Malondialdehyde (MDA) Colorimetric Assay Kit (Plant Samples)

Catalog No: E-BC-K027-M

Method: Colorimetric method

Specification: 96T (Can detect 80 samples without duplication)

Instrument: Microplate reader

Sensitivity: 0.17 nmol/mL

Detection range: 0.17-50 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

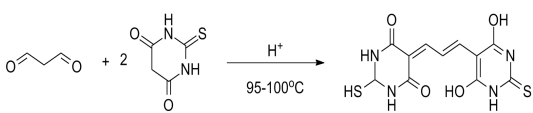
This kit can be used to measure the Malondialdehyde (MDA) content in plant tissue samples.

▲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	1.5 mL × 2 vials	2-8℃ , 6 months
Reagent 2	Acid Reagent	45 mL × 2 vials	2-8℃ , 6 months
Reagent 3	Chromogenic Agent	30 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 Extracting Solution	40 mL × 1 vial	2-8℃ , 6 months
	Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	
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

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

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. 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.

Pre-assay preparation

▲Reagent preparation

1. Reagent 1 will be solidification when the weather is cold, please warm it in 37℃ 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. Prepare the fresh solution before use.
3. Preparation of reagent 5 working solution:
   Dilute the 10×concentrated extracting solution with double distilled water for 10 times before use.

▲Sample preparation

Weigh the tissue accurately and cut into pieces, add reagent 5 at the ratio of reagent 5 (mL): the weight of the tissue (g) =9:1, then homogenize the sample in ice water bath. The tissue homogenate is centrifuged at 10000 g for 15 min. Take the supernatant for detection.

▲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 pre-experiment and the detection range (0.17-50 nmol/mL).

Assay protocol	
Ambient temperature	25-30℃
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

▲Plate set up

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S1	S9	S17	S25	S33	S41	S49	S57	S65	S73
B	B	B	S2	S10	S18	S26	S34	S42	S50	S58	S66	S74
C	C	C	S3	S11	S19	S27	S35	S43	S51	S59	S67	S75
D	D	D	S4	S12	S20	S28	S36	S44	S52	S60	S68	S76
E	E	E	S5	S13	S21	S29	S37	S45	S53	S61	S69	S77
F	F	F	S6	S14	S22	S30	S38	S46	S54	S62	S70	S78
G	G	G	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79
H	H	H	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80

Note: A-H, standard wells; S1-S80, sample wells.

▲Operating steps

1. The preparation of standard curve

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.

2. The measurement of samples

(1) Standard tube: Take 100 μL of standard solution with different concentrations to the 1.5 mL EP tubes.

Sample tube: Take 100 μL of sample to the sample wells.

(2) Add 600 μL of working solution into the wells 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℃ water bath for 40 min.

(4) Cool the tubes to room temperature with running water. Centrifuge at 2000 g for 10 min.

(5) Take 250 μL of supernatant to microplate and measure the OD value at 532 nm with microplate reader.

▲Operation table

	Standard tube	Sample tube
Standards with different concentrations (μL)	100	
Sample (μL)		100
Working solution (μL)	600	600
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. Cool the tubes to room temperature with running water. Centrifuge at 2000 g for 10 min. Take 250 μL of supernatant to microplate and measure the OD value at 532 nm with microplate reader.		

▲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. If the sample have been diluted, the concentration calculated from the standard curve must be multiplied by the dilution factor. The actual concentration is the calculated concentration multiplied by dilution factor.

The standard curve is: y= ax + b.

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

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}: 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 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	0.17-50 nmol/mL	Average intra-assay CV (%)	4.6
Sensitivity	0.17 nmol/mL	Average inter-assay CV (%)	6.4
Average recovery rate (%)	100		

▲Example analysis

Take 0.1 g of carrot and cut into pieces, add 0.9 mL of reagent 5 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:

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