Amplite™ Colorimetric Malondialdehyde (MDA) Quantitation Kit *Blue Color*

Ordering Information	*Storage Conditions*	*Instrument Platform*
Cat#: 10070 (200 Assays)	Keep in freezer and protect from light	Absorbance microplate readers

Introduction

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 90-100ºC. 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.

Kit Components

Components	**Amount**
Component A: MDA Blue™	1 vial
Component B: Dilution Buffer	1 bottle (10 mL)
Component C: MDA Standard	1 vial (lyophilized powder)
Component D: Reaction Solution	1 bottle (10 mL)

Assay Protocol for One 96-Well Plate

Brief Summary

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

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

1. Prepare serially diluted MDA standards and test samples:

1.1 Add 100 µL of ddH_2O into MDA Standard vial (**Component C**) to make 100 mM MDA stock solution.

1.2 Prepare MDA standard dilutions: Add 4 µL of 100 mM MDA standard (from step 1.1) into 996 µL of Dilution Buffer (**Component B**) to get 400 µM MDA solution. 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.

1.3 Add MDA containing samples and serially diluted MDA standards into a 96-well clear bottom microplate according to Tables 1 and 2.

Table 1. Layout of MDA standards and test samples in a 96-well clear bottom microplate

BL	BL	TS	TS	….	….						
MDA 1	MDA 1	….	….	….	….						
MDA 2	MDA 2										
MDA 3	MDA 3										
MDA 4	MDA 4										
MDA 5	MDA 5										
MDA 6	MDA 6										
MDA 7	MDA 7										

Note 1: MDA= MDA Standard, BL=Blank Control (dilution buffer), TS=Test Sample.
Note 2: Add the serial dilutions of MDA standards from 6.25 µM to 400 µM into wells from MDA 1 to MDA 7.

Table 2. Reagent composition for each well

MDA Standard	Blank Control	Test Sample
Serial Dilutions: 50 µL	Assay Buffer: 50 µL	50 µL

2. Run MDA assay:

2.1 Add 10 µL MDA Blue™ (**Component A**) solution into each well of MDA standard, blank control, and test samples (see Step 1.3).
Note: For a 384-well plate, add 25 µL of sample, 5 µL of MDA Blue™ stock solution into each well.
Note: Please aliquot Component A into single use size and store unused at -20ºC and avoid light!

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

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

2.4 Incubate the final reaction mixture at room temperature for 30-60 minutes.

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

Data Analysis

The absorbance reading in blank wells (with dilution buffer only) is used as a control, and is subtracted from the values of those wells with the standards and test samples. The standard curve of MDA is shown in Figure 1. Calculate the MDA concentrations of the samples according to the standard curve:

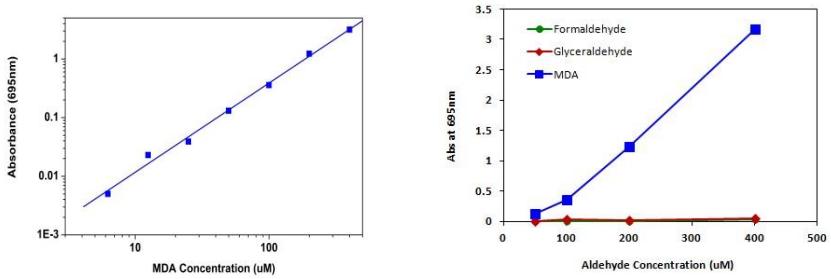

Figure1. MDA dose response was measured with Amplite™ Colorimetric Malondialdehyde (MDA) Quantitation Kit on a 96-well clear bottom microplate using a SpectraMax microplate reader (Molecular Devices).
A: Standard curve of MDA. B: Signal Comparison of MDA, Formaldehyde, and Glyceraldehyde.

References

1. X.L. Zheng, Y. Kitamoto and J.E. Sadler. (2009) Enteropeptidase, a type II transmembrane serine protease. Frontiers in Bioscience E1, pp. 242-249.
2. B. Hadorn, M.J. Tarlow, J.K. Lloyd and O.H. Wolff. (1969) Intestinal enterokinase deficiency. The Lancet, 7599: pp. 812-813.
3. G. Luengo-Gil, M.I. Calvo, E. Martín-Villar, S. Águila, N. Bohdan, A.I. Antón, S. Espín, F.A. de la Peña, V. Vicente, J. Corral, M. Quintanilla and I. Martínez-Martínez. (2016) Antithrombin controls tumor migration, invasion and angiogenesis by inhibition of enteropeptidase. Scientific reports, 27544