Malondialdehyde (MDA)
TBARs Assay Kit

KB-03-016

100/200/400 tests

BQCkit

A brand of Bio QuChem

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All chemicals should be handled with care

- **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	4ºC
Reagent B	1 bottle	4ºC
Reagent C	2 vials	4ºC
Reagent D	1 vial	4ºC
Reagent E	1 bottle	4ºC
Standard	1 vial	4ºC

BQCkit Malondialdehyde Assay Kit ***KB03016-200 tests*** contains:

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

Materials

BQCkit Malondialdehyde Assay Kit ***KB03016-400 tests*** contains:

Product	Quantity	Storage
Reagent A	2 bottles	4ºC
Reagent B	1 bottles	4ºC
Reagent C	1 bottles	4ºC
Reagent D	1 vials	4ºC
Reagent E	1 bottles	4ºC
Standard	2 vials	4ºC

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.

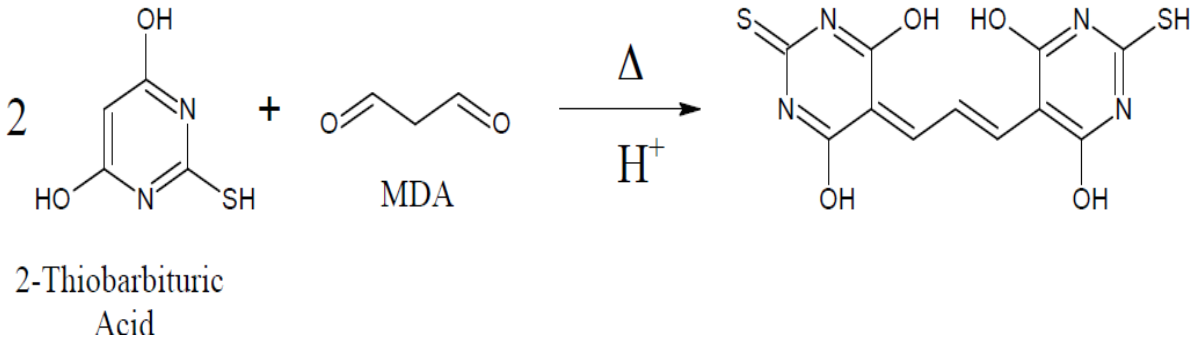

Figure 1. *Principle of the assay reaction*

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 °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 °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 °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.

Standard solution:

Colorimetric assay: In a plastic tube, pipette 100 µL of Standard vial with 700 µL of ddH_2O. This produces a stock solution of 125 µM.

Fluorometric assay: In a plastic tube, pipette 10 µL of Standard vial with 790 µL of ddH_2O. 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.*

		Colorimetric Assay	Fluorometric Assay
Standard (µL)	**ddH_2O (µL)**	**[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 µL of standard (See table 1 for standard curve preparation) or sample with 25 µL of Reagent D.
2. Incubate for 30 minutes at 60ºC.
3. Add 100 µL of Reagent E and 50 µL of Reagent C to each vial.
4. Centrifuge at 10,000 rpm x 10 minutes.
5. Carefully remove 200 µL of supernatant to clean vial and add 100 µL of the Working Solution (See Reagent Preparation).
6. Incubate for 60 minutes at 90ºC.
7. Cool the vials with ice to stop the reaction. Add 150 µL 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:

$$A = A\ 532\ nm\ (sample/standard) - A532\ nm\ (blank)$$

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

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 $A_{532\ nm}$ or the $RFU_{532/553nm}$ values for each sample.

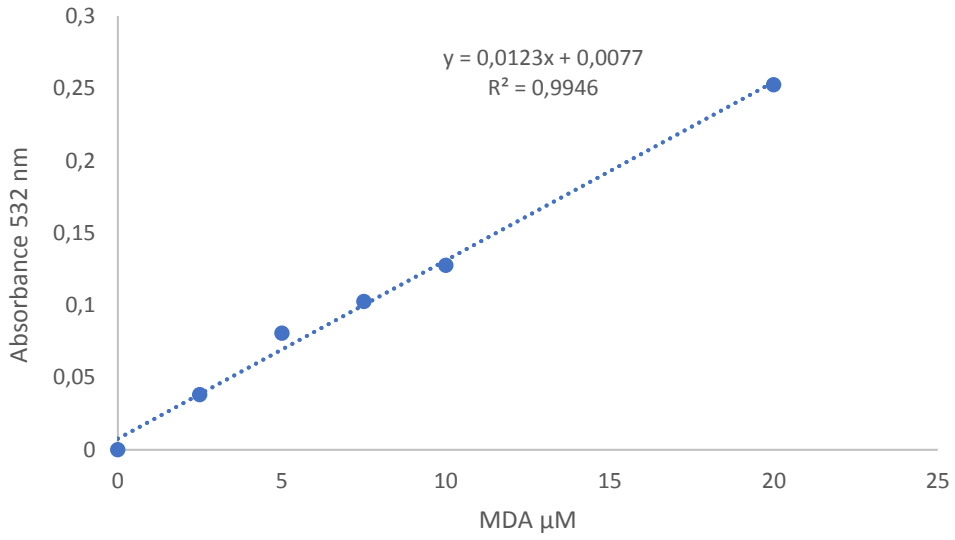

Figure 2. *Example of the colorimetric standard representation*

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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

For further details, please refer to our website **www.bqckit.com**.