Amplite™ Colorimetric Malondialdehyde (MDA) Quantitation Kit

Catalog number: 10070
Unit size: 200 Tests

Component	Storage	Amount
Component A: MDA Blue™	Freeze (<-15 °C), Avoid Light	1 vial
Component B: Dilution Buffer	Freeze (<-15 °C), Avoid Light	1 bottle (10 mL)
Component C: MDA Standard	Freeze (<-15 °C), Dessicated, Avoid Light	1 vial (lyophilized powder)
Component D: Reaction Solution	Freeze (<-15 °C), Avoid Light	1 bottle (10 mL)

OVERVIEW

Malondialdehyde (MDA) is natural byproduct of lipid peroxidation and is widely used as a key indicator to determine the oxidative stress and free radical formation. Measurement of MDA has historically relied on the reaction with thiobarbituric acid (TBA) to results in a compound that can be measured colorimetrically at 532 nm or fluorimetrically at Ex/Em = 530 nm/550 nm. But this assay is not specific to MDA and also takes place under acidic conditions at 90-100 °C. There have been a number of commercial ELISA kits, which makes it more expensive and tedious. The Amplite™ Colorimetric Malondialdehyde (MDA) Quantitation Kit offers the most rapid and convenient method to measure MDA without the TBARS heating steps. MDA Blue™ reacts with MDA to generate a blue color product which is measured at 695 nm with absorbance microplate reader. This assay is very fast and specific to MDA with little interference from other aldehydes.

AT A GLANCE

Protocol summary

1. Prepare test samples along with serially diluted MDA standards (50 µL)
2. Add MDA Blue™ stock solution (10 µL)
3. Incubate at room temperature for 10 - 30 minutes
4. Add Reaction Solution (40 µL)
5. Monitor OD increase at 695 nm

Important Thaw all the components at room temperature before starting the experiment.

KEY PARAMETERS

Instrument:	Absorbance microplate reader
Absorbance:	695 nm
Recommended plate:	Clear bottom

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles.

1. MDA standard solution (100 mM):
Add 100 µL of ddH_2O into MDA Standard vial (Component C) to make 100 mM MDA stock solution.

PREPARATION OF STANDARD SOLUTION

MDA standard

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/10070

Add 4 µL of 100 mM MDA standard into 996 µL of Dilution Buffer (Component B) to get 400 µM MDA solution (MDA7). Then perform 1:2 serial dilutions in dilution buffer to get serially diluted MDA standards (MDA6 - MDA1).

SAMPLE EXPERIMENTAL PROTOCOL

Table 1. Layout of MDA standards and test samples in a 96-well clear bottom microplate. MDA= MDA Standard (MDA1 - MDA7, 6.25 to 400 µM), BL=Blank Control, TS=Test Sample.

BL	BL	TS	TS
MDA1	MDA1	...	...
MDA2	MDA2	...	...
MDA3	MDA3		
MDA4	MDA4		
MDA5	MDA5		
MDA6	MDA6		
MDA7	MDA7		

Table 2. Reagent composition for each well.

Well	Volume	Reagent
MDA1 - MDA7	50 µL	Serial Dilution (6.25 to 400 µM)
BL	50 µL	Dilution Buffer (Component B)
TS	50 µL	test sample

1. Prepare MDA standards (MDA), blank controls (BL), and test samples (TS) according to the layout provided in Tables 1 and 2. For a 384-well plate, use 25 µL of reagent per well instead of 50 µL.
2. Add 10 µL MDA Blue™ (Component A) solution into each well of MDA standard, blank control, and test samples. For a 384-well plate, use 5 µL MDA Blue™ solution into each well.
3. Incubate the reaction mixture at room temperature for 10 - 30 minutes.
4. Add 40 µL of Reaction Solution (Component D) to make the total assay volume of 100 µL/well. For a 384-well plate, use 20 µL of Reaction Solution, for a total assay volume of 50 µL/well.
5. Incubate the final reaction mixture at room temperature for 30 - 60 minutes.
6. Monitor absorbance increase with an absorbance plate reader with path-check correction at OD of 695 ~ 700 nm.

Tel: 408-733-1055 | Fax: 408-733-1304 | Email: support@aatbio.com

EXAMPLE DATA ANALYSIS AND FIGURES

The reading (Abs) obtained from the blank standard well is used as a negative control. Subtract this value from the other standards' readings to obtain the base-line corrected values. Then, plot the standards' readings to obtain a standard curve and equation. This equation can be used to calculate Aldehyde Concentration samples. We recommend using the Online Linear Regression Calculator which can be found at:

https://www.aatbio.com/tools/linear-logarithmic-semi-log-regression-online-calculator/

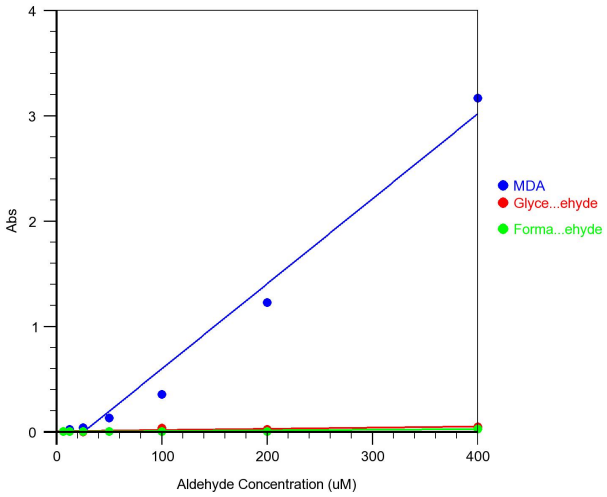

Figure 1. MDA dose response was measured with Amplite™ Colorimetric Malondialdehyde (MDA) Quantitation Kit on a 96-well clear bottom microplate using a SpectraMax microplate reader (Molecular Devices).

DISCLAIMER