TBARS Assay Kit

Item No. 10009055

www.caymanchem.com
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

Item Number	Item	Quantity
10009199	Thiobarbituric Acid	1 vial
10009200	TBA Acetic Acid	2 vials
10009201	TBA Sodium Hydroxide (10X)	1 vial
10009202	TBA Malondialdehyde Standard	1 vial
10009203	TBA SDS Solution	1 vial
400014	96-Well Solid Plate (Colorimetric Assay)	1 plate
400017	96-Well Solid Plate (black)	1 plate
400012	96-Well Cover Sheet	2 covers

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: 734-971-3641

Email: techserv@caymanchem.com

Hours: M-F 8:00 AM to 5:30 PM EST

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. 5 ml polypropylene screw-cap centrifuge tubes (*i.e.*, VWR Item No. 16465-262)
6. Centrifuge capable of spinning 5 ml centrifuge tubes at 1,600 x g at 4°C

Alternate Assay

Cayman Chemical also offers an alternative assay kit for TBARS assessment (TBARS (TCA Method) Assay Kit; Item No. 700870). While maintaining the reliability and accuracy of the of the original assay, this assay offers the advantages of smaller working volumes (1 ml *vs.* 4 ml) and improved sample processing resulting from the formation of harder protein pellets during TCA precipitation.

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 synthase also catalyzes the conversion of PGH_2 to thromboxane A_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 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. Although this reaction has a much higher sensitivity when measured fluorometrically, protocols for both methods are provided (see Figure 1 below).

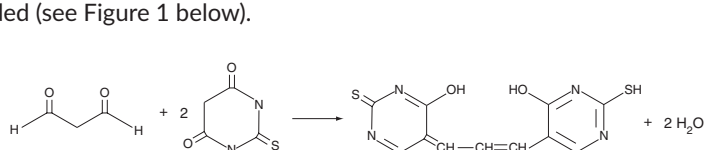

Figure 1.

PRE-ASSAY PREPARATION

Reagent Preparation

1. **Thiobarbituric Acid - (Item No. 10009199)**

   The vial contains 2 g of thiobarbituric acid (TBA). It is ready to use to prepare the Color Reagent.

2. **TBA Acetic Acid - (Item No. 10009200)**

   Each vial contains 20 ml of concentrated acetic acid. Slowly add both vials (40 ml) of TBA Acetic Acid to 160 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. **TBA Sodium Hydroxide (10X) - (Item No. 10009201)**

   The vial contains a solution of sodium hydroxide (NaOH). Dilute 20 ml of TBA NaOH 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.

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

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

5. **TBA SDS Solution - (Item No. 10009203)**

   The vial contains a solution of sodium dodecyl sulfate (SDS). 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. Adjust the volumes accordingly if more or less samples are going to be assayed. Weigh 530 mg of TBA (Item No. 10009199) and add to ≥150 ml beaker containing 50 ml of diluted TBA Acetic Acid Solution. Add 50 ml of diluted TBA 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 1.86-3.94 µM.[1,8]

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. Pipette off the top yellow plasma layer without disturbing 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 1.86-3.94 µM.[1]

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. Pipette off the top yellow serum layer without disturbing 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 20).
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 22).

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S1	S1	S9	S9	S17	S17	S25	S25	S33	S33
B	B	B	S2	S2	S10	S10	S18	S18	S26	S26	S34	S34
C	C	C	S3	S3	S11	S11	S19	S19	S27	S27	S35	S35
D	D	D	S4	S4	S12	S12	S20	S20	S28	S28	S36	S36
E	E	E	S5	S5	S13	S13	S21	S21	S29	S29	S37	S37
F	F	F	S6	S6	S14	S14	S22	S22	S30	S30	S38	S38
G	G	G	S7	S7	S15	S15	S23	S23	S31	S31	S39	S39
H	H	H	S8	S8	S16	S16	S24	S24	S32	S32	S40	S40

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 SDS Solution will take at least one hour to equilibrate to room temperature if stored at 2-8°C. Briefly heating the SDS Solution at 37°C will re-dissolve the precipitated SDS. The SDS Solution can then be stored at room temperature.
- 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.

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 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.
9. Vials are stable at room temperature for 30 minutes.
10. Load 150 µl (in duplicate) from each vial 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.

ANALYSIS

Colorimetric Calculations

1. Calculate the average absorbance of each standard and sample.
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 corrected absorbance values (from step 2 above) of each standard as a function of MDA concentration (see Table 1, on page 13).
4. Calculate the values of MDA for each sample from the standard curve. An example of the MDA standard curve is shown on page 17 in Figure 3.

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

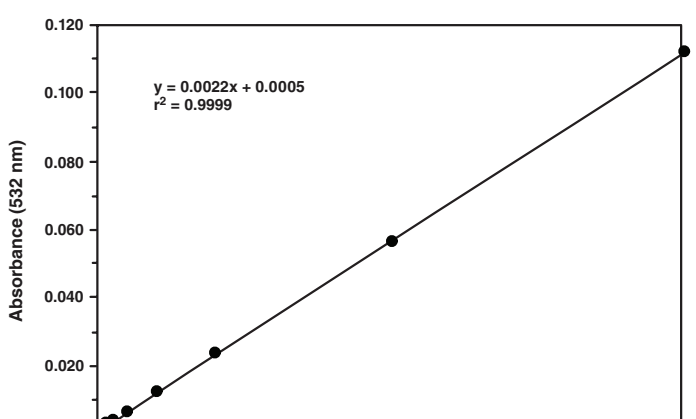

Figure 3. MDA colorimetric standard curve

Fluorometric Calculations

1. Calculate the average fluorescence of each standard and sample.
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 corrected fluorescence values (from step 2 above) of each standard as a function of MDA concentration (see Table 2, 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 on page 19 in Figure 4.

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

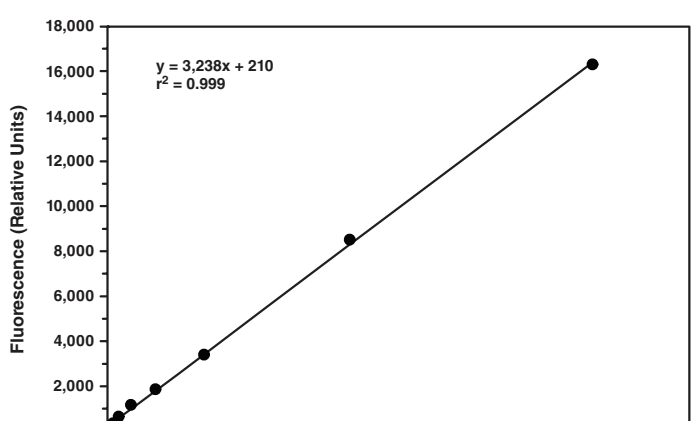

Figure 4. MDA fluorometric standard curve

Performance Characteristics

Precision:

When a series of ten human plasma and sixteen human urine samples were assayed on the same day, the intra-assay coefficient of variation was 5.5% and 7.6%, respectively. When a series of eight human plasma and sixteen human urine samples were assayed on seven different days under the same experimental conditions, the inter-assay coefficient of variation was 5.9% and 5.1%, respectively.

Assay Range:

Under the standardized conditions of the assay described in this booklet, the dynamic range of the kit is 0-50 µM (Colorimetric) or 0-5 µM (Fluorometric) (µM = µmole/liter = nmol/ml) MDA equivalents.

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
	Triton X-100 (≤1%)	No
	Polysorbate 20 (≤1%)	No
Protease Inhibitors/ Chelators:	Antipain (≤0.1 mg/ml)	No
	Chymostatin (≤10 µg/ml)	No
	Leupeptin (≤10 µg/ml)	No
	PMSF (≤200 µM)	No
	Trypsin (≤10 µg/ml)	No
	EDTA (≤1 mM)	No
	EGTA (≤1 mM)	No
Others:	Sucrose (250 mM)	Yes
	Glycerol (≤10%)	No

Troubleshooting

Problem	Possible Causes	Recommended Solutions
Erratic values; dispersion of duplicates/triplicates	A. Poor pipetting/ technique B. Bubble in the well(s)	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
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
The fluorometer exhibited 'MAX' values for the wells	The GAIN setting is too high	Reduce the GAIN and re-read

References

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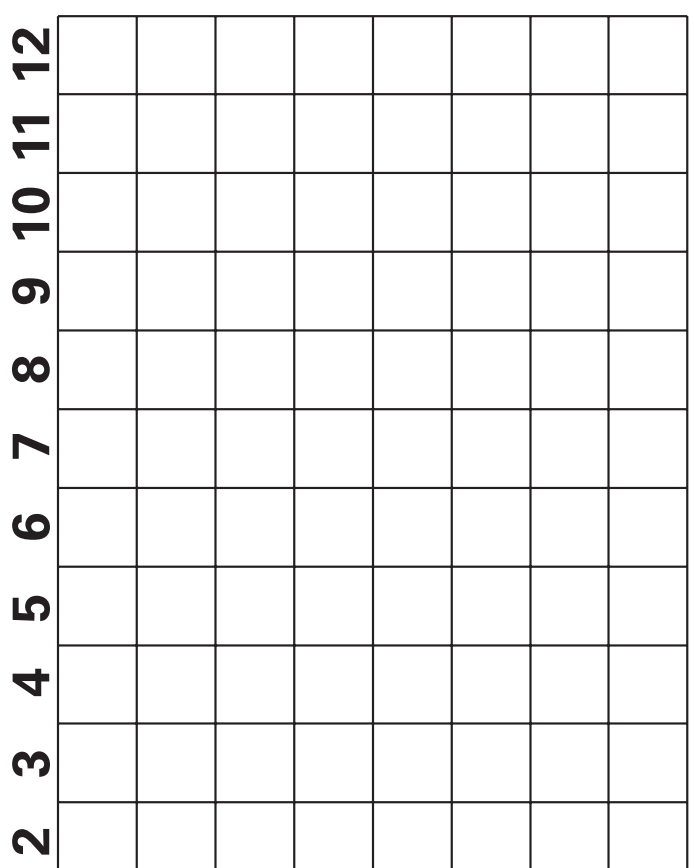

NOTES

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.