Amplite™ Fluorimetric Malondialdehyde (MDA) Quantitation Kit

Catalog number: 10071
Unit size: 200 Tests

Component	Storage	Amount
Component A: Monoaldelite™ Blue	Freeze (<-15 °C), Minimize light exposure	2 vials (lyophilized powder)
Component B: Assay Buffer	Freeze (<-15 °C)	1 bottle (20 mL)
Component C: MDA Standard	Freeze (<-15 °C), Minimize light exposure	1 vial (lyophilized powder)
Component D: Reaction Solution	Freeze (<-15 °C), Minimize light exposure	1 bottle (5 mL)
Component E: DMSO	Freeze (<-15 °C)	1 bottle (100 µL)

OVERVIEW

Malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE) are natural byproducts of lipid peroxidation. As the most popular and reliable biomarker for lipid peroxidation, MDA has been widely used for many years to determine oxidative stress in clinical situations. Therefore, quantification of MDA is essential to assess oxidative stress in pathophysiological processes. The Amplite™ Fluorimetric Malondialdehyde (MDA) Quantitation Kit offers a quick and convenient method to measure MDA without heating steps that are required for the commercial MDA assay kits from other vendors. Monoaldelite™ Blue itself is nearly non-fluorescent, but generates strong blue fluorescence upon reacting with MDA.

AT A GLANCE

Protocol summary

1. Prepare and add MDA standards or test samples (50 µL)
2. Prepare and add MDA working solution (50 µL)
3. Incubate at room temperature for 10 to 30 minutes
4. Add Reaction Solution (25 µL)
5. Incubate at room temperature for 30 to 60 minutes
6. Monitor fluorescence intensity at Ex/Em = 365/435 nm

Important Thaw all the kit components to room temperature before starting the experiment.

KEY PARAMETERS

Instrument:	Fluorescence microplate reader
Excitation:	365 nm
Emission:	435 nm
Cutoff:	420nm
Recommended plate:	Solid black

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 the vial of MDA Standard (Component C) to make 100 mM MDA standard solution.

2. Monoaldelite™ Blue stock solution (250X):
Add 20 µL of DMSO (Component E) into one vial of Monoaldelite™ Blue (Component A) to make Monoaldelite™ Blue stock solution.

Note This Monoaldelite™ Blue stock solution is enough for one 96-well plate. It is not stable, use it promptly.

PREPARATION OF STANDARD SOLUTION

MDA standard

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/10071

Add 10 µL of 100 mM MDA standard solution into 990 µL of Assay Buffer (Component B) to generate 1000 µM MDA standard solution (MDA7). Take 1000 µM MDA standard solution (MDA7) and perform 1:3 serial dilutions to get serially diluted MDA standards (MDA6 - MDA1) with Assay Buffer (Component B).

PREPARATION OF WORKING SOLUTION

Monoaldelite™ Blue working solution:
Add 20 µL of 250X Monoaldelite™ Blue stock solution into 5 mL of Assay Buffer (Component B) and mix them well.

SAMPLE EXPERIMENTAL PROTOCOL

Table 1. Layout of MDA standards and test samples in a solid black 96-well microplate. MDA= MDA Standards (MDA1 - MDA7, 1.37 to 1000 µM), BL=Blank Control, TS=Test Samples.

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 Dilutions (1.37 to 1000 µM)
BL	50 µL	Assay Buffer (Component B)
TS	50 µL	test sample

1. Add 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 50 µL of MDA working solution to each well of MDA standard, blank control, and test samples to make the total assay volume of 100 µL/well. For a 384-well plate, add 25 µL of MDA working solution into each well instead, for a

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total volume of 50 µL/well.

3. Incubate the reaction at room temperature for 10 - 30 minutes.

4. Add 25 µL of Reaction Solution (Component D) to each well to make the total assay volume of 125 µL/well. For a 384-well plate, add 12.5 µL of Reaction Solution (Component D) into each well instead, for a total volume of 62.5 µL/well.

5. Incubate the reaction at room temperature for 30 - 60 minutes.

6. Monitor the fluorescence increase with a fluorescence plate reader at Ex/Em = 365/435 nm (Cutoff = 420 nm).

EXAMPLE DATA ANALYSIS AND FIGURES

The reading (RFU (Ex/Em= 365/435nm)) 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 MDA Con. 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

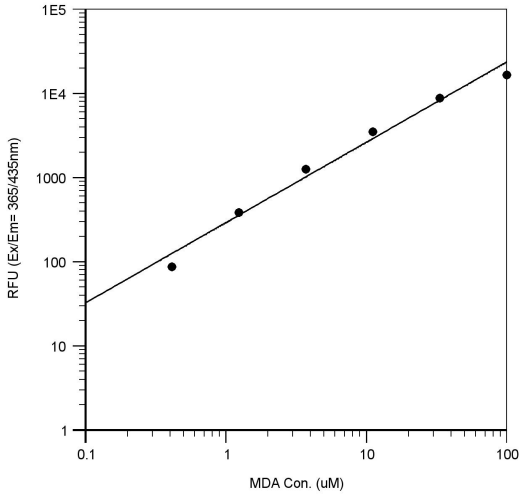

Figure 1. MDA dose response was measured with Amplite™ Fluorimetric Malondialdehyde (MDA) Quantitation Kit on a 96-well solid black microplate using a Gemini microplate reader (Molecular Devices) at Ex/Em=365/435 nm, cutoff=420 nm.

DISCLAIMER