TBARS (TCA Method) Assay Kit

Item No. 700870

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Customer Service 800.364.9897
Technical Support 888.526.5351
1180 E. Ellsworth Rd · Ann Arbor, MI · USA

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GENERAL INFORMATION

Materials Supplied

Kit will arrive packaged as a 4°C kit. For best results, store the kit as supplied or remove components and store as stated below.

Item Number	Item	Quantity/Amount	Storage
700871	Thiobarbituric Acid Assay Reagent	1 vial/1 g	RT
700872	TBA Acetic Acid	1 vial/10 ml	RT
700017	Sodium Hydroxide Assay Reagent (3.5 M)	1 vial/10 ml	RT
10009202	TBA Malondialdehyde Standard	1 vial/1 ml	4°C
700016	TCA Assay Reagent (10%)	1 vial/10 ml	RT
400014	96-Well Solid Plate (Colorimetric Assay)	1 plate	RT
400017	96-Well Solid Plate (black)	1 plate	RT
400012	96-Well Cover Sheet	2 covers	RT

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.

WARNING: THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

Safety Data

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent *via* email to your institution.

Precautions

Please read these instructions carefully before beginning this assay.

It is recommended to take appropriate precautions when using the kit reagents (*i.e.*, lab coat, gloves, eye goggles, etc.), as some of them may be harmful.

The sodium hydroxide and acid solutions are corrosive and harmful if swallowed. Contact with skin may cause burns. In case of contact with skin or eyes, rinse immediately with plenty of water for 15 minutes.

Care should be exercised when removing samples from boiling water.

If You Have Problems

Technical Service Contact Information

Phone: 888-526-5351 (USA and Canada only) or 734-975-3888

Fax: 734-971-3640

Email: techserv@caymanchem.com

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

Storage and Stability

This kit will perform as specified if stored at 4°C and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

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
2. Adjustable pipettes and a repeating pipettor
3. A source of pure water; glass distilled water or HPLC-grade water is acceptable
4. Container sufficient to boil samples and standards
5. 1.5 ml Microcentrifuge tubes with a clasp or 2 ml plastic centrifuge tubes with screw-on lids
6. Centrifuge capable of spinning 1.5 ml microcentrifuge tubes or 2 ml plastic centrifuge tubes at 1,600 x g at 4°C

INTRODUCTION

Background

Malondialdehyde (MDA) is a naturally occurring product of lipid peroxidation. Lipid peroxidation is a well-established mechanism of cellular injury in both plants and animals and is used as an indicator of oxidative stress in cells and tissues.[1,2] Lipid peroxides, derived from PUFAs, are unstable and decompose to form a complex series of compounds, which include reactive carbonyl compounds, such as MDA. In human platelets, thromboxane (TX) synthase also catalyzes the conversion of prostaglandin H_2 to TXA_2, 12(S)-HHTrE, and MDA in a ratio of 1:1:1.[3]

The measurement of Thiobarbituric Acid Reactive Substances (TBARS) is a well-established method for screening and monitoring lipid peroxidation.[1,2] Modifications of the TBARS assay by many researchers have been used to evaluate several types of samples including human and animal tissues and fluids, drugs, and foods.[4-8] 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.[2] If lipoprotein fractions are first acid precipitated from the sample, interfering soluble TBARS are minimized, and the test becomes quite specific for lipid peroxidation.[2] Lipids with greater unsaturation will yield higher TBARS values. It is recommended that if high TBARS values are obtained, a more specific assay such as HPLC should be performed.

About This Assay

Cayman's TBARS (TCA Method) Assay Kit provides a simple, reproducible, and standardized tool for assaying lipid peroxidation in plasma, serum, urine, tissue homogenates, and cell lysates. The MDA-TBA adduct formed by the reaction of MDA and TBA under high temperature (90-100°C) and acidic conditions is measured colorimetrically at 530-540 nm or fluorometrically at an excitation wavelength of 530 nm and an emission wavelength of 550 nm (see Figure 1 below). Although this reaction has a much higher sensitivity when measured fluorometrically, protocols for both methods are provided.

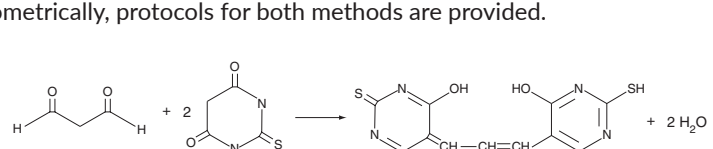


Figure 1. Assay scheme

PRE-ASSAY PREPARATION

Reagent Preparation

1. **Thiobarbituric Acid Assay Reagent - (Item No. 700871)**

   The vial contains 1 g of thiobarbituric acid (TBA). It is ready to use to prepare the Color Reagent.

2. **TBA Acetic Acid - (Item No. 700872)**

   The vial contains 10 ml of concentrated acetic acid. Carefully dilute 10 ml of TBA Acetic Acid into 40 ml of HPLC-grade water. This diluted Acetic Acid Solution is used in preparing the Color Reagent. The diluted Acetic Acid Solution is stable for at least three months at room temperature.

3. **Sodium Hydroxide Assay Reagent (3.5 M) - (Item No. 700017)**

   The vial contains 10 ml of 3.5 M sodium hydroxide (NaOH). Dilute 10 ml of Sodium Hydroxide Assay Reagent (3.5 M) with 40 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.

4. **TBA Malondialdehyde Standard - (Item No. 10009202)**

   The vial contains 1 ml of 500 µM malondialdehyde (MDA) in water. It is ready to use to prepare the standard curve.

5. **TCA Assay Reagent (10%) - (Item No. 700016)**

   The vial contains 10 ml of 10% trichloroacetic acid (TCA). The solution is ready to use as supplied.

6. **To prepare the Color Reagent:**

   The following amount of Color Reagent is sufficient to evaluate 24 samples/standards. Adjust the volumes accordingly if more or less samples are going to be assayed. Weigh 106 mg of TBA (Item No. 700871) and add to a beaker containing 10 ml of diluted TBA Acetic Acid Solution. Add 10 ml of diluted Sodium Hydroxide and mix until the TBA is completely dissolved. The solution is stable for 24 hours.

Sample Preparation

Plasma

Typically, normal human plasma has a lipid peroxide level (expressed in terms of MDA) of 0.26-3.94 µM.[1,8,11]

1. Collect blood using an anticoagulant such as heparin, EDTA, or citrate.
2. Centrifuge the blood at 700-1,000 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 the same day, freeze at -80°C. The plasma sample will be stable for one month while stored at -80°C.
3. Plasma does not need to be diluted before assaying.

Serum

Typically, normal human serum has a lipid peroxide level (expressed in terms of MDA) of 0.23-3.94 µM.[1,12]

1. Collect blood without using an anticoagulant.
2. Allow blood to clot for 30 minutes at 25°C.
3. 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 the same day, freeze at -80°C. The serum sample will be stable for one month while stored at -80°C.
4. Serum does not need to be diluted before assaying.

Urine

Typically, normal human urine has a lipid peroxide level (expressed in terms of MDA) of 0.8-2 µmol/g creatinine.[9,10]

1. Urine does not require any special treatments. If not assaying the same day, freeze at -80°C.

Tissue Homogenates

1. Weigh out approximately 25 mg of tissue into a 1.5 ml centrifuge tube.
2. Add 250 µl of RIPA Buffer (prepared from Item No. 10010263) containing protease inhibitors of choice (see **Interferences** section on page 22).
3. Homogenize or sonicate the tissue on ice.
4. 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 the same day, freeze at -80°C. The sample will be stable for one month.
5. Tissue homogenates do not need to be diluted before assaying.

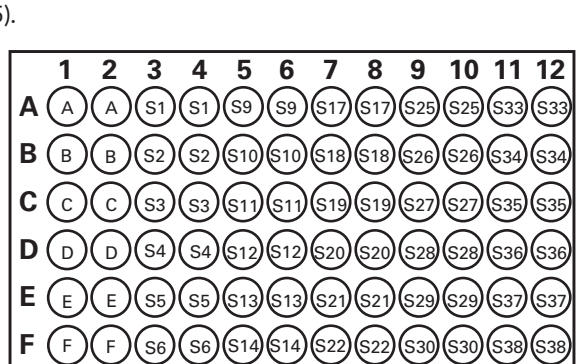
Cell Lysates

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

ASSAY PROTOCOL

Plate Set Up

There is no specific pattern for using the wells on the plate. A typical layout of standards and samples to be measured in duplicate is shown below in Figure 2. We suggest you record the contents of each well on the template sheet provided (see page 25).



A-H = Standards
S1-S40 = Sample Wells

Figure 2. Sample plate format

Pipetting Hints

- It is recommended that an adjustable pipette be used to deliver reagents to the wells.
- Before pipetting each reagent, equilibrate the pipette tip in that reagent (*i.e.*, slowly fill the tip and gently expel the contents, repeat several times).
- Do not expose the pipette tip to the reagent(s) already in the well.

General Information

- All reagents except samples must be equilibrated to room temperature before beginning the assay.
- The final volume of the assay is 200 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. For the fluorometric determination, it is recommended that the sensitivity be set at high with the excitation and emission bandwiths set to 10 nm.

Colorimetric Standard Preparation

Dilute 250 µl of the MDA Standard (Item No. 10009202) with 750 µl of water to obtain a stock solution of 125 µM. Take eight clean glass test tubes and label them A-H. Add the amount of 125 µM MDA stock solution and water to each tube as described in Table 1.

Tube	MDA (µl)	Water (µl)	MDA Concentration (µM)
A	0	1,000	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

Table 1. MDA colorimetric standards

Fluorometric Standard Preparation

Dilute 25 µl of the MDA Standard (Item No. 10009202) with 975 µl of water to obtain a stock solution of 12.5 µM. Take eight clean glass test tubes and label them A-H. Add the amount of 12.5 µM MDA stock solution and water to each tube as described in Table 2.

Tube	MDA (µl)	Water (µl)	MDA Concentration (µM)
A	0	1,000	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

Table 2. MDA fluorometric standards

Performing the Assay

1. Label 1.5 ml microcentrifuge (or similar size screw cap vial) vial caps with standard number or sample identification number.
2. Add 100 µl of sample or standard to appropriately labeled vial.
3. Add 100 µl of TCA Assay Reagent (10%) to vial and swirl to mix.
4. Add 800 µl of the Color Reagent to each vial and vortex.
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. *NOTE: Vial caps may occassionally pop open during boiling. Close cap immediately to avoid sample evaporation. Screw cap vials may be more appropriate for this assay than flip cap vials.*
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.
9. Vials are stable at room temperature for 30 minutes.
10. Carefully remove 200 µl (in duplicate) from each vial without disturbing the pellet and transfer to either the clear plate (colorimetric version) or to the black plate (fluorometric version).
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.

ANALYSIS

Colorimetric Calculations

1. Calculate the average absorbance of Standard A.
2. Subtract the absorbance value of the standard A (0 µM) from itself and all other values (both standards and samples). This is the corrected absorbance.
3. Plot the average corrected absorbance values (from step 2 above) of each standard as a function of MDA concentration (see Table 1, on page 14).
4. Calculate the values of MDA for each sample from the standard curve. An example of the MDA standard curve is shown below in Figure 3.

$$\text{MDA (µM)} = \left[\frac{\text{(Corrected absorbance) - (y-intercept)}}{\text{Slope}}\right]$$

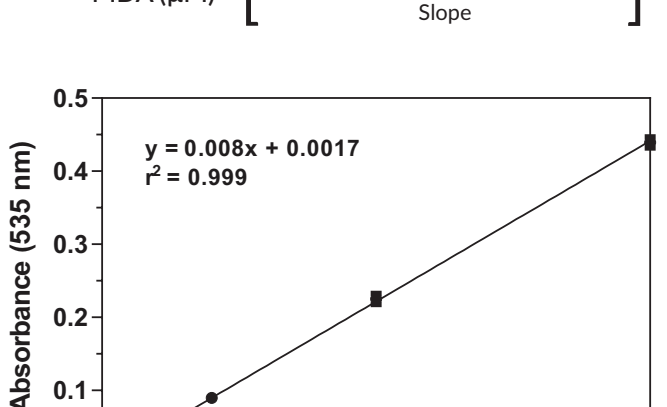


Figure 3. MDA colorimetric standard curve

Fluorometric Calculations

1. Calculate the average fluorescence of Standard A.
2. Subtract the fluorescence value of the standard A (0 µM) from itself and all other values (both standards and samples). This is the corrected fluorescence.
3. Plot the average corrected fluorescence values (from step 2 above) of each standard as a function of MDA concentration (see Table 2, on page 15).
4. Calculate the values of MDA for each sample from the standard curve. An example of the MDA standard curve is shown below in Figure 4.

$$\text{MDA (µM)} = \left[\frac{\text{(Corrected fluorescence) - (y-intercept)}}{\text{Slope}}\right]$$

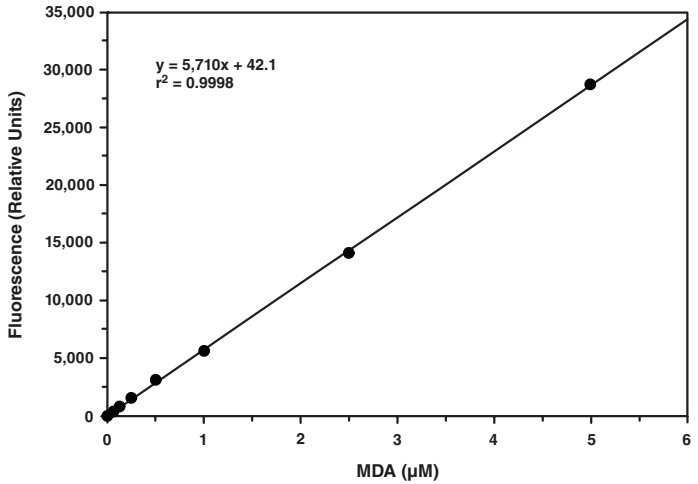


Figure 4. MDA fluorometric standard curve

Performance Characteristics

Precision:

When a series of three human plasma and twenty human urine samples were assayed on the same day, the intra-assay coefficient of variation was 4.9% and 5.8%, respectively. When a series of three human plasma samples were assayed on three different days under the same experimental conditions, the inter-assay coefficient of variation was 2.5%.

Comparison between colorimetric and fluorometric detections:

Samples	Colorimetric MDA (µM)	Fluorometric MDA (µM)
Serum 1	1.61	1.48
Serum 2	0.81	0.73
Serum 3	1.1	0.92
Urine 1	0.86	0.78
Urine 2	0.79	0.74
Urine 3	0.88	0.84

Table 3. MDA concentration in human serum and human urine samples using colorimetric and fluorometric detection.

Assay Recovery:

Human urine was spiked with various concentrations of MDA. The data in Figures 5 and 6 represent the amount of MDA added to urine *versus* the calculated amount of MDA using the colorimetric and fluorometric detection methods.

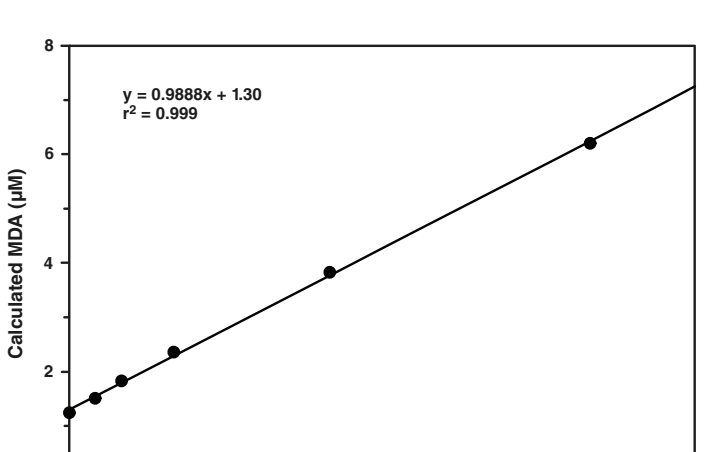


Figure 5. Spike/Recovery results using the colorimetric detection

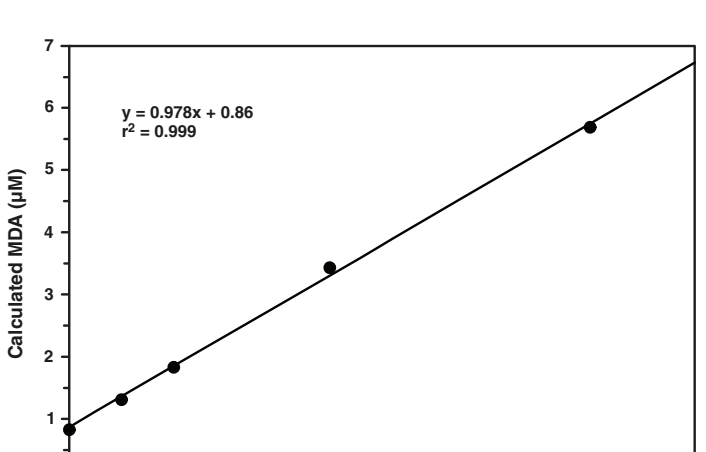


Figure 6. Spike/Recovery results using the fluorometric detection

RESOURCES

Interferences

The following reagents were tested for interference in the assay.

	Reagent	Will Interfere (Yes or No)
Buffers:	Borate (50 mM)	No
	HEPES (100 mM)	No
	Phosphate (100 mM)	No
	Tris (25 mM)	No
Detergents:	CHAPS (≤1%)	No
	Polysorbate 20 (≤1%)	No
	Triton X-100 (≤1%)	No
Protease Inhibitors/ Chelators:	Antipain (≤0.1 mg/ml)	No
	Chymostatin (≤10 µg/ml)	No
	EDTA (≤1 mM)	No
	EGTA (≤1 mM)	No
	Leupeptin (≤10 µg/ml)	No
	PMSF (≤200 µM)	No
	Trypsin (≤10 µg/ml)	No
Others:	BHT (0.01%)	Yes
	BHT (0.005%)	No
	Glycerol (≤10%)	No
	Sucrose (250 mM)	Yes

Troubleshooting

Problem	Possible Causes	Recommended Solutions
Erratic values; dispersion of duplicates/triplicates	A. Poor pipetting/technique B. Bubble in the well(s) C. Bandwidths are too high	A. Be careful not to splash the contents of the wells 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 detected in the sample	A. MDA concentration was too low B. The sample was too dilute	A. Process more tissue (50-100 mg) B. Harvest more cells (2×10^8) and re-assay C. Use a lower dilution
The fluorometer exhibited 'MAX' values for the wells	The GAIN setting is too high	Reduce the GAIN and re-read; Excitation and Emission bandwidths have to be set at 10 nm

References

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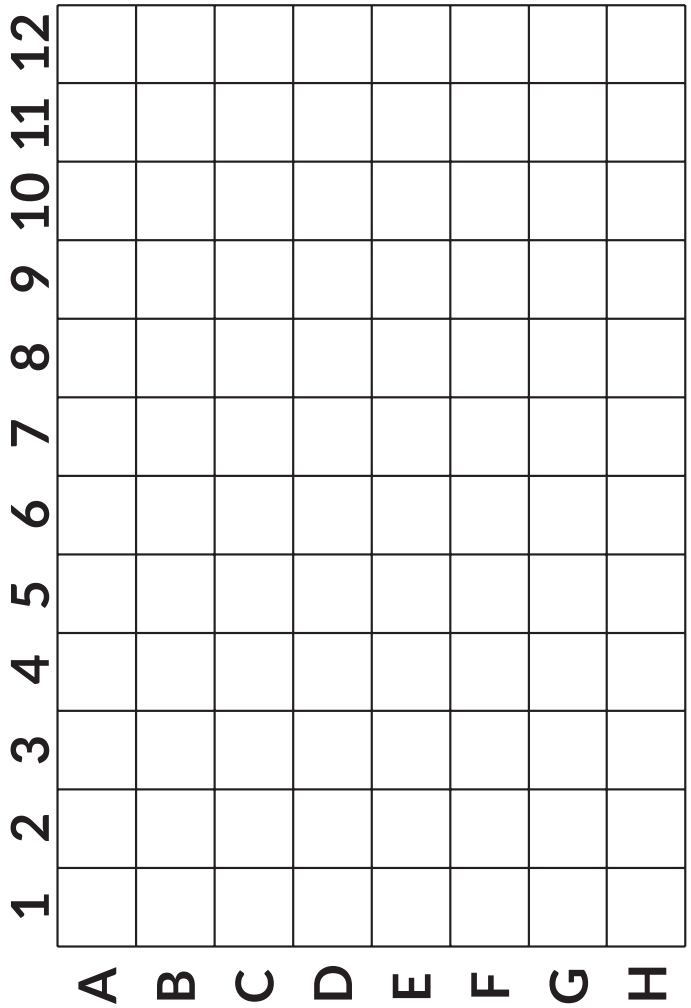

NOTES

Warranty and Limitation of Remedy

Buyer agrees to purchase the material subject to Cayman's Terms and Conditions. Complete Terms and Conditions including Warranty and Limitation of Liability information can be found on our website.