Malondialdehyde (MDA)

Cat# 1503

Malondialdehyde (MDA) Assay Kit

Quantitative Colorimetric Determination

DESCRIPTION

Polyunsaturated lipids are susceptible to an oxidative attack, typically by reactive oxygen species, resulting in a well-defined chain reaction with the production of malondialdehyde (MDA). Lipid peroxidation may contribute to the pathology of many diseases including atherosclerosis, diabetes, and Alzheimer's. Simple, direct and high-throughput assays for Malondialdehyde (MDA) find wide applications in research, Physiology, Nutrition, Botany, Food industry, environmental sciences, reproduction, and drug discovery.
In this kit, lipid peroxidation is determined by the reaction of MDA with thiobarbituric acid (TBA) to form a colorimetric (532 nm) proportional to the MDA present.

KEY FEATURES

Sensitive and accurate. 2.5 nmol MDA. Equiv./L
Simple and high-throughput.

APPLICATIONS

Direct Assays: serum, plasma, urine, saliva and other biological samples, food and beverages.
Drug Discovery/Pharmacology: effects of drugs on MDA.KIT
CONTENTS 100 Reaction Kit

- **R1 (reaction solution1):** 3 ml
- **R2 (reaction solution2)**: 20 ml
- **R3 (reaction solution3)**: 20 ml
- **R4 (Distil water)**: 10 ml
- **R5 (Dying Reagent):** 65 ml
- **Standard: Malondialdehyde solution:** 1 ml

Storage conditions. The kit is shipped at room temperature. Store all components at -20°C to ensure shelf life of 12 months.
Precautions: Reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

SAMPLE PREPARATION

Serum sample: Blood should be collected in a gel tube, then centrifuged at 4000 rpm for 5 min, and obtained clear solution on top of the cellular material. Separate it into a new Eppendorf tube and use it for further processing.
Tissue homogenate: Cell lysate is prepared by homogenizing tissue (25mg) or sonicating cells in ice-cold 1 x PBS (1mL) and centrifugation for 10 min at 14,000 rpm to pellet any debris. Use the clear supernatant for the assay. If not assayed immediately, freeze supernatant at -80°C (stable for 1 month).

ASSAY PROCEDURE

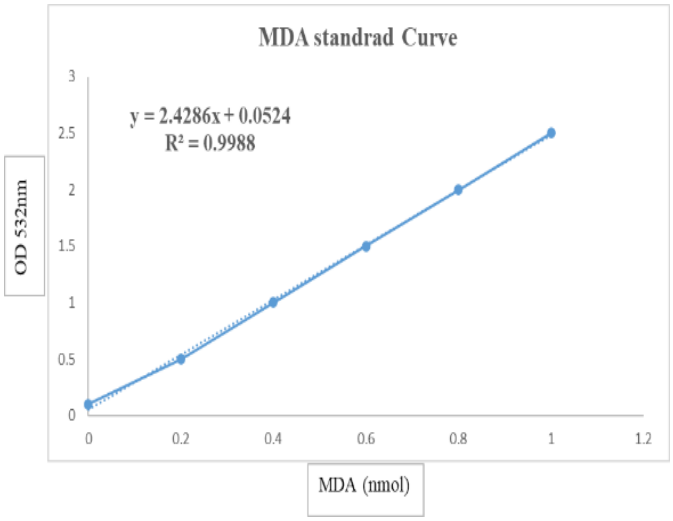
1. To begin, label two parallel set of eppendorf tubes based on the total number of samples.
2. Subsequently, in first set of labelled eppendorf tubes, introduce the following components following sequence (R1, R2, R3, R4), one after the other along with sample. (Quantity in above-mentioned Table.1)

Table.1

Sr.	Reagents	Sample((µl)
1	Sample	25 µl
2	**R1** (reaction solution1)	25 µl
3	**R2** (reaction solution2)	190 µl
4	**R3** (reaction solution3)	190 µl
5	**R4** (Distil water)	75 µl
Incubation ninety degrees Celsius for 1 hour in a water bath		
6	**R5** (Dying Reagent)	625 µl
centrifugation at 4000 g for 10 min		
Shift supernatant into 2nd Eppendorf tubes already labeled and determine OD at 532 nm.		

4. Following the addition of the specified components (R1, R2, R3, R4), all Eppendorf tubes were incubated in a water bath maintained at a constant temperature of ninety degrees Celsius for 1 hour.
   a. After a 1-hour incubation, the Eppendorf tubes were permitted to cool to ambient temperature naturally.
   b. Subsequently, 625 µl of R5 was added to all reaction tubes.
   c. The Eppendorf tubes were thereafter agitated vigorously and then subjected to centrifugation at 4000 g for roughly 10 minutes.
   d. The supernatant was carefully shifted into 2nd set of eppendorf tubes already labeled to determine OD at 532 nm.

Measure absorbance of sample and Blank at 532 nm as values of the sample and H_2O blank (standard #4). *n* is the sample dilution factor. Y is the absorbance value. Calculate X for the unknown value of the sample showing linearity of R^2=0.9988.
Y=2.4286x+ 0.0524, x= y-0.2524/ 2.4286



MDA standard for calorimetric detection

Take 100 µL of a 2 mM MDA stock solution of the given standard and dilute it with 900 µL of purified water to obtain a 0.2 mM MDA solution. Prepared the MDA standard solution according to Table 2.

Table.2

well	2mM MDA standard	Purified water	MDA (nm/well)
1	**10µl**	**190µl**	**2**
2	**8 µl**	**192µl**	**1.6**
3	**6 µl**	**194µl**	**1.2**
4	**4 µl**	**196µl**	**0.8**
5	**2 µl**	**198µl**	**0.4**
6	**-**	**200µl**	**0**

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipetting devices, water / dry bath, centrifuge tubes, clear flat-bottom uncoated 96-well plates, plate reader capable of reading optical density at 532 nm, homogenizer or sonicator etc.

PUBLICATIONS

1. Saleem, S., Mukhtar, I., Aati, H. Y., Muzaffar, H., Anwar, H., Hussain, M., Ahmad, M., & Umair, M. (2024). Effects of Withania somnifera (L.) Dunal in acute pulmonary pathophysiology in a rat model of smoke-induced lung injury and role of IRS-1 and SOX-2. *South African Journal of Botany*, *171*, 757-767.
2. Liza, Hussain, G., Malik, A., Akhtar, S., & Anwar, H. (2024). Artemisia vulgaris Extract as a Novel Therapeutic
3. Approach for Reversing Diabetic Cardiomyopathy in a Rat Model. *Pharmaceuticals*, *17*(8), 1046.