# Malondialdehyde (MDA) TBARs Assay

## (Cat. # BAQ068, BAQ069, BAQ070)

## 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).

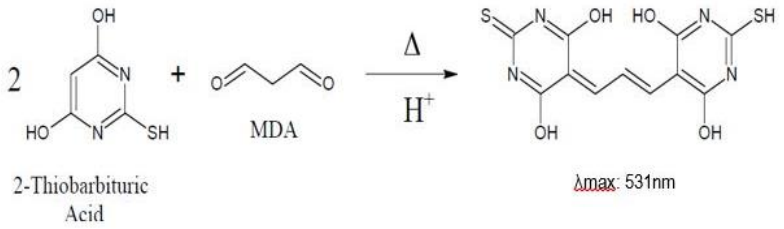
This method, is convenient to determine the relative lipid peroxide content of samples, including plasma, serum, cell culture supernatants and urine samples. Multi-unsaturated lipids are most likely to form peroxides, and so they are the most reactive lipids in the TBARs assay. TBARs assay is commonly 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.

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.




## ITEM(S) SUPPLIED

| Description | Cat. # BAQ068 100 tests (96 well plate) | Cat. # BAQ069 200 tests (96 well plate) | Cat. # BAQ070 400 tests (96 well plate) |
|---|---|---|---|
| MDA Reagent A | 1 bottle | 2 bottles | 4 bottles |
| MDA Reagent B | 1 bottle | 1 bottle | 2 bottles |
| MDA Reagent C | 2 bottles | 3 bottles | 6 bottles |
| MDA Reagent D | 1 bottle | 2 bottles | 4 bottles |
| MDA Reagent E | 1 bottle | 2 bottles | 4 bottles |
| MDA Standard | 1 vial | 2 vials | 4 vials |

## STORAGE CONDITIONS

This kit is shipped on blue ice. Store entire kit at 4°C upon arrival. If stored and used as directed this kit is stable for 12 months.

## ADDITIONAL ITEMS REQUIRED

- Double distilled water ($ddH_2O$)
- Spectrophotometer microplate reader that can measure at 532 nm or a fluorometer with 532 nm excitation and 553 nm emission
- 96 well microtiter plate for microplate assay.
- 1.5ml Tubes

## SAMPLE PREPARATION

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

**Cell Culture Supernatants:** 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.

## PREPARATION BEFORE USE

Bring all reagents and samples to room temperature before use.

***Working solution:***

For BAQ068 and BAQ069, add 12.5 mL of MDA Reagent B to the powdered contents of a bottle of MDA 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 50 µL of Standard vial in to 950 µL of $ddH_2O$. This produces a stock solution of 50 µM.

**Fluorometric assay:** In a plastic tube, pipette 5 µL of Standard vial in to 995 µL of $ddH_2O$. This produces a stock solution of 5 µM.

Prepare calibration curves in tubes as shown below.

| | | Colorimetric Assay | Fluorometric Assay |
|---|---|---|---|
| **Standard (µL)** | **ddH2O (µL)** | **[Standard] µM** | **[Standard] µM** |
| 0 | 500 | 0 | 0 |
| 5 | 495 | 0.5 | 0.05 |
| 10 | 490 | 1.0 | 0.10 |
| 25 | 475 | 2.5 | 0.25 |
| 50 | 450 | 5.0 | 0.50 |
| 100 | 400 | 10 | 1.0 |
| 250 | 250 | 25 | 2.5 |
| 500 | 0 | 50 | 5.0 |

## PROTOCOL

1. In 1.5 ml tubes, mix 100 µL of standard or sample with 25 µL of MDA Reagent D.
2. Incubate for 30 minutes at 60°C.
3. Add 100 µL of MDA Reagent E and 50 µL of MDA 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 prepared earlier.
6. Incubate for 60 minutes at 90°C.
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 (fluorometric method) using a microplate reader.

## DATA ANALYSIS

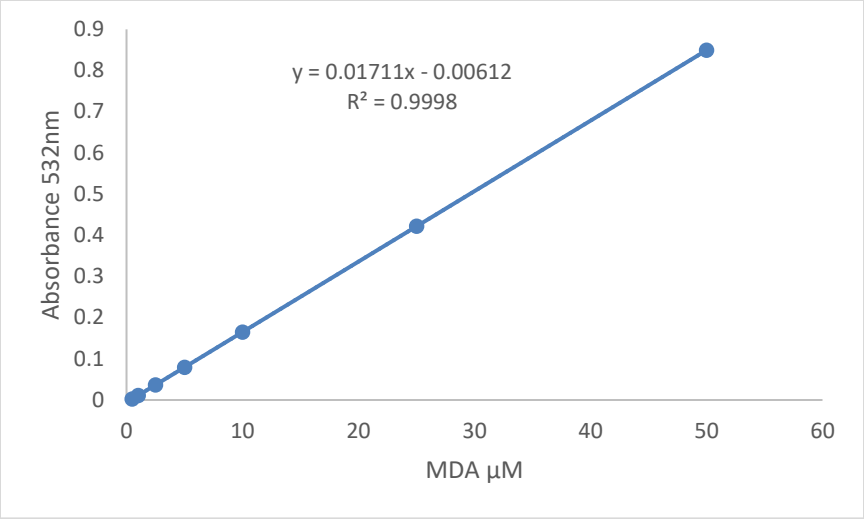
Zero the absorbance/fluorescence values:

A = A 532 nm (sample/standard) – A532 nm (blank)

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

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.

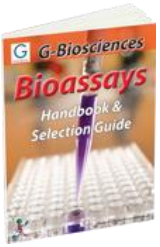
Calculate the MDA concentration of the samples using the equation obtained from the linear regression of the standard curve replacing the A532 nm or the RFU532/553nm values for each sample.




*This standard curve is only an example, **DO NOT USE** this standard curve to calculate for your samples. A new standard curve must be performed by the end user.

## RELATED PRODUCTS

Download our Bioassays Handbook.




http://info2.gbiosciences.com/complete-bioassay-handbook

*For other related products, visit our website at www.GBiosciences.com or contact us.*


**www.GBiosciences.com**