Cell Meter™ Intracellular Colorimetric Lipid Peroxidation (MDA) Assay Kit

Catalog number: 15991
Unit size: 200 tests

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

OVERVIEW

Lipid peroxidation is characterized by the oxidative degradation of unsaturated fatty acids, phospholipids, glycolipids, cholesterol esters and cholesterol. Malondialdehyde (MDA) is one of the most commonly used biomarkers for lipid peroxidation. Measurement of MDA has historically relied on a reaction with thiobarbituric acid (TBA) to generate a product that can be measured colorimetrically or fluorimetrically. However, TBA assay has quite a few limitations. (1). The reaction is not specific to MDA. (2). TBA-MDA reaction need be run under acidic conditions. (3). The assays need be run under high temperature, commonly at 90-100 ºC. The commercial TBA-based MDA assays are extremely tedious to run due to these limitations. This Cell Meter™ Colorimetric Lipid Peroxidation (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 and add MDA standards and/or test samples (50 µL)
2. Prepare and add MDA Blue™ (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 kit 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.

MDA standard stock solution (100 mM):
Add 100 µL of ddH_2O into MDA Standard (Component C) and mix them well.

Note The unused MDA stock solution should be stored at -20°C in single use aliquots.

PREPARATION OF STANDARD SOLUTION

MDA standard

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/15991

Add 4 µL of MDA standard (100 mM) into 996 µL of Dilution Buffer (Component B) to get MDA standard solution (400 µM). Then perform 1:2 serial dilutions in dilution buffer to get 200, 100, 50, 25, 12.5, 6.25 and 0 µM serially diluted MDA standards.

SAMPLE EXPERIMENTAL PROTOCOL

Table 1. Layout of MDA standards and test samples in a clear bottom 96-well microplate. MDA = MDA standard (MDA1-MDA7= 400 to 6.25 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 (400 to 6.25 µM)
BL	50 µL	Dilution Buffer (Component B)
TS	50 µL	Test Sample

MDA assay

1. Add 50 µL of MDA standard, blank control, and test samples to clear bottom 96-well microplate (As shown in Table 1 and Table 2).

2. Add 10 µL/well of MDA Blue™ (Component A) into each well of MDA standard, blank control and test samples. *Note:* For a 384-well plate, add 25 µL of sample, 5 µL of MDA Blue™ solution into each well.

   Note Please aliquot Component A into single use size and store unused at -20°C and avoid light.

3. Incubate the reaction at room temperature for 10 - 30 minutes, protected from light.

4. Add 40 µL of Reaction Solution (Component D) to make the total assay volume of 100 µL/well.

   Note For a 384-well plate, add 20 µL of Reaction Solution (Component D) to make the total assay volume of 50 µL/well.

5. Monitor absorbance increase with an absorbance plate reader with path-check correction at OD of 695~700 nm.

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EXAMPLE DATA ANALYSIS AND FIGURES

The reading (Absorbance(695nm)) 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

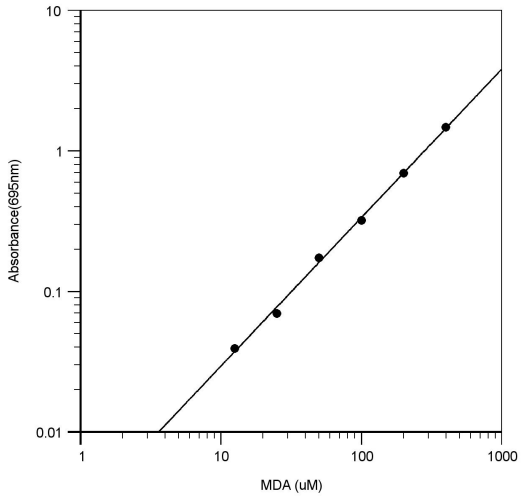

Figure 1. MDA dose response was measured with Amplite™ Colorimetric Lipid Peroxidation (MDA) Quantitation Kit on a 96-well clear bottom microplate using a SpectraMax microplate reader (Molecular Devices).

DISCLAIMER