Cat. No. 309

TBARS®

Thiobarbituric Acid Reactive Substances Assay Kit
Product Information

Quick Facts

1. Storage conditions before opening： **4 ℃, protected from light.**
2. The volume of **Samples**(**Standards**)/**Clarificants**/**Color working reagents**： **100/100/800 µL**.
3. The reaction temperature and time：**95 ℃/1 Hour**.
4. After centrifugation, transfer the supernatant to a non-coated 96-well plate and measure the **OD 530 nm**. **OR**
5. Transfer the supernatant to a black 96-well plate and measure the fluorescence intensity at **550 nm** (Excitation at **530 nm**).

Introduction

Malondialdehyde (MDA) is a natural product occurring metabolite of lipid peroxidation. Lipid peroxidation is a mechanism of damage in plant and animal cells and is considered an indicator of oxidative stress in cells and tissues. After peroxidation, polyunsaturated fatty acids (PUFAs) decompose into a complex series of compounds, including the reactive carbonyl compound, MDA. As shown in Fig. 1, the assay method for measuring MDA concentration by reacting with thiobarbituric acid (TBA), is a well-established technique. Numerous researchers have adapted this Thiobarbituric Acid Reactive Substances (TBARS) assay for the evaluation of a variety of sample types, including human and animal tissues, body fluids, pharmaceuticals, and foods. Although there is a debate in the literatures regarding TBA's reactivity with many types of compounds, not just with MDA (e.g., the TBA has the specificity for MDA ?), TBARS remains the most widely used assay for determining lipid peroxidation. If lipoprotein fractions are acid precipitated from the sample first, such as treatment the sample with the Clarificants that provided in this kit (Reagent E), interfering soluble TBARS are minimized, and the test becomes quite specific for lipid peroxidation. In addition, if your ELISA reader has a fluorescent detection function, it is suggested to use fluorescent detection because the fluorescent signal is more sensitive. Meanwhile, as shown in Fig. 1, the 2TBA-MDA product has a special long-chain conjugated structure (highlight with green color), which can emit a unique fluorescent signal, making the fluorescent signal more representative (Toxicol Res. 2014 Mar;30(1):7-11.).

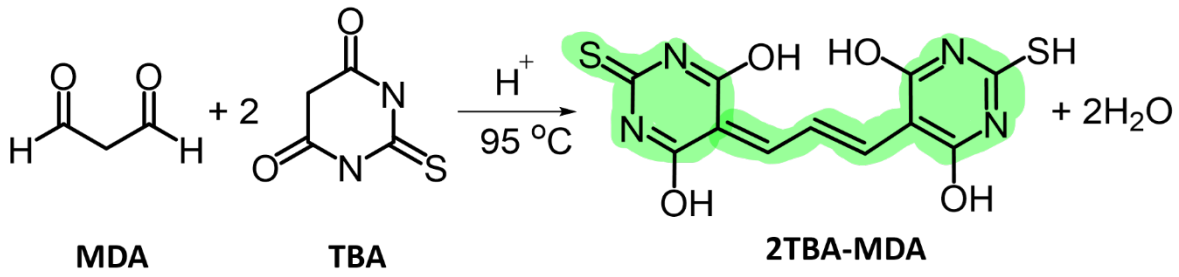

Fig. 1. The reaction of TBA and MDA.

Kit Contents, Shipping, and Storage Information

Reagents	Description	100 tests	Shipping	Storage
A	Colorant powder	1.5 mL brown plastic vial X1	4°C.with protection from light.	4 °C with protection from light.
B	Acidifying agent	50 mL white plastic bottle X1		
C	Neutralizing solution	50 mL white plastic bottle X1		
D	MDA Standard	200 µM, 3 mL brown glass vial X1		
E	Clarifying solution	20 mL transparent glass bottle X1		
1.5 mL microcentrifuge sample vials X35 pc				

Protocol

1. Materials Required but not Provided：

1.1 A plate reader capable of measuring absorbance between 530-540 nm or a fluorometer with the capacity to measure fluorescence using an excitation wavelength of 530 nm and an emission wavelength of 550 nm.
1.2 Adjustable pipettes and a repeating pipettor.
1.3 ddH_2O.
1.4 Tube rack for 1.5 mL sample vials. (can be replaced by a home-made polystyrene foam with suitable holes).
1.5 Heater to boil 1.5 mL sample vials sufficiently (95 ℃/1 Hour).
1.6 Centrifuge with capable of spinning 1.5 mL microcentrifuge tubes at 2,000 x g at 4°C

2. Experimental Protocols：

2.1 Sample Preparation：

2.1.1 Plasma：Typically, normal human plasma has a lipid peroxide level around 0.26-3.94 μM (expressed in terms of MDA).

A. Collect blood using an anticoagulant such as heparin, EDTA, or citrate.
B. Centrifuge the blood at 1,000 x g for 10 minutes at 4 °C. Transfer the plasma (upper layer) to a clean test tube being careful not to disrupt the white buffy layer. Store plasma on ice. If not assaying in the same day, freeze at -80 °C. The plasma sample will be stable for one month while stored at -80 °C. Avoid repeated freeze-thaw cycles.
C. Plasma does not need to be diluted before assaying.

2.1.2 Serum：Typically, normal human serum has a lipid peroxide level around 0.23-3.94 μM (expressed in terms of MDA).

A. Collect blood without using an anticoagulant.
B. Allow blood to clot for 30 minutes at 25 °C.
C. Centrifuge the blood at 2,000 x g for 15 minutes at 4 °C. Transfer the serum (upper layer) to a clean test tube being careful not to disrupt the white buffy layer. Store serum on ice. If not assaying in the same day, freeze at -80 °C. The serum sample will be stable for one month while stored at -80 °C. Avoid repeated freeze-thaw cycles.
D. Serum does not need to be diluted before assaying.

2.1.3 Urine: Typically, the MDA concentration in human urine is approximately 2.8 μM (measured by fluorescence).

A. Aseptically collect the first urine of the day (mid-stream), voided directly into a sterile container.
B. Centrifuge at 1,000 x g for 10 minutes at 4 °C to remove particulate matter, and assay immediately. If not assaying in the same day, freeze at -80 °C. The urine sample will be stable for one month while stored at -80 °C. Avoid repeated freeze-thaw cycles.
C. Urine does not need to be diluted before assaying.

2.1.4 Tissue Homogenates

A. Weigh out approximately 25 mg of tissue into a 1.5 mL centrifuge tube.
B. Add 250 μL of RIPA buffer solution containing protease inhibitors of choice (see section **4**.：interferences section).
C. Homogenize or sonicate the tissue on ice
D. Centrifuge the tube at 1,600 x g for 10 minutes at 4°C. Use the supernatant for analysis. Store supernatant on ice. If not assaying in the same day, freeze at -80°C. The sample will be stable for one month. Avoid repeated freeze-thaw cycles.

E. Tissue homogenates do not need to be diluted before assaying.

2.1.5 Cell Lysates

A. Collect 2 x 10^7 cells in 1 ml of cell culture medium or buffer of choice, such as PBS.
B. Add 250 µl of RIPA Buffer containing protease inhibitors of choice (see section **4**. : interferences section)
C. Homogenize or sonicate the tissue on ice
D. Centrifuge the tube at 2,000 x g for 10 minutes at 4°C. Use the supernatant for analysis. Store supernatant on ice. If not assaying in the same day, freeze at -80°C. The sample will be stable for one month. Avoid repeated freeze-thaw cycles.
E. Being sure to use the culture medium and RIPA buffer solution as a sample blank.
F. Cell lysates do not need to be diluted before assaying.

2.2 Reagent Preparation：

2.2.1 To prepare the color working solution:

Weigh 106 mg of colorant powder (Reagent **A**) and add it to a 50 mL plastic centrifuge tube containing 10 mL of Acidifying agent (Reagent **B)**. Vortex to mix thoroughly. Separate 10 mL of neutralizing solution (Reagent **C**) in several parts, add one part (about 2 mL) at a time to the same 50 mL plastic centrifuge tube, vortex to completely dissolve the colorant powder. This 20 mL of color working solution is sufficient for measuring 24 samples. Users can adjust the volume as needed. Although this color working solution is stable at 4°C for 24 hours, it is suggested to use it as soon as possible.

2.2.2 To prepare the Standard Preparation:

Please follow the table to prepare 0~50 µM MDA standard solution:

No.	Solution (Concentration)	Addition Volume (µL)	Volume of ddH_2O addition (µL)	MDA Concentration (µM)	Volume of preparation (µL)
1	Reagent **D** (200 µM)	80	240	**50**	320
2	Reagent **D** (200 µM)	40	280	**25**	320
3	Reagent **D** (200 µM)	35	665	**10**	700
4	No.3 (10 µM)	160	160	**5**	320
5	No.3 (10 µM)	80	240	**2.5**	320
6	No.3 (10 µM)	40	280	**1.25**	320
7	No.3 (10 µM)	20	300	**0.625**	320
8	--	--	320	**0**	320

2.3 Performing the Assay：

2.3.1 Label 1.5 ml microcentrifuge sample vials (provided in the this kit) with standard number or sample identification number.

<**Note**> *If you use the other type of 1.5 mL sample tubes (made with PP), please note that in step **2.3.6**, heating for the color-development step, you must clamp the tube tightly with a tube clamp to prevent the tube pop open occasionally during the heating, which could cause water loss and inaccurate analyzing results.*

2.3.2 Add 100 µL of sample or standard to appropriately labeled vial.

2.3.3 Add 100 µL of Clarificants solution (Reagent **E**) to vial and vortex.

2.3.4 Add 800 µL of the color working solution (step **2.2.1**) to each vial and vortex.

2.3.5 Tighten the vial cap.

2.3.6 Place vials in home-made foam or other holder to keep the vials upright during heating. Add all vials to 95℃ hot water for 1 hour.

<**Note**> *Vial caps may occassionally pop open during heating. Close cap immediately to avoid water evaporation.*

2.3.7 Immediately remove the vials and place in ice bath to stop reaction. Incubate on ice for 10 minutes.

2.3.8 Centrifuge the vials for 10 minutes at 1,600 x g at 4 °C.

2.3.9 Stablize vials at room temperature for 30 minutes.

2.3.10 Carefully transfer 200 µL supernatant from each vial to each well of microplate (96 wells) without disturbing the pellet and transfer to either the non-coated clear microplate (for colorimetric assay) or to the black microplate (for fluorometric assay).

2.3.11 Read the absorbance at 530-540 nm or read fluorescence at an excitation wavelength of 530 nm and an emission wavelength of 550 nm with the sensitivity set to high and the excitation and emission bandwiths set no higher than 10 nm.

3. Product Performance Test Results：

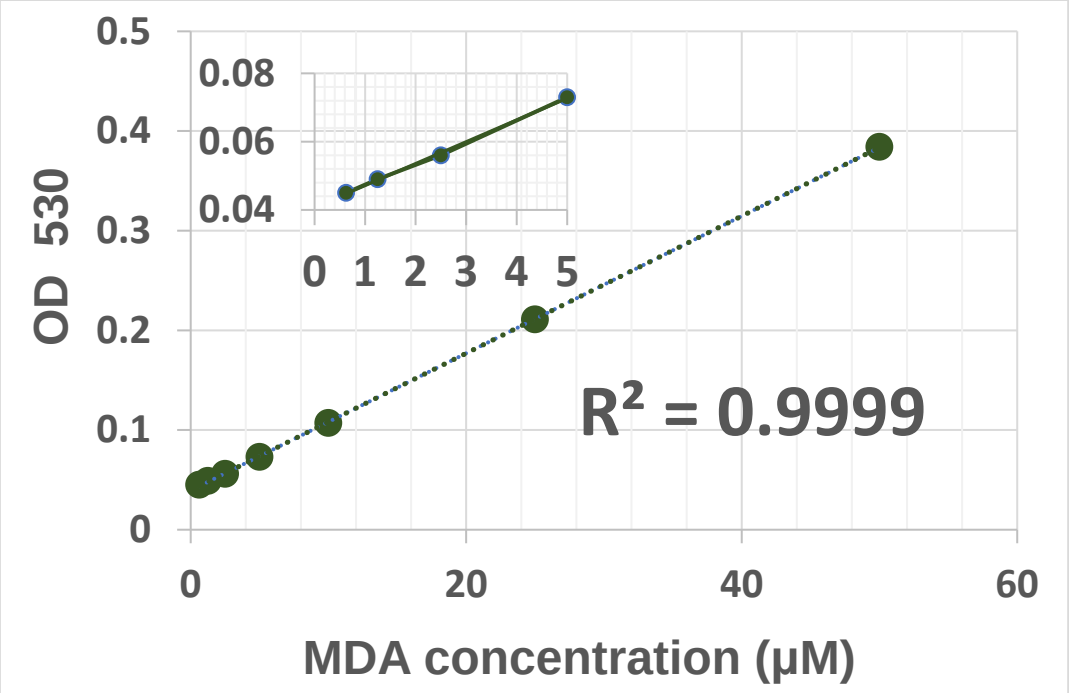

Fig 2. The test results of this product. The inset shows the test results for low concentrations.

4. Interferences：

The following reagents were tested for interference in this assay：

Type	Reagents	Will interfere (Yes / No)
Surfactants	Triton X-100 (≦1%)	N

	Polysorbate 20 (≦1%)	N
	CHAPS (≦1%)	N
Protease Inhibitors/ Chelators	Chymostatin (≤10 μg/ml)	N
	Leupeptin (≤10 μg/ml)	N
	Antipain (≤0.1 mg/ml)	N
	EGTA (≤1 mM)	N
	EDTA (≤1 mM)	N
	PMSF (≤200 μM)	N
	Trypsin (≤10 μg/ml)	N
Buffers	Tris (25 mM)	N
	HEPES (100 mM)	N
	Borate (50 mM)	N
	Phosphate (100 mM)	N
Compounds with hydroxy group	Glycerol (≤10%)	N
	BHT (0.005%)	N
	BHT (0.01%)	Y
	Sucrose (250 mM)	Y

5. Troubleshooting :

Problem	Causes	Recommended Solutions
Erratic values; dispersion of duplicates/triplicates	A. Improper use of the pipetting device or abnormality of the pipetting device. B. Bubble in the well(s). C. Bandwidths are too high	A. Please use the normal pipetting device and ask experienced person to operate.。 B. Carefully tap the side of the plate with your finger to remove bubbles。 C. Set bandwidths on fluorimeter to ≤10 nm and re-read.
No MDA was can be detected in the sample	A. The MDA concentration in the samples was too low。 B. The sample was over diluted。	A. Observe the standard sample for a color change (turn to pink) and use a spectrometer to check for normal readings. If the color changed and readings are normal, the reagents

		are good and preparations are correct. B. Harvest more cells(2 x 10^8) and re-assay; Use a lower dilution ratio.
The fluorometer exhibited "MAX values	The GAIN setting of fluorometer is too high.	Reduce the GAIN setting and re-read; Excitation and Emission bandwidths have to be set at 10 nm.

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