（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-K027-M

Specification：48T(32 samples)/96T(80 samples)

Measuring instrument：Microplate reader（525-535 nm）

Detection range：0.17-50 nmol/mL

Elabscience® Malondialdehyde (MDA) Colorimetric Assay Kit (Plant Samples)

This manual must be read attentively and completely before using this product.

If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623

Tell: 1-832-243-6086

Fax: 1-832-243-6017

Email: techsupport@elabscience.com

Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

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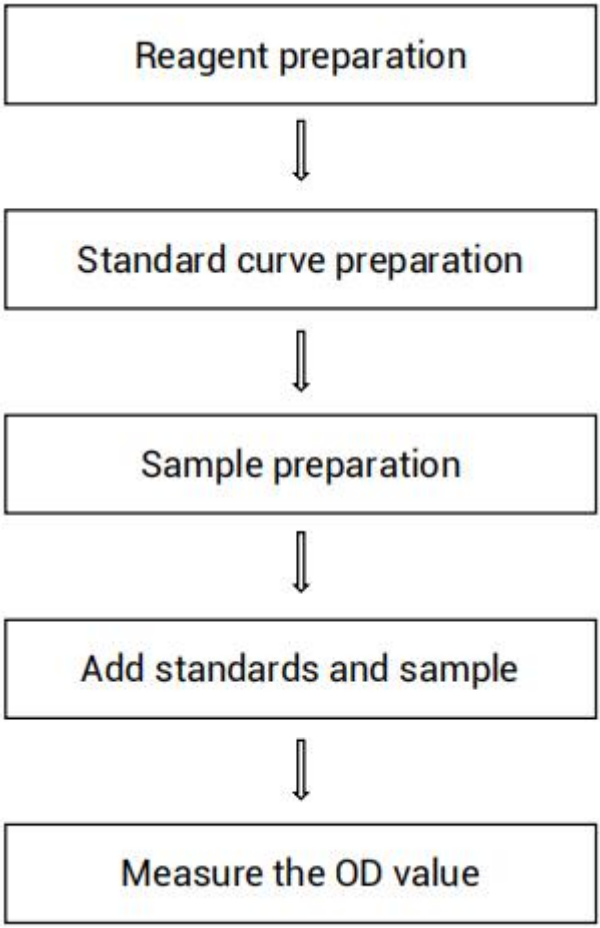
Assay summary

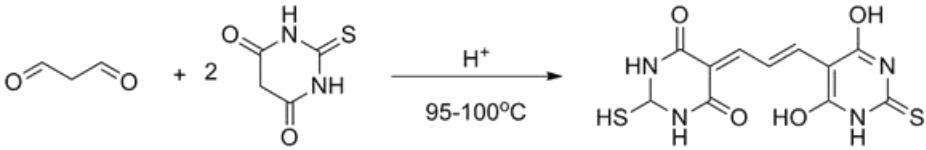


Intended use

This kit can be used to measure the MDA content in plant tissue samples.

Detection principle

MDA in the catabolite of lipid peroxide can react with thiobarbituric acid (TBA) and produce red compound, which has a maximum absorption peak at 532 nm.



Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Clarificant	1.5 mL × 1 vial	1.5 mL × 2 vials	2-8 ℃ , 12 months
Reagent 2	Acid Reagent	45 mL × 1 vial	45 mL × 2 vials	2-8℃, 12 months
Reagent 3	Chromogenic Agent	15 mL × 1 vial	30 mL × 1 vial	2-8℃, 12 months shading light
Reagent 4	200 nmol/mL Standard	5 mL × 1 vial	5 mL × 1 vial	2-8℃, 12 months
Reagent 5	10×Concentrated Extracting Solution	40 mL × 1 vial	40 mL × 1 vial	2-8℃, 12 months
	Microplate	48 wells	96 wells	No requirement
	Plate Sealer	2 pieces		
	Sample Layout Sheet	1 piece		

Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other. For a small volume of reagents, please centrifuge

before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

Microplate reader (525-535 nm), Micropipettor, Vortex mixer, Incubator, Centrifuge

Reagents:

Double distilled water, Absolute ethanol

Reagent preparation

① Equilibrate all reagents to room temperature before use.

② Clarificant will be solidification when the weather is cold, please warm it in 37℃ water bath until the liquid turns to transparent before the experiment.

③ The preparation of working solution：
For each tube, prepare 615 μL of working solution (mix well 15 μL of clarificant, 450 μL of acid reagent and 150 μL of chromogenic agent). The working solution should be prepared on spot.

④ The preparation of 10×concentrated extraction working solution：
For each tube, prepare 180 μL of 10×concentrated extraction working solution (mix well 18 μL of 10×concentrated extraction solution and 162 μL of double-distilled water).

⑤ The preparation of standard curve：

Always prepare a fresh set of standards. Discard working standard dilutions after use.

Dilute 200 nmol/mL standard solution with absolute ethanol to a serial concentration. The recommended dilution gradient is as follows: 0, 5, 10, 15, 20, 30, 40, 50 nmol/mL. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (nmol/mL)	**0**	**5**	**10**	**15**	**20**	**30**	**40**	**50**
200 nmol/mL (μL)	0	25	50	75	100	150	200	250
Absolute ethanol (μL)	1000	975	950	925	900	850	800	750

Sample preparation

Sample preparation

Tissue sample:

① Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).

② Wash tissue in cold PBS (0.01 M, pH 7.4), and cut into pieces.

③ Homogenize 20 mg tissue in 180 μL 10×concentrated extraction working solution with a dounce homogenizer at 4℃.

④ Centrifuge at 10000×g for 15 min at 4℃ to remove insoluble material. Collect supernatant and keep it on ice for detection.

The key points of the assay

① It is recommended to fasten the glass tube mouth with preservative film and make a small hole in the film.
② The temperature of water-bath and the time of incubation should be stabilized (95-100℃, 40 min). Cool the tubes with running water immediately once the incubation finished.
③ The supernatant for assay should not contain sediment, otherwise it will affect the OD values. It is recommended to use a pipette to take the supernatant.
④ The sampling quantity of blank tube, standard tube and sample tube can be increased to 100 μL if the MDA content of samples is low.
⑤ Accurately take 250 μL reaction solution into the 96-wells and without bubble.

Operating steps

① Standard tube: add 100 μL of standard solution with different concentrations to the 1.5 mL EP tubes.
Sample tube: add 100 μL of sample to the 1.5 mL EP tubes.
② Add 600 μL of working solution into each tube.
③ Mix fully with a vortex mixer. Tighten the tubes with preservative film and make a hole in the film. Incubate the tubes in 95℃ water bath for 40 min.
④ Cool the tubes to room temperature with running water. Centrifuge at 2000 g for 10 min.
⑤ Take 250μL the supernatant of each tube to the microplate with a micropipette.
⑥ Measure the OD values of each well at 532 nm with microplate reader.

Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean OD value of the blank (Standard # ①) from all standard readings. This is the absoluted OD value.

3. Plot the standard curve by using absoluted OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (**y = ax + b**) with graph software (or EXCEL).

The sample:

$$\underset{(\text{nmol/g})}{\text{MDA}} = (\Delta A_{532}\text{-b}) \div a \times f \div \frac{m}{V}$$

[Note]

ΔA_{532}: Absoluted OD, $OD_{Sample} - OD_{Blank}$.

f: Dilution factor of sample before test.

m: The weight of plant tissue, g.

V: The volume of added 10×concentrated extraction working solution, mL.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three carrot samples were assayed in replicates of 20 to determine precision within an assay (CV = Coefficient of Variation).

Parameters	Sample 1	Sample 2	Sample 3
Mean (nmol/mL)	0.50	15.50	35.60
%CV	4.9	4.5	4.4

Intra-assay Precision

Three carrot samples were assayed 17 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (nmol/mL)	0.50	15.50	35.60
%CV	6.2	6.7	6.3

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 100%.

	Standard 1	Standard 2	Standard 3
Expected Conc. (nmol/mL)	8.5	18.3	33.5
Observed Conc. (nmol/mL)	8.6	18.1	33.5
Recovery rate (%)	101	99	100

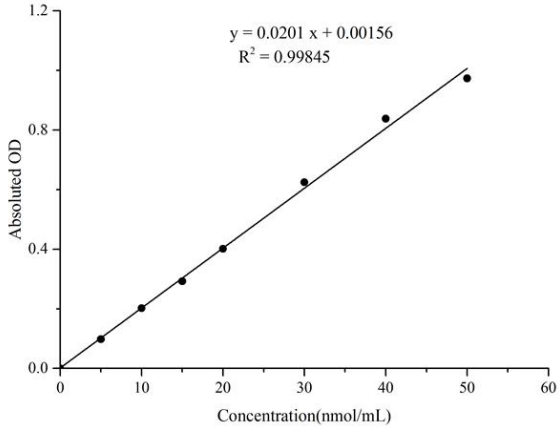
Sensitivity

The analytical sensitivity of the assay is 0.17 nmol/mL MDA. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

2. Standard curve:

As the OD value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), so the standard curve and data are provided as below for reference only：

Concentration (nmol/mL)	**0**	**5**	**10**	**10**	**20**	**30**	**40**	**50**
Average OD	0.040	0.138	0.242	0.333	0.442	0.665	0.878	1.014
Absoluted OD	0	0.098	0.202	0.293	0.402	0.625	0.838	0.974



Appendix Π Example Analysis

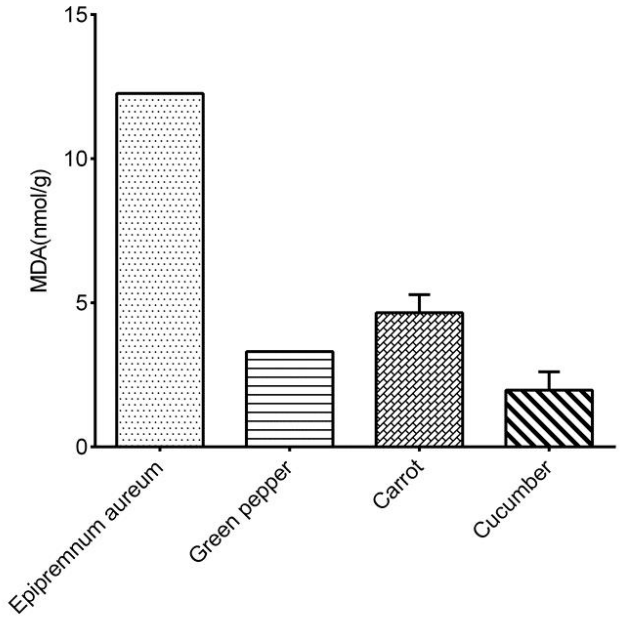
Example analysis：

Take 0.1 g of carrot and cut into pieces, add 0.9 mL of 10×concentrated extraction working solution, then homogenize (60 Hz, 90 s, 5 times) the sample in ice water bath, centrifuge at 1000 g for 15 min, then take 100 μL of the supernatant and carry the assay according to the operation table. The results are as follows:

standard curve: y = 0.0201 x + 0.00156, the average OD value of the sample is 0.052, the average OD value of the blank is 0.040, and the calculation result is:

$$\underset{(\text{nmol/g})}{\text{MDA}} = (0.052 - 0.040 - 0.00156) \div 0.0201 \times 1 \div \frac{0.1}{0.9} = 4.67 \text{ nmol/g}$$

Detect epipremnum aureum leaf， green pepper, daucus carota, cucumis sativus according to the protocol, the result is as follows：



Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and not be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.