Amplite™ Fluorimetric Malondialdehyde (MDA) Quantitation Kit *Enhanced Selectivity*

Catalog number: 10072
Unit size: 200 Tests

Component	Storage	Amount
Component A: MDA Green™	Freeze (<-15 °C), Minimize light exposure	2 vials
Component B: MDA Assay Buffer	Freeze (<-15 °C), Minimize light exposure	1 bottle (20 mL)
Component C: MDA Standard	Freeze (<-15 °C), Minimize light exposure	1 vial
Component D: MDA Stopping Solution	Freeze (<-15 °C), Minimize light exposure	1 bottle (10 mL)
Component E: DMSO	Freeze (<-15 °C)	1 vial (100 uL)

OVERVIEW

Malondialdehyde (MDA) is one of the natural byproducts during lipid peroxidation. It is widely used as a reliable biomarker to determine oxidative stress, and the quantification of MDA is an essential way to assess oxidative stress in pathophysiological processes. Amplite™ Fluorimetric Malondialdehyde (MDA) Quantitation Kit offers a new method to measure MDA without the heating steps required for the TBARS-based MDA assay. The MDA Green™ can react with MDA to generate a considerable enhancement of green fluorescence signal in a convenient 96-well or 384-well microtiter-plate format. Unlike other commercial MDA assay kits, this assay is robust and specific to MDA with little interference from other aldehydes.

AT A GLANCE

Protocol summary

1. Prepare and add MDA standards or test samples (50 uL)
2. Prepare and add MDA Green™ working solution (50 uL)
3. Incubate at room temperature for 6 minutes
4. Add MDA Stopping Solution (50 uL)
5. Monitor fluorescence intensity at Ex/Em=480/555 nm

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

KEY PARAMETERS

Instrument:	Fluorescence microplate reader
Excitation:	480 nm
Emission:	555 nm
Cutoff:	530 nm
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 uL of ddH_2O into the vial of MDA Standard (Component C) to make 100 mM MDA standard solution.

2. MDA Green™ stock solution (250 X):
Add 20 uL of DMSO (Component E) into one vial of MDA Green™ (Component A) to make vial of MDA Green™ stock solution.

Note All stock solutions should be stored at -20°C after preparation. Avoid repeated freeze-thaw cycles.

PREPARATION OF STANDARD SOLUTION

MDA standard

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/10072

Add 10 uL of 100 mM MDA standard solution into 990 uL of MDA Assay Buffer (Component B) to generate 1000 uM MDA standard solution (MDA1). Take 1000 uM MDA standard solution (MDA1) and perform 1:3 serial dilutions to get serially diluted MDA standards (MDA2 - MDA7) with MDA Assay Buffer (Component B).

PREPARATION OF WORKING SOLUTION

MDA Green™ working solution:
Add 20 uL of 250 X MDA Green™ stock solution into 5 mL of MDA Assay Buffer (Component B), and mix well to make MDA Green™ working solution.

Note This MDA Green™ working solution should be prepared before the experiment, and kept from light.

SAMPLE EXPERIMENTAL PROTOCOL

Table 1. Layout of MDA standards and test samples in a solid black 96-well microplate. MDA= MDA Standards (MDA1 - MDA7, 1000 to 1.37 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 (1000 to 1.37 µM)
BL	50 µL	MDA Assay 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

uL of reagent per well instead of 50 uL.

2. Add 50 uL of MDA Green™ working solution to each well of MDA standard, blank control, and test samples. For a 384-well plate, add 25 uL of MDA Green™ working solution into each well instead.

3. Incubate the reaction at room temperature for 5-6 minutes, protected from light.

4. Add 50 uL of MDA Stopping Solution (Component D) to each well of the reaction. For a 384-well plate, add 25 uL of MDA Stopping Solution (Component D) into each well instead.

5. Monitor the fluorescence intensity with a fluorescence plate reader at Ex/Em=480/555 nm (Cutoff= 530 nm).

EXAMPLE DATA ANALYSIS AND FIGURES

The reading (RFU) 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 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

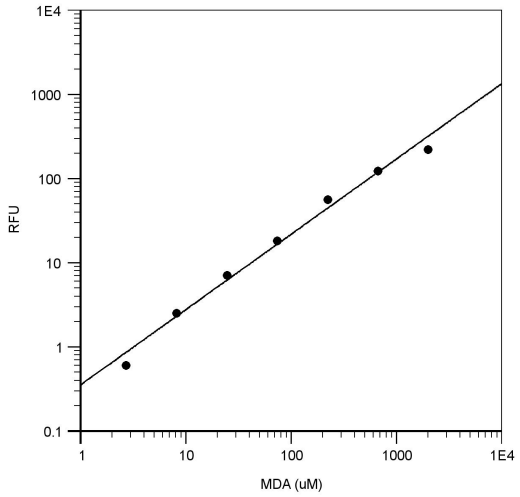

Figure 1. MDA dose response was measured with Amplite™ Fluorimetric Malondialdehyde (MDA) Quantitation Kit on a 96-well solid black microplate using a Gemini microplate reader (Molecular Devices) at Ex/Em=480/555 nm, cutoff=530 nm.

DISCLAIMER