



Dynamic Test Kits for R&D
and Quality Control

Malondialdehyde (MDA) TBARs Assay Kit KB-03-016

100/200/500 tests

BOCKit

A brand of  BioQuoChem

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All chemicals should be handled with

➤ This kit is for R&D use only

Introduction

Malondialdehyde (MDA), can be generated by oxidizing agents that alters lipid structure, creating lipid peroxides. MDA can be measured as Thiobarbituric Acid Reactive Substances (TBARS).

This method, is convenient to determine the relative lipid peroxide content of samples, including plasma, serum, cell culture supernatants and urine samples.

Multi-unsaturated lipids, are most likely to form peroxides, and so they are the most reactive lipids in the TBARs assay. TBARs assay is commonly used to compare one set of samples to another.

Lipid oxidation is reported to increase with age and correlates with some clinical features of cardiovascular disease, ischemia/reperfusion and cerebrovascular disorders among others.

Materials

BQCKit Malondialdehyde Assay Kit KB03016-100 tests contains:

| Product | Quantity | Storage |
|-----------|----------|---------|
| Reagent A | 1 bottle | 4°C |
| Reagent B | 1 bottle | 4°C |
| Reagent C | 2 vials | 4°C |
| Reagent D | 1 vial | 4°C |
| Reagent E | 1 bottle | 4°C |
| Standard | 1 vial | 4°C |

BQCKit Malondialdehyde Assay Kit KB03016-200 tests contains:

| Product | Quantity | Storage |
|-----------|-----------|---------|
| Reagent A | 2 bottles | 4°C |
| Reagent B | 1 bottle | 4°C |
| Reagent C | 1 bottle | 4°C |
| Reagent D | 2 vials | 4°C |
| Reagent E | 1 bottles | 4°C |
| Standard | 1 vial | 4°C |

Materials

BQCkit Malondialdehyde Assay Kit KB03016-500 tests contains:

| Product | Quantity | Storage |
|-----------|-----------|---------|
| Reagent A | 2 bottles | 4°C |
| Reagent B | 1 bottles | 4°C |
| Reagent C | 1 bottles | 4°C |
| Reagent D | 1 vials | 4°C |
| Reagent E | 1 bottles | 4°C |
| Standard | 1 vial | 4°C |

Assay Principle

The Thiobarbituric Acid Reactive Substances (TBARS) Assay Kit is a tool for the direct quantitative measurement of MDA in biological samples. The samples containing MDA or the MDA standards are reacted with TBA.

After the incubation, the samples and standards can be read spectrophotometrically or fluorometrically.

The MDA concentration in the samples is then calculated by comparison with the MDA standard curve.

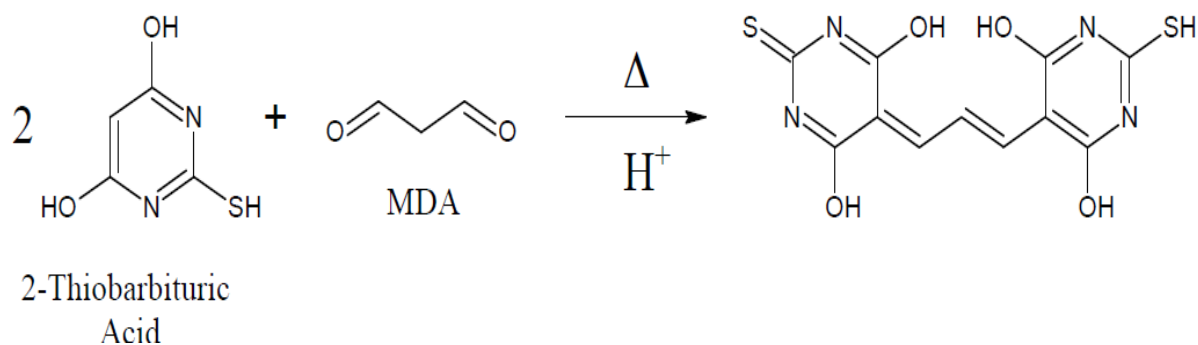


Figure 1. Principle of the assay reaction

Sample Preparation

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell Culture Supernates: Remove particulates by centrifugation. Assay immediately or aliquot and store samples at $-20\text{ }^{\circ}\text{C}$. Avoid repeated freeze-thaw cycles.

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 or aliquot and store samples at $-20\text{ }^{\circ}\text{C}$. Avoid repeated freeze-thaw cycles.

Plasma does not need to be diluted before assaying.

Urine: Centrifuge to remove particulate matter, and assay immediately or aliquot and store at $-20\text{ }^{\circ}\text{C}$. Avoid repeated freeze-thaw cycles.

Reagent Preparation

Working solution:

-For KB-03-016 100 test format: Add 12.5 mL of Reagent B to the powdered contents of bottle of Reagent A and shake until completely dissolved.

-For KB-03-016 200 test format: Add 12.5 mL of Reagent B to the powdered contents of each bottle of Reagent A and shake until completely dissolved.

-For KB-03-016 400 test format: Add 25 mL of Reagent B to the powdered contents of each bottle of Reagent A and shake until completely dissolved.

Sonication can be used to assist dissolution if necessary. Store at room temperature and use within 1 week of preparation.

Reagent Preparation

Standard solution:

Colorimetric assay: In a plastic tube, pipette 100 μL of Standard vial with 700 μL of ddH₂O. This produces a stock solution of 125 μM .

Fluorometric assay: In a plastic tube, pipette 10 μL of Standard vial with 790 μL of ddH₂O. This produces a stock solution of 12.5 μM .

Prepare calibration curves in tubes as shown in Table1.

Table1. Reagent volumes needed to carry out the standard curve.

| Standard (μL) | ddH ₂ O (μL) | Colorimetric Assay | Fluorometric Assay |
|-------------------------------|---|-----------------------------|-----------------------------|
| | | [Standard] μM | [Standard] μM |
| 0 | 500 | 0 | 0 |
| 10 | 490 | 2.5 | 0.25 |
| 20 | 480 | 5 | 0.5 |
| 30 | 470 | 7.5 | 0.75 |
| 40 | 460 | 10 | 1 |
| 80 | 420 | 20 | 2 |
| 200 | 300 | 50 | 5 |

Assay Protocol

Bring all reagents and samples to room temperature before use.

1. In 1 ml tubes mix 100 μ L of standard (See table 1 for standard curve preparation) or sample with 25 μ L of Reagent D.
2. Incubate for 30 minutes at 60°C.
3. Add 100 μ L of Reagent E and 50 μ L of Reagent C to each vial.
4. Centrifuge at 10,000 rpm x 10 minutes.
5. Carefully remove 200 μ L of supernatant to clean vial and add 100 μ L of the Working Solution (See Reagent Preparation).
6. Incubate for 60 minutes at 90°C.
7. Cool the vials with ice to stop the reaction. Add 150 μ L to each well of a 96 well microplate and determine the OD at 532 nm (colorimetric method) or the RFU at 532 nm excitation vs 553 nm emission (fluorimetric method) using a microplate reader.

Data Analysis

1. Zeroed the absorbance/fluorescence values:

$$A = A_{532 \text{ nm}} (\text{sample/standard}) - A_{532 \text{ nm}} (\text{blank})$$

$$\text{RFU} = \text{RFU}_{(532/553\text{nm})} (\text{sample/standard}) - \text{RFU}_{(532/553\text{nm})} (\text{blank})$$

2. Create a standard curve by plotting the mean absorbance/fluorescence for each well on a linear y-axis against the concentration on a linear x-axis.

3. Calculate the MDA concentration of the samples using the equation obtained from the linear regression of the standard curve replacing the $A_{532 \text{ nm}}$ or the $\text{RFU}_{532/553\text{nm}}$ values for each sample.

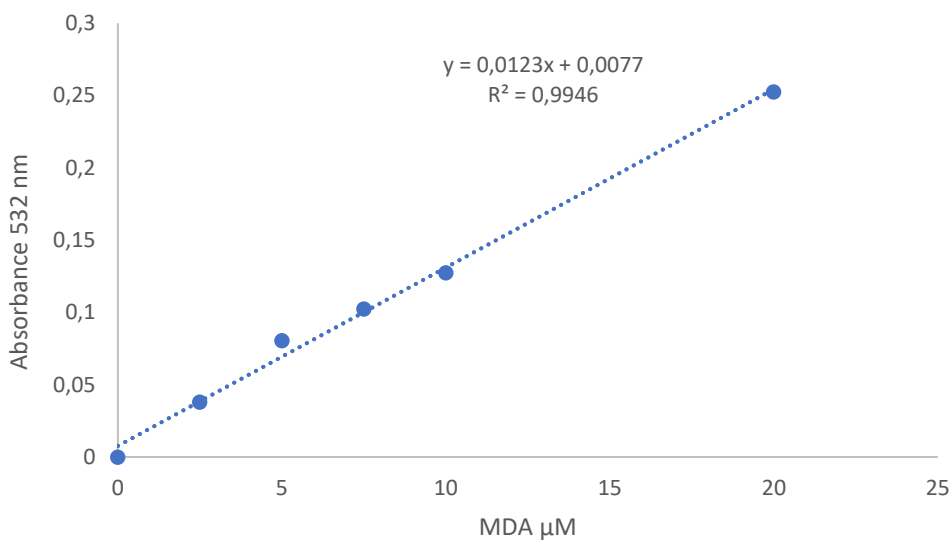


Figure 2. Example of the colorimetric standard representation

Warranties and Limitation of Liability

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Buyer's exclusive remedy and Bioquochem's sole liability hereunder shall be limited to a refund of the purchase price, or the replacement of all material that does not meet our specifications.

Said refund or replacement is conditioned on buyer giving written notice to Bioquochem within 30 days after arrival of the material at its destination.

Expiration date: 1 year from the date of delivery

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