Malondialdehyde (MDA)/ Thiobarbituric Acid Reactive Substances (TBARS) Colorimetric Assay kit

(96 Tests)

Zellbio GmbH (Germany)

CAT No. ZX-44116-96

www.zellx.de

Sample Types Validated for:

Serum, Plasma, Urine, Tissue and Cell Lysates, Food Extracts, and Buffers

!!! Caution: This product is for Research Use Only. Not for *in-vitro* Diagnostics !!!

Table of Contents

Please read this insert completely prior to using the product.

Introduction

Background

Malondialdehyde (MDA) is an organic compound ($C_3H_4O_2$) generated by lipid peroxidation of polyunsaturated fatty acids after degradation by reactive oxygen species (ROS). Lipid peroxidation is a well-established mechanism of cellular injury in plants and animals, used as an indicator of oxidative stress in cells and tissues. Lipid peroxidation products derived from polyunsaturated fatty acids decompose to form a diverse mixture of compounds including MDA. MDA is the most commonly used biomarker of oxidative stress in many health disorders such as cancer, cardiovascular diseases, asthma, and chronic obstructive pulmonary disease.

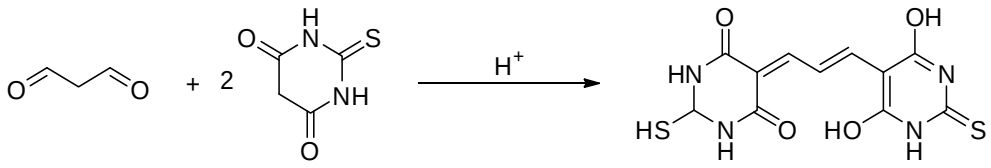
Malondialdehyde

In addition to MDA, other reactive aldehydes such as 2-alkenals and 2,4-alkedienals, are also formed by these oxidative mechanisms, which can react with Thiobarbituric Acid (TBA) to generate a measurable signal in a quantitative assay. Total MDA combined with other reactive substances are termed as Thiobarbituric Acid Reactive Substances (TBARS). Modifications of the TBARS assay have been widely used to evaluate the MDA levels in different types of samples, including mammalian tissues, serum, plasma and urine along with food samples. The reactivity of acidified TBA towards other reactive aldehydes has led to some sort of ambiguity surrounding the use of TBARS for different sample types under various oxidative stress conditions; nevertheless, the assay is extensively used to determine the level of lipid peroxidation. In general, lipids with increased degree of unsaturation will show higher TBARS values.

Assay principle

The ZellX® TBARS/MDA Colorimetric assay Kit is designed to quantitatively assess the level of MDA in a variety of samples. An MDA standard is provided to generate a standard curve for the assay, and all samples should be read off the standard curve.

MDA is conveniently measured by the reaction of TBA in an acidic environment according to the following reaction. Samples are diluted in Sample Diluent and mixed with the Substrate. The reaction is incubated at 37°C for one hour. TBA and MDA chemically react and produce pinked color MDA-TBA adduct which can easily be measured at 535 nm. Increasing levels of MDA or other reactive aldehydes cause a linear increase in color.



MDA **TBA** **MDA-TBA**

General information

Materials supplied in the Kit

Component	*Quantity*
MDA Standard (2000 µM)	45 µL
Sample Diluent	50 mL
TBA Substrate	5.5 mL
Clear Half Area 96 Well Plate	1 plate
Plate sealer	1 sealer

Storage instruction

All reagents should be stored at 4° C until the expiration date of the kit.

Materials required but not supplied

Deionized water (diH_2O)

High quality 35–38% concentrated HCl (We suggest using high-quality concentrated hydrochloric acid (35–38%) such as Sigma-Aldrich, catalog number 320331 or similar)

Cell lysis buffer for cells and tissues. We recommend ThermoFisher RIPA Buffer, Catalog Number 89900

Microplate/ELISA Reader capable of reading optical absorption at 535 nm (Acceptable Range 530-545 nm)

Precision pipettes, multichannel pipette and disposable pipette tips

Plate shaker capable of heating to 37°C

Precautions

This kit should only be used by qualified personnel who have had laboratory safety instruction. The complete insert should be read and understood before attempting to use the product.

The MDA Standard of this kit is supplied in Propanol. Propanol is flammable and care should be taken to avoid sparks or flames.

Sample Diluent is diluted hydrochloric acid. (Should be used carefully).

TBA Substrate is supplied in diluted base. (Should be used carefully).

General remarks

- The instruction must be strictly followed. The reading of Microplate/ELISA Reader must be set as at the appropriate wavelength for determining the experiment result.
- Pipette tips should not be used more than once to prevent cross contamination.
- Reagents of different batches should not be mixed or used after their expiration dates.

Assay protocol

Sample preparation

Samples containing visible particulate should be centrifuged prior to conducting the assay. Moderate to severely hemolyzed samples should not be used for this assay.

I. **Serum, Plasma:**

- Collect fresh serum or plasma with Heparin or EDTA.
- Leave it for 30 minutes at room temperature to clot.
- Centrifuge at 1000 g for 15 min at 4˚C.
- Separate serum or plasma from red blood cells, and transfer into fresh tubes.
- Sample must be used immediately or frozen at ≤ -70°C for later use.
- Add 200 µL of serum or plasma into a microtube, and mix with 10 µL of concentrated hydrochloric acid (12.1 M).
- Vortex and centrifuge at 14000 rpm for 10 minutes.
- Supernatant should be diluted ≥ 1:10 by taking one part of sample and adding 9 or more parts of Sample Diluent prior to conducting the assay.
- Multiply the obtained MDA concentration by 1.0476 to adjust for the addition of concentrated hydrochloric acid, followed by further multiplications for any dilutions with Sample Diluent.

II. **Urine:**

- Collect urine sample in a sterile container.
- Sample must be used immediately or frozen at ≤ -70°C for later use.
- Add 200 µL of urine sample into a microtube, and mix with 10 µL of concentrated hydrochloric acid (12.1 M)
- Urine should be diluted ≥ 1:5 by taking one part of sample and adding 4 or more parts of Sample Diluent prior to conducting the assay.
- Multiply the obtained MDA concentration by 1.0476 to adjust for the addition of concentrated hydrochloric acid, followed by further multiplications for any dilutions with Sample Diluent.
- **Normal urinary MDA levels are approximately 1 µM.**

III. **Cell & Tissue Lysates:**

- Wash cells or tissue by suspension in cold PBS.
- Centrifuge at 5000 rpm in a microtube.
- Weigh out 25 mg of tissue or 10^6 cells into a tube.
- Add 1 mL of appropriate Lysing Buffer (we recommend Thermo Fisher Scientific RIPA Buffer, Catalog Number 89900).
- Vortex vigorously and freeze at ≤ -70°C for 10 minutes. Thaw, and repeat the freeze-thaw cycle.
- For hardy cells, sonication in a sonicator bath for 10 minutes may be needed at this stage.
- Centrifuge at 1600 g for 10 minutes at 4°C, and collect the supernatant in a clean tube.
- Supernatant must be used immediately or frozen at ≤ -70°C for later use.
- Add 200 µL of supernatant into a microtube, and mix with 10 µL of concentrated hydrochloric acid (12.1 M).
- Multiply the obtained MDA concentration by 1.0476 to adjust for the addition of concentrated hydrochloric acid, followed by further multiplications for any dilutions with Sample Diluent.

IV. **Food Samples:**

- Due to the variability of food samples, each sample may require a different method of preparation based on the nature and polarity of its lipids and the sample type (liquid or solid sample). For further information regarding the preparation method please contact us at technical@zellx.de.

V. **Tissue Culture Media:**

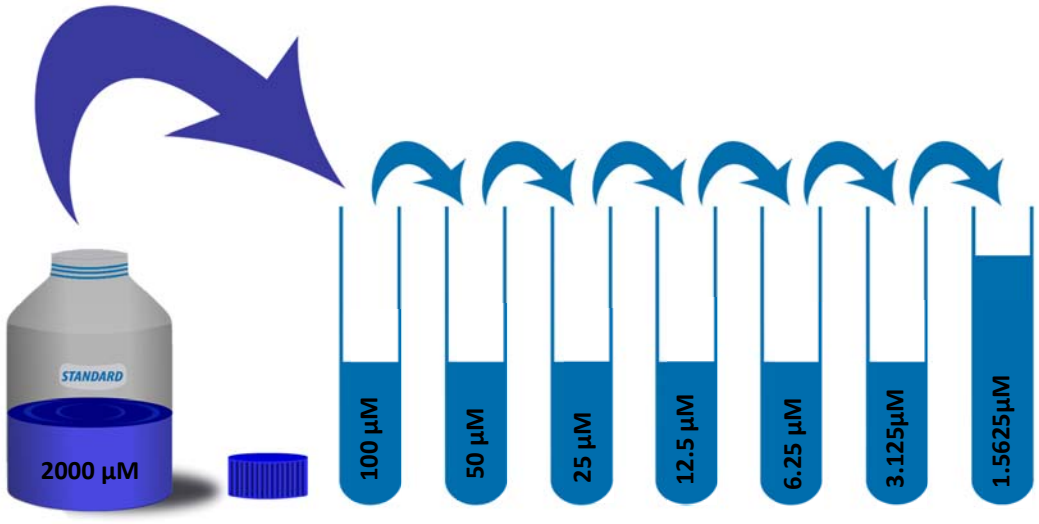
- For measuring MDA in tissue culture media (TCM), samples should be read off a standard curve generated in TCM. Samples may need to be diluted further in TCM. The assay has been validated using RPMI-1640.

All the samples must be used immediately after dilution or stored at -70°C or lower, preferably after being frozen in liquid nitrogen.

Standard preparation

- Prepare a 1:20 dilution of MDA Standard with Sample Diluent (mix 15 µL of standard with 285 µL of Sample Diluent), and label as the Standard No.7 (100 µM).
- Make series of lower dilutions as described in the table.
- The Sample Diluent is used as the 0 µM Standard.

No.	*Concentration*	*Material needed*
Standard No.7	100 µM	15 µL MDA Standard + 285 µL Sample Diluent
Standard No.6	50 µM	150 µL Standard No.7 + 150 µL Sample Diluent
Standard No.5	25 µM	150 µL Standard No.6 + 150 µL Sample Diluent
Standard No.4	12.5 µM	150 µL Standard No.5 + 150 µL Sample Diluent
Standard No.3	6.25 µM	150 µL Standard No.4 + 150 µL Sample Diluent
Standard No.2	3.125 µM	150 µL Standard No.3 + 150 µL Sample Diluent
Standard No.1	1.5625 µM	150 µL Standard No.2 + 150 µL Sample Diluent
Standard No.0	0 µM	150 µL Sample Diluent



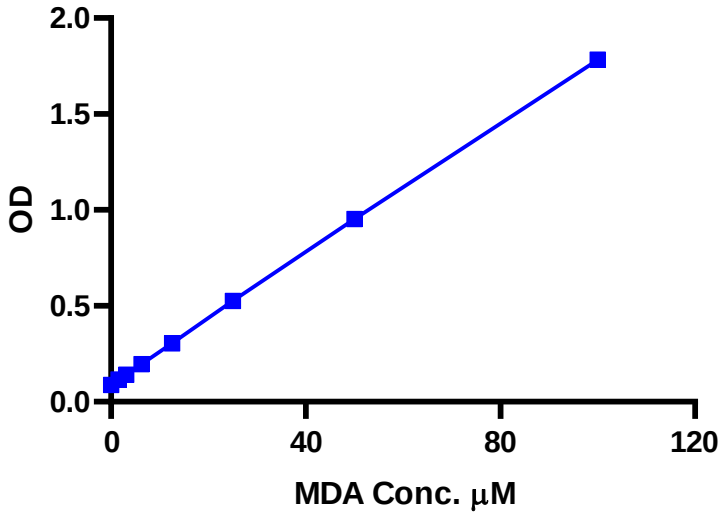
All standard must be used within 2 hours of preparation

Assay Procedure

1. Pipette 50 µL of either samples or standards into duplicate wells in the plate.
2. Pipette 50 µL of Sample Diluent into duplicate wells as the Zero standard.
3. Add 50 µL of TBA Substrate to each well using a multichannel/repeater pipette.
4. Incubate at 37°C for 60 minutes with shaking. If the plate is not shaken, signals will be approximately 25 % lower.
5. Read the optical density at 535 nm. (530-545 nm)

Calculation

- Average the duplicate optical density (OD) readings for each standard and sample.
- Create a standard curve by reducing the data using the four parameter logistic curve (4PLC) fitting routine on the plate reader using the adjusted OD values
- The obtained concentrations should be multiplied by 1.0476 to adjust for the addition of concentrated HCl, as well as other dilution factors to obtain the correct sample values.



A typical standard curve of ZellX® MDA-TBRAS Assay kit

Run your own standard curves for calculation of results

Assay range

The limit of detection of ZellX® MDA assay was determined as 0.62 µM.

Sensitivity

The sensitivity of the ZellX® MDA assay was determined as 0.36 µM.

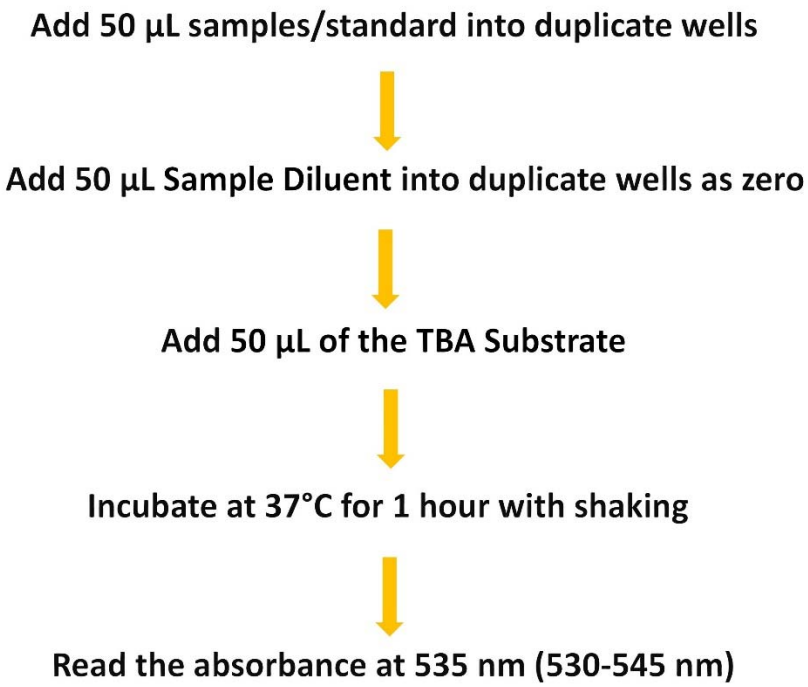
Precision

Intra-Assay Precision (Precision within an assay): 3 samples were tested 16 times in an assay.

Inter-Assay Precision (Precision between assays): 3 samples were tested in duplicate on 20 different assays over multiple days.

Item	*%CV*
Intra assay	12.7, 12.9, 3.5
Inter assay	13.8, 11.6, 4.5

Protocol summary



References

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