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

Catalog No：E-BC-K027-S

Specification：50Assays (36 samples)/ 100Assays (86 samples)

Measuring instrument：Spectrophotometer（532 nm）

Detection range：0.17-120 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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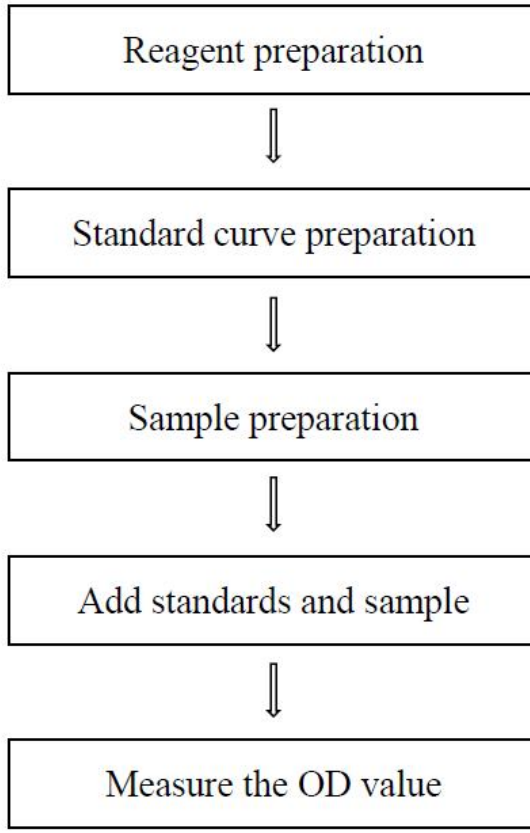
Assay summary

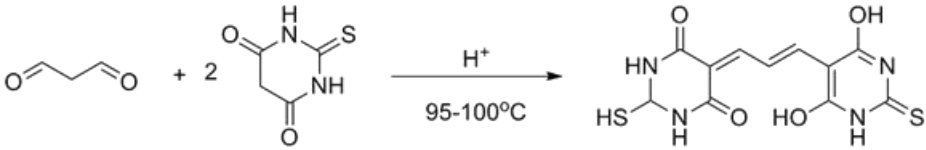


Intended use

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 (50 assays)	Size 2 (100 assays)	Storage
Reagent 1	Clarificant	3 mL × 1 vial	6 mL× 1 vial	2-8℃, 12 months
Reagent 2	Acid Reagent	45 mL × 2 vials	60 mL× 3 vials	2-8℃, 12 months
Reagent 3	Chromogenic Agent	30 mL × 1 vial	60 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 Extraction Solution	60 mL × 1 vial	60 mL×1 vial	2-8℃, 12 months

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:

Spectrophotometer (532 nm), Micropipettor, Vortex mixer, Centrifuge, Water bath, Incubator

Reagents:

Double distilled water, Absolute ethanol

Reagent preparation

① Equilibrate all the 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 2000 μL of working solution (mix well 48.8 uL of clarificant, 1463.4 uL of acid reagent and 487.8 uL of chromogenic agent).

③ The preparation of 10×concentrated extraction working solution：

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

④ 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 (self-prepared) to a serial concentration. The recommended dilution gradient is as follows: 0, 15, 35, 55, 75, 100, 120 nmol/mL. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦
Concentration (nmol/mL)	**0**	**15**	**35**	**55**	**75**	**100**	**120**
200 nmol/mL (μL)	0	75	175	275	375	500	600
Absolute ethanol (μL)	1000	925	825	725	625	500	400

Sample preparation

① 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.

② Dilution of sample

The recommended dilution factor for different samples is as follows (for reference only):

Sample type	Dilution factor
10% Epipremnum aureum tissue homogenate	1
10% Green pepper tissue homogenate	1
10% Eggplant tissue homogenate	1

Note: The diluent is 10×concentrated extraction working solution. For the dilution of other sample types, please do pretest to confirm the dilution factor

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 Water-bath temperature (95-100℃) and incubation time (40 min) should be stabilized. 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.

Operating steps

① Standard tube: add 100 μL of standard solution with different concentrations to the 5 mL EP tubes.

Sample tube: add 100 μL of sample to the 5 mL EP tubes.

② Add 2000 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 and take the supernatant.

⑤ Set the spectrophotometer to zero with double distilled water and measure the OD value of each tube at 532 nm with a 1 cm optical path cuvette.

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 epipremnum aureum tissue 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)	3.50	35.40	84.20
%CV	2.5	2.1	2.0

Intra-assay Precision

Three epipremnum aureum tissue samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (nmol/mL)	3.50	35.40	84.20
%CV	5.8	6.2	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 95%.

	Standard 1	Standard 2	Standard 3
Expected Conc. (nmol/mL)	28	65	96
Observed Conc. (nmol/mL)	27.7	61.1	88.3
Recovery rate (%)	99	94	92

Sensitivity

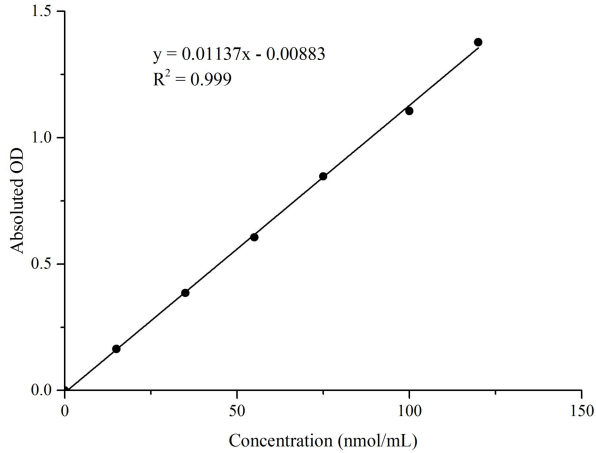
The analytical sensitivity of the assay is 0.17 nmol/mL MDA. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding

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**	**15**	**35**	**55**	**75**	**100**	**200**
Average OD	0.004	0.168	0.389	0.609	0.85	1.109	1.381
Absoluted OD	0	0.164	0.385	0.605	0.846	1.105	1.377



Appendix II Example Analysis

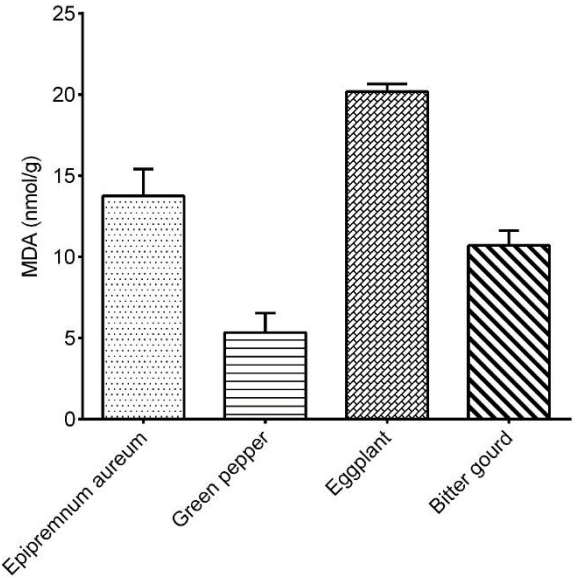
Example analysis:

Take 10% Epipremnum aureum tissue homogenate and carry the assay according to the operation steps. The results are as follows:

standard curve: y = 0.0114 x - 0.0041, the average OD value of the blank is 0.008, the average OD value of the blank is 0.021, and the calculation result is:

$$\underset{(\text{nmol/g})}{\text{MDA}} = (0.021 - 0.008 + 0.0041) \div 0.0114 \div \frac{0.1}{0.9} = 13.5 \text{ nmol/g}$$

Detect 10% Epipremnum aureum tissue homogenate, 10% green pepper tissue homogenate, 10% eggplant tissue homogenate and 10% bitter gourd tissue homogenate 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.