CheKine™ Lipid Peroxidation (MDA) Assay kit

Item NO.	Product Name
KTB1050	CheKine™ Lipid Peroxidation (MDA) Assay kit

ATTENTION

For laboratory research use only. Not for clinical or diagnostic use

Version 201901
Abbkine, Inc • China • ***www.abbkine.com***

TABLE OF CONTENTS

INTRODUCTION

Background

Oxidative stress has been implicated in the etiology of many chronic diseases, including cardiovascular disease. Lipid peroxidation is the degradation of lipids that occurs as a result of oxidative damage and is a useful marker for oxidative stress. Polyunsaturated lipids are susceptible to an oxidative attack, typically by reactive oxygen species, resulting in a well-defined chain reaction with the production of end products such as malondialdehyde (MDA). Lipid peroxidation may contribute to the pathology of many diseases including atherosclerosis, diabetes and Alzheimer's.

Assay principle

Lipid Peroxidation (MDA) Assay Kit provides a convenient tool for detection of the malondialdehyde (MDA) present in a variety of samples. MDA, together with 4-hydroxynonenal (4-HNE), is a natural bi-product of lipid peroxidation and its quantification is generally used as marker for lipid peroxidation. The MDA in the sample reacts with thiobarbituric acid (TBA) to generate a MDA-TBA adduct，which can be easily quantified colorimetrically at OD 532 nm. Meanwhile, the absorbance at 600 nm was measured and MDA was calculated by the difference between the absorbance at 532 nm and 600 nm (To avoid the interference of saccharose).

Storage/Stability

Storage at 4^{o}C. Kit has a storage time of 6 months from receipt. Refer to list of materials supplied for storage conditions of individual components.

Assay Restrictions

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

PRODUCT INFORMATION

Materials supplied and Storage conditions

Kit components	Quantity		Storage conditions
	48T	96T	
Lysis Buffer	50 mL	100 mL	4ºC
Reaction Mix	15 mL	30 mL	4ºC
1 mM Standard	1mL	1mL	4ºC

Other supplies required, Not Supplied

- Standard microplate reader capable of measuring absorbance at 532 nm and 600 nm
- Precision pipettes, disposable pipette tips
- Distilled or deionized water
- Assorted glassware for the preparation of reagents and buffer solutions
- 96 well plate with clear flat bottom
- Dounce homogenizer (for tissue samples)

Technical hints

- Avoid foaming or bubbles when mixing or reconstituting components.
- To avoid cross-contamination, change pipette tips between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.
- Samples generating values that are greater than the most concentrated standard should be further diluted in the appropriate sample dilution buffer.
- Make sure all necessary equipment is switched on and set at the appropriate temperature.

ASSAY PROTOCOL

Reagent preparation

Lysis Buffer: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4ºC.
Reaction Mix: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4ºC. In case of precipitate formation, dissolved in a 70ºC water bath.

Sample preparation

Note: If you cannot perform the assay at the same time, we suggest that you freeze the sample at -80°C. The sample will be stable for at least one month. If determine the amount of MDA remaining in sample Based on protein concentration, please determination the concentration of protein firstly.

Tissue homogenates - For general information, hemolysis blood may affect the result, so you should rinse the tissues with ice-cold PBS (0.01 M, pH=7.4) to remove excess blood thoroughly. Tissue pieces should be weighed and then minced to small pieces which will be homogenized on ice in MDA Lysis Buffer (the volume depends on the weight of the tissue, 10 mL Lysis Buffer would be appropriate for 1 g tissue pieces). To further break the cells, you can sonicate the suspension with an ultrasonic cell disrupter. The homogenates are then centrifuged (13,000 X g, 10 min.) to get the supernatant.

Cell culture and Bacterium – Harvest the necessary amount of cells (adherent/suspension cells) or bacterium for each assay. For adherent cells, do not harvest using proteolytic enzymes; rather use a rubber policeman. Wash cells or bacterium with cold PBS. Resuspend cells or bacterium in ice-cold Lysis Buffer (1×10^6 cells or bacterium can be homogenized on ice in 200 µL Lysis Buffer). Homogenize quickly by pipetting up and down a few times. Centrifuge for 10 minutes at 4ºC, 13,000 X g, in a cold microcentrifuge to get the supernatant.

Plasma, Serum and Urine (and other biological fluids) - Tested directly by adding samples to the microcentrifuge tubes. However, to find the optimal values and ensure your readings will fall within the standard values, we recommend performing several dilutions of the samples.

Assay procedure

1. Preparation of the MDA Standards: Dilute 100 µl of 1 mM Standard with 0.9 ml of ddH_2O to obtain a 100 µM Standard. Dilute100 µM Standard solution with ddH_2O as described in the table in a microplate or microcentrifuge tubes:

Standard	100 µM Standard (µL)	ddH2O (µL)	Final Concentration of Standard (µM)
1	0	300	0
2	3	297	1
3	6	294	2
4	15	285	5
5	30	270	10
6	75	225	25
7	150	150	50
8	300	0	100

2. Prepare the sample as suggested and add 100 µl to a microcentrifuge tube.
3. Add 300 µl of Reaction Mix to each 100 µl sample and Standard solution.
4. Mix by inversion and incubate 30 minutes at 95^{o}C.
5. Then cool to room temperature in an ice bath for 10 minutes.
6. Centrifuge for 10 minutes at 25^{o}C at 10000x g.
7. Transfer 200 µl aliquot of the supernatant into a 96 well plate for analysis.
8. Measure the absorbance at 532 nm and 600 nm.

DATA ANALYSIS

Calculation of results

Calculate the change in measurement between A532 and A600 and make a standard curve (Figure 1):

$\Delta A_{standard} = A_{standard}532 - A_{standard}600$

$\Delta A_{sample} = A_{sample}532 - A_{sample}600$

Determine the amount of MDA (D) in the Colorimetric Reaction mixture using the MDA standard curve.

a. Determine the amount of MDA remaining in the Plasma, Serum and Urine (and other biological fluids).

MDA Concentration (nmol/mL)= $(V_R \times D) / V_S$

b. Determine the amount of MDA remaining in Tissues, Cells and bacterium Based on protein concentration.

MDA Concentration (nmol/mg)= $(V_R \times D) / (V_S \times C_P)$

c. Determine the amount of MDA remaining in Tissues Based on Tissue Weigh.

MDA Concentration (nmol/g)= $(V_R \times D) / (W \times V_S / V_T)$

D = The amount of MDA in the Colorimetric Reaction, μM;
V_R = Total volume of reaction system, 4×10^{-4}L;
V_S = Volume (mL) added into the wells, 0.1 mL;
C_P = Protein Concentration of sample;
W= Weigh of sample (g);
V_T= The total volume of sample (mL);

Typical data

Typical standard curve - data provided for demonstration purposes only. A new standard curve must be generated for each assay performed.

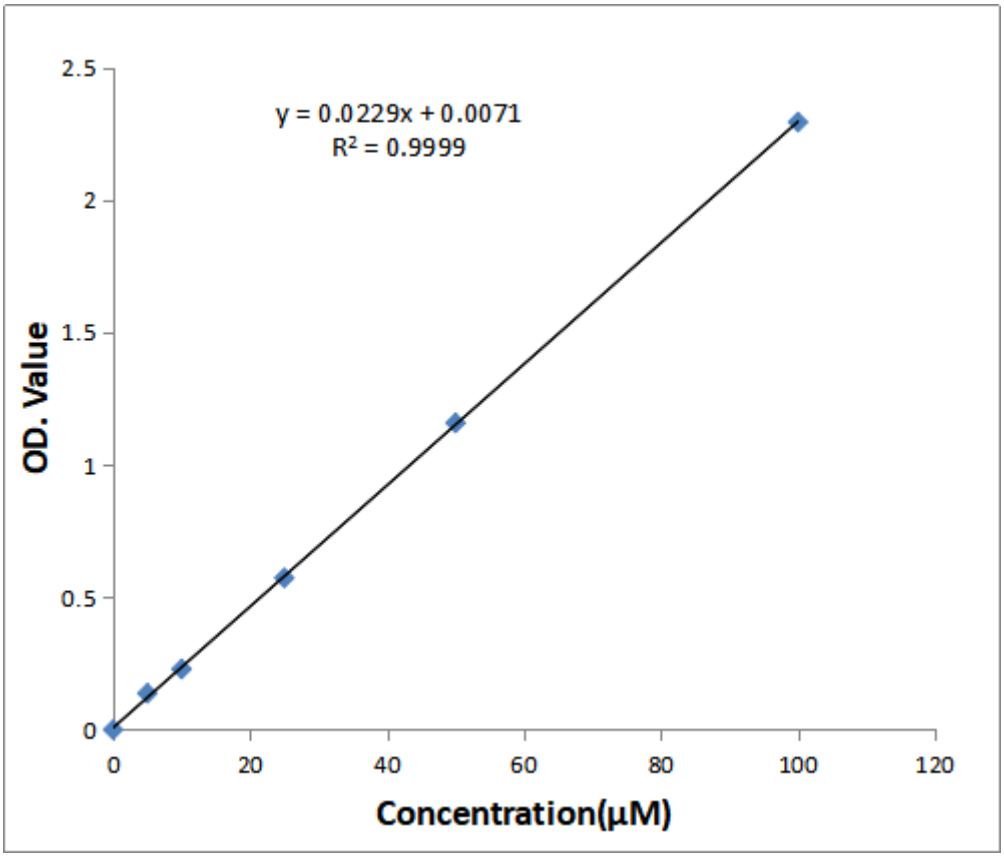

Figure 1 Typical MDA standard calibration curve.