CheKine™ Pro Malondialdehyde (MDA) Fluorometric Assay Kit

Cat #: KTB9050 Size: 48 T/48 S 96 T/96 S

	Malondialdehyde (MDA) Fluorometric Assay Kit		
REF	**Cat #:** KTB9050	LOT	**Lot #:** Refer to product label
	Detection range: 0.04-10 μM		**Sensitivity:** 0.04 μM
	Applicable samples: Animal and Plant Tissues, Cells, Serum, Plasma		
	Fluorescence Excitation/ Emission: Ex/Em=520/550 nm		
	Storage: Stored at 4℃ for 6 months, protected from light		

Assay Principle

Malondialdehyde (MDA) is an organic compound formed after decomposition of lipid peroxides. Mda is used to measure lipid oxidation levels and has been widely used in studies of oxidative stress and iron death. CheKine™ Pro Malondialdehyde (MDA) Fluorometric Assay Kit can be used to detect biological samples such as animal tissue, cells, serum, plasma, saliva. In the kit, MDA can be condensed with thiobarbituricacid (TBA) to form TBA complex under acidic and high temperature environment, and its fluorescence intensity can be measured to detect MDA in samples..

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer Ⅰ	60 mL	120 mL	4℃
Extraction Buffer Ⅱ	24 mL	48 mL	4℃
Reagent Ⅰ	300 μL	600 μL	4℃, protected from light
Reagent Ⅱ	52 mL	104 mL	4℃
Reagent Ⅲ	36 mL	72 mL	4℃, protected from light
Reagent Ⅳ	Powder×1 vial	Powder×1 vial	4℃, protected from light
Reagent Ⅴ	20 mL	40 mL	4℃
Standard (4.05 mmol/mL)	400 μL	400 μL	4℃, protected from light

Note: Before formal testing, it is recommended to select 2-3 samples with large expected differences for pre-experiment.

Materials Required but Not Supplied

- Fluorescence microplate reader (Ex/Em=520/550 nm)
- 96-well black plate, precision pipettes, disposable pipette tips, 1.5 mL EP tube
- Incubator, ice maker, freezing centrifuge
- Deionized water
- Homogenizer or mortar (for tissue samples)

Reagent Preparation

Extraction Buffer Ⅰ : Ready to use as supplied; Equilibrate to room temperature before use; Store at 4℃.

Extraction Buffer Ⅱ: Ready to use as supplied; Store at 4℃.

Reagent Ⅰ: Ready to use as supplied; Equilibrate to room temperature before use; Store at 4℃, protected from light.

Reagent Ⅱ: Ready to use as supplied; Equilibrate to room temperature before use; Store at 4℃.

Reagent Ⅲ: Ready to use as supplied; Equilibrate to room temperature before use; Store at 4℃, protected from light.

Working Reagent Ⅳ: Prepared before use. Transfer all Reagent Ⅱ to Reagent Ⅳ and dissolve fully. The remaining reagent can also be stored at -20℃ and protected from light after aliquoting to avoid repeated freezing and thawing.

Reagent Ⅴ: Ready to use as supplied; Equilibrate to room temperature before use; Store at 4℃.

Working Reagent Ⅰ : For each well, prepare 600 μL Working Reagent Ⅰ which is freshly prepared. Mix evenly according to the ratio of Reagent Ⅲ: Working Reagent Ⅳ=200 μL: 400 μL.

Working Reagent Ⅱ: For each well, prepare 600 μL Working Reagent Ⅱ which is freshly prepared. Mix evenly according to the ratio of Reagent Ⅲ: Reagent Ⅴ=200 μL: 400 μL.

Standard (4.05 mmol/mL): Ready to use as supplied; Equilibrate to room temperature before use; Store at 4℃, protected from light.

Note: Reagent Ⅰ, Reagent Ⅲ, Reagent Ⅳ and Standard has a pungent odor, so it is recommended to experiment in a fume hood.

Standard preparation:

100 nmol/mL Standard: Prepare 1 mmol/mL Standard by diluting 20 μL 4.05 mmol/mL Standard into 61 μL deionized water. Prepare 10 μmol/mL Standard by diluting 10 μL 1 mmol/mL Standard into 990 μL deionized water. Prepare 100 nmol/mL Standard by diluting 10 μL 10 μmol/mL Standard into 990 μL deionized water. Using 100 nmol/mL Standard, prepare standard curve dilution as described in the table:

Num.	Standard Volume (μL)	Deionized water (μL)	Concentration (nmol/mL)
Std.1	100	900	10
Std.2	80	920	8
Std.3	60	940	6
Std.4	40	960	4
Std.5	20	980	2
Std.6	10	990	1
Std.7	5	995	0.5
Blank	0	1,000	0

Notes: Always prepare fresh Standards per use; Diluted Std. solution is unstable and must be used within 4 h.

Sample Preparation

Note: We recommend that you use fresh samples. If not assayed immediately, samples can be stored at -80℃ for one month.When measuring, the temperature and time of thawing should be controlled. When thawing at room temperature, the sample should be thawed within 4 h.

1. Tissues: Weigh 0.1 g tissue, add 1 mL Extraction Buffer Ⅰ and homogenize or mortar on ice. Centrifuge at 10,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.
2. Cells: Collect 5×10^6 cells into the centrifuge tube, wash cells or bacteria with cold PBS, discard the supernatant after centrifugation; add 0.4 mL Extraction Buffer Ⅱ. After mixing, the cells were placed on ice for lysis for 5 min, mixed once every 2 min, and placed directly on ice to be tested.
3. Plasma, Serum or other Liquid samples: Test directly.

Note: If the protein concentration of the sample is need to determined, it is recommended to use Abbkine Cat #: KTD3001 Protein Quantification Kit (BCA Assay) to measure the protein concentration of the sample.

Assay Procedure

1. Preheat the fluorescence microplate reader for more than 30 min, and adjust the excitation wavelength to 520 nm and the emission wavelength to 550 nm.
2. Sample measurement. (The following operations are operated in the 1.5 mL EP tube)

Reagent	Blank Well (μL)	Standard Well (μL)	Test Well (μL)	Control Well (μL)
Sample	0	0	200	200
Standard	0	200	0	0
Deionized water	200	0	0	0
Reagent Ⅰ	2	2	2	2
Working Reagent Ⅰ	600	600	600	0
Working Reagent Ⅱ	0	0	0	600

3. Mix well, incubate in water bath for 50 min at 95℃. Remove and cool under running water, centrifuge at 10,000 g for 10 min, take 200 μL of supernatant in a 96-well black plate. Detect the fluorescence value RFU at Ex/Em=535/590 nm. The Blank Well is recorded as RFU_{Blank}, the standard Well is marked as $RFU_{Standard}$, the Test Well is marked as RFU_{Test}., the Control Well is marked as $RFU_{Control}$. Finally calculate $\Delta RFU_{Test}=RFU_{Test}-RFU_{Control}$, $\Delta RFU_{Standard}=RFU_{Standard}-RFU_{Blank}$.

Note: The Blank Well and the Standard Well only need to be done 1-2 times. If the sample does not have hemolysis and lipemia, the Control Well can be detected, and the Blank Well can be used to replace the Control Well. In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples to be diluted into different concentrations. According to the results of the pre-experiment, combined with the linear range of this kit: 0.04-10 μM, the appropriate dilution ratio was selected for sample detection.

Data Analysis

Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

1. Drawing of standard curve

With the concentration of the standard solution as the x-axis and the $\Delta RFU_{Standard}$ as the y-axis, draw the standard curve and obtain the standard equation. The determination of ΔRFU_{Test} is brought into the equation to get x (nmol/mL).

2. Calculation of the MDA content

(1) Calculated by protein concentration

MDA (nmol/mg prot)**=x÷Cpr**

(2) Calculated by fresh weight of samples

MDA (nmol/g fresh weight)=x÷(W÷V_1)**=x÷W**

(3) Calculated by bacteria or cells

MDA (nmol/10^4 cell)=x÷(n÷V_2)×F**=0.4x÷n**

(4) Calculated by volume of liquid samples

MDA (nmol/mL)**=x**

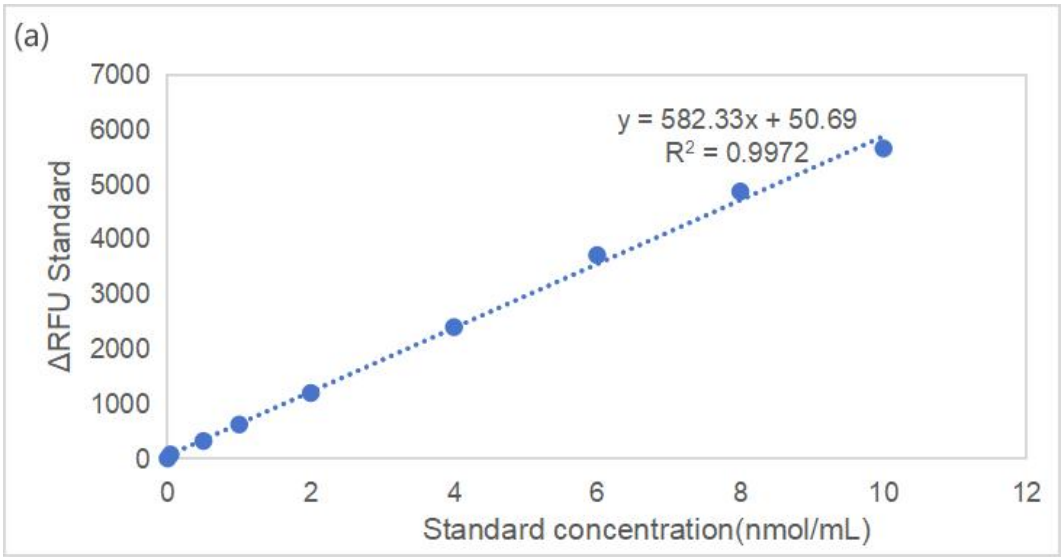
V_1: Added the Extraction Buffer Ⅰ volume, 1 mL; V_2: Added the Extraction Buffer Ⅱ volume, 0.4 mL; Cpr: sample protein concentration, mg/mL; W: Sample weight, g; n: Number of cells, calculated in units of ten thousand.

Precautions

1. If there is flocculent in the homogenate of the tissue sample to be measured, the supernatant can be obtained by multiple centrifuges to avoid the instability of the measured value and the large difference of multiple pores.
2. A new standard curve needs to be established for each test.
3. If the RFU_{Test} is low or the ΔRFU_{Test} is negative, the sample size can be increased or the reaction time can be extended, but it should not exceed 100 min.
4. If no value is found in the sample, it is recommended to take a fresh sample and test it again.

Typical Data

The following data are for reference only. And the experimenters need to test the samples according to their own experiments.



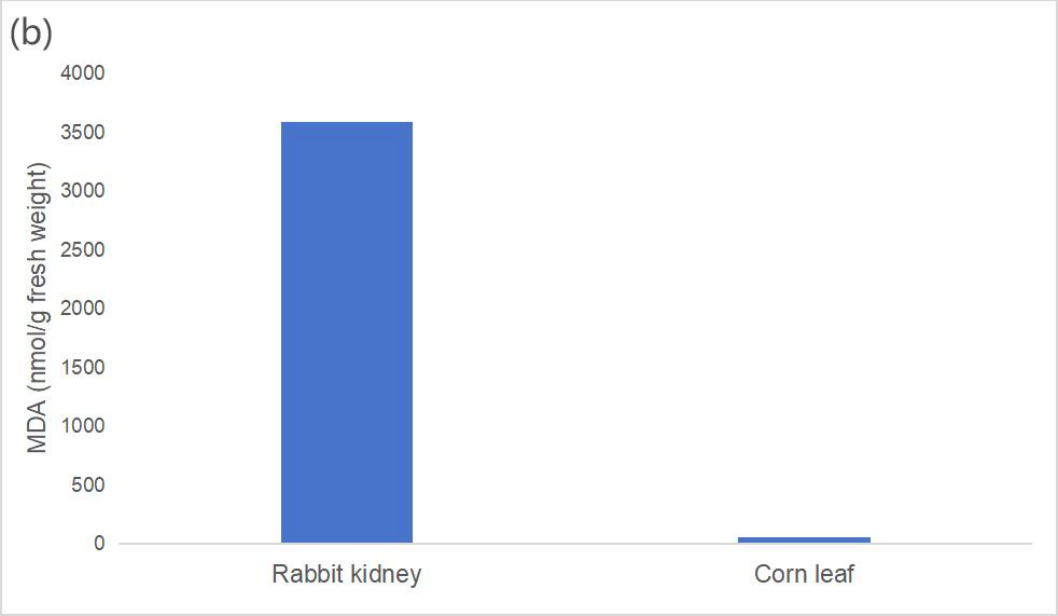


Figure 1. (a) MDA standard curve. (b) Determination of MDA content in rabbit kidney and corn leaf by this kit.

Recommended Products

Catalog No.	Product Name
KTB9300	CheKine™ Pro Glucose Fluorometric Activity Assay Kit
KTB9041	CheKine™ Pro Hydrogen Peroxide (H_2O_2) Fluorometric Assay Kit
KTB9042	CheKine™ Pro Catalase (CAT) Fluorometric Activity Assay Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes. For your safety and health, please wear a lab coat and disposable gloves.