Malondialdehyde (MDA) Colorimetric Assay Kit (Plant Samples)

Catalog No: E-BC-K027-S

Method: Colorimetric method

Specification: 100Assays (Can detect 86 samples without duplication)

Measuring instrument: Spectrophotometer

Sensitivity: 0.17 nmol/mL

Detection range: 0.17-120 nmol/mL

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

General information

▲Intended use

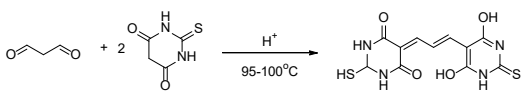
This kit can be used to measure the malondialdehyde (MDA) content in plant tissue samples.

▲Background

The body produce oxygen free radicals through the enzyme system and non-enzyme system, which can attack unsaturated fatty acid on biofilm and lead to lipid peroxidation and form lipid peroxide, such as aldehyde group (MDA), keto-, hydroxyl, carbonyl, etc. Oxygen free radicals cause cell damage not only by peroxidation of polyunsaturated fatty acids in biofilm, but also by decomposition products of lipid hydroperoxide. Detection of the MDA content can reflect the level of lipid peroxidation in cells and reflect level of cellular damage indirectly.

▲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	Specification	Storage
Reagent 1	Clarificant	6 mL× 1 vial	2-8℃ , 6 months
Reagent 2	Acid Reagent	60 mL× 3 vials	2-8℃ , 6 months
Reagent 3	Chromogenic Agent	60 mL× 1 vial	2-8℃ , 6 months, shading light
Reagent 4	200 nmol/mL Standard	5 mL× 1 vial	2-8℃ , 6 months
Reagent 5	10×Concentrated Extraction Solution	60 mL× 1 vial	2-8℃ , 6 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.			

▲Materials prepared by users

Instruments

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

Consumptive material

Tips (10 μL, 200 μL, 1000 μL), EP tubes (1.5 mL, 2 mL)

Reagents

Double distilled water, Absolute ethanol

▲Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the skin and clothing. Wash immediately with plenty of water if touching it carelessly. All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

▲Precautions

Before the experiment, please read the instructions carefully, and wear gloves and work clothes.

▲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.

Pre-assay preparation

▲Reagent preparation

1. Reagent 1 will be solidification when the weather is cold, please warm it in 37°C water bath until the liquid turns to transparent before the experiment
2. Preparation of working solution:
   Mix the reagent 1, reagent 2, reagent 3 at a ratio of 0.1: 3: 1 fully. Prepare the fresh solution before use.
3. Preparation of reagent 5 working solution:
   Dilute the 10×concentrated extraction solution with double distilled water for 10 times before use. Prepare the fresh solution before use.

▲Sample preparation

Plant tissue: weigh the tissue accurately and add reagent 5 working solution at the volume of reagent 5 working solution (mL): the weight of the tissue (g) =9:1, then homogenize on ice. The tissue homogenate is centrifuged at 10000 g for 15 min. Collect the supernatant for detect.

▲Dilution of sample

It is recommended to take 2~3 samples with expected large difference to do pre-experiment before formal experiment and dilute the sample according to the result of the pre-experiment and the detection range (0.17-120 nmol/mL).

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
10% Bitter gourd tissue homogenate	1

Note: The diluent is reagent 5 working solution.

Assay protocol	
Ambient temperature	25-30°C
Optimum detection wavelength	532 nm

Instructions for the use of transferpettor:

(1) Equilibrate the pipette tip in that reagent before pipetting each reagent.

(2) Don't add the liquid outside the tips into the reaction system when pipetting each reagent.

Assay protocol

▲Operating steps

1. The preparation of standard curve

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.

2. The measurement of samples

1) Standard tube: Take 100 μL of standard solution with different concentrations to the 5 mL EP tubes.
Sample tube: Take 100 μL of sample to the 5 mL EP tubes.

2) Add 2000 μL of working solution into the tubes of Step 1.

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°C 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.

▲Operation table

	Standard tube	Sample tube
Standard solution with different concentration (μL)	100	
Sample (μL)		100
Working solution (μL)	2000	2000
Mix fully with a vortex mixer. Tighten the tubes with preservative film and make a hole in the film. Incubate the tubes in 95°C water bath for 40 min. Cool the tubes to room temperature with running water. Centrifuge at 2000 g for 10 min and take the supernatant. 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

Plot the standard curve by using OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve with graph software (or EXCEL). The concentration of the sample can be calculated according to the formula based on the OD value of sample.

The standard curve is: $y = ax + b$.

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

Note:

y: $OD_{Standard} - OD_{Blank}$. ($OD_{Blank}$ is the OD value when the standard concentration is 0).

x: The concentration of standard.

a: The slope of standard curve.

b: The intercept of standard curve.

ΔA_{532}: $OD_{Sample} - OD_{Blank}$.

f: Dilution factor of sample before test.

m: The weight of plant tissue , g.

V: The volume of added reagent 5 working solution, mL.

▲Notes

1. This kit is for research use only.
2. Instructions should be followed strictly, changes of operation may result in unreliable results.
3. The validity of kit is 6 months.
4. Do not use components from different batches of kit.

Appendix I Performance characteristics

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Detection range	0.17-120 nmol/mL	Average intra-assay CV (%)	2.2
Sensitivity	0.17 nmol/mL	Average inter-assay CV (%)	6.1
Average recovery rate (%)	95		

▲Example analysis

For epipremnum aureum sample, take 0.1 mL of 10% epipremnum aureum tissue homogenate and carry the assay according to the operation table.

The results are as follows:

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

MDA content (nmol/g)=(0.021-0.008+0.0041)÷0.0114÷(0.1÷0.9)

=13.5 nmol/g