Reagent and is stable for at least three months at room temperature.
Diluent 2: The bottle contains a solution of sodium hydroxide (20 mL). Dilute 20 mL of Diluent 2 with 180 mL of HPLC-grade water. This diluted NaOH solution is used in preparing the Color Reagent. The diluted NaOH solution is stable for at least three months at room temperature. Store the diluted NaOH solution in a plastic container suitable for corrosive materials.
Malondialdehyde Standard: The vial contains 500 µM Malondialdehyde (MDA) in water. It is ready to use to prepare the standard curve.
SDS Solution: The bottle contains a solution of sodium dodecyl sulfate (SDS). The solution is ready to use as supplied.

To prepare the **Color Reagent:**

The following amount of Color Reagent is sufficient to evaluate 24 samples. Adjust the volumes accordingly if more or less samples are going to be assayed. Weigh 530 mg of TBA and add to ≥150 mL beaker containing 50 mL of diluted **Diluent 1**. Add 50 mL of diluted **Diluent 2** and mix until the TBA is completely dissolved. The solution is stable for 24 hours.

Sample Collection & Storage

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately. Typically, normal human serum has a lipid peroxide level (expressed in terms of MDA) of 1.86-3.94 µM.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately. Typically, normal human plasma has a lipid peroxide level (expressed in terms of MDA) of 1.86-3.94 µM.

Urine - Aseptically collect the first urine of the day (mid-stream), voided directly into a sterile container. Centrifuge to remove particulate matter, and assay immediately. Typically, normal human urine has a lipid peroxide level (expressed in terms of MDA) of 0.8-2 µmol/g creatinine.

Tissue Homogenates - Weigh out approximately 25 mg of tissue into a 1.5 mL centrifuge tube. Add 250 µL of RIPA Buffer containing protease inhibitors. Homogenize or sonicate the tissue on ice. Centrifuge the tube at 1,600 x g for 10 minutes at 4°C. Use the supernatant for analysis. Tissue homogenates do not need to be diluted before assaying.

Cell Lysates - Collect 2 x 10^7 cells in 1 mL of cell culture medium or PBS. Homogenize or sonicate the cells on ice. Use the whole homogenate in the assay, being sure to use the culture medium as a sample blank. Cell lysates do not need to be diluted before assaying.

Food Samples -
Meat Products, Cheese, and Dry Nuts: Weigh out approximately 5g of sample into a 15 mL centrifuge tube. Add 5 ml of 10% TCA (with 0.1% EDTA 0.1% of propyl gallate) and homogenize the sample to a smooth suspension. Centrifuge the tube at 1,600 x g for 10 minutes at 4°C. Use the supernatant for analysis.

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Oil Samples: Add 1g of the oil sample to 5 mL of deionized water in a 15 mL centrifuge tube. Vortex the mix vigorously for 2 minutes. Centrifuge at 5000 x g for 5 minutes. Collect the aqueous layer for analysis. Repeat the procedure again.

Standard Curve

Standard Preparation

Malondialdehyde is provided as a solution of the Malonaldehyde bis(dimethyl acetal). When mixed with the Color Reagent, the MDA-TBA molecule is acidified and generates MDA quantitatively.

1. Prepare 125µM MDA Standard Stock (Colorimetric): Add 250 µL of 500 µM MDA Standard to 750 µl dH2O. Prepare immediately prior to use.

Table 1: Colorimetric Standard Curve Preparation

Tube	MDA Standard Stock (ul)	Water (ul)	MDA Concentration (uM)
A	0	1000	0
B	5	995	0.625
C	10	990	1.25
D	20	980	2.5
E	40	960	5
F	80	920	10
G	200	800	25
H	400	600	50

2. Prepare 12.5 µM MDA Standard Stock (Fluorometric): Add 25 µL of 500 µM MDA to 975 µL dH2O. Prepare immediately prior to use.

Table 2: Fluorometric Standard Curve Preparation

Tube	MDA Standard Stock (ul)	Water (ul)	MDA Concentration (uM)
A	0	1000	0
B	5	995	0.0625
C	10	990	0.125
D	20	980	0.25
E	40	960	0.5
F	80	920	1
G	200	800	2.5
H	400	600	5

Assay Procedure

- All reagents except samples must be equilibrated to room temperature before beginning the assay.
- The final volume of the assay is 150 µL in all wells.
- The assay is performed at room temperature.
- It is not necessary to use all the wells on the plate at one time.
- It is recommended that the samples and standards be assayed at least in duplicate.
- It is recommended that the samples and standards be kept at 4°C after preparation to increase sensitivity and reproducibility.
- Monitor the absorbance at 530-540 nm or read fluorescence at an excitation wavelength of 530 nm and an emission wavelength of 550 nm.

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1. Label vial caps with standard number or sample identification number.
2. Add 100 µL of sample or standard to appropriately labeled 5 mL vial.
3. Add 100 µL of SDS solution to vial and swirl to mix.
4. Add 4 mL of the Color Reagent forcefully down side of each vial.
5. Cap vials and place vials in foam or some other holder to keep the tubes upright during boiling.
6. Add vials to vigorously boiling water. Boil vials for one hour.
7. After one hour, immediately remove the vials and place in ice bath to stop reaction. Incubate on ice for 10 minutes.
8. After 10 minutes, centrifuge the vials for 10 minutes at 1,600 x g at 4°C. Vials may appear clear or cloudy. Cloudiness will clear upon warming to room temperature. Vials are stable at room temperature for 30 minutes.
9. 96 well Plate: Load 150 µL (in duplicate) from each vial to either the clear plate (colorimetric version) or to the black plate (fluorometric version).
   Spectrophotometric cuvettes (0.5 mL or 1 mL volume): Transfer enough of the solution (ca. 1.0 mL) into a cuvette.
10. Read the absorbance at 530-540 nm or read fluorescence at an excitation wavelength of 530 nm and an emission wavelength of 550 nm.

Data Analysis

Calculations (Colorimetric/fluorometric):

1. Calculate the average absorbance (or fluorescence) of each standard and sample.
2. Subtract the absorbance value (or fluorescence value) of the standard A (0 µM) from itself and all other values (both standards and samples). This is the corrected absorbance (or fluorescence).
3. Plot the corrected absorbance values(or fluorescence value) (from step 2 above) of each standard as a function of MDA concentration.
4. Calculate the values of MDA for each sample from the standard curve.

$$\text{MDA}(\mu\text{M}) = \left(\frac{(\text{Corrected absorbance/fluorescence}) - (\text{y-intercept})}{\text{Slope}} \right)$$