TECHNICAL MANUAL

Lipid Peroxidation (MDA) Colorimetric Assay Kit (Plant Samples)

- **SKU CODE:** MAES0039
- **SIZE:** 48 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Intended use

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

2. Detection principle

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

3. 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°C, 12 months
Reagent 2	Acid Reagent	45 mL × 1 vial	45 mL × 2 vials	2-8°C, 12 months
Reagent 3	Chromogenic Agent	15 mL × 1 vial	30 mL × 1 vial	2-8°C, 12 months, protect from light
Reagent 4	200 nmol/mL Standard	5 mL × 1 vial	5 mL × 1 vial	2-8°C, 12 months
Reagent 5	10× Concentrated Extracting Solution	40 mL × 1 vial	40 mL × 1 vial	2-8°C, 12 months
	Microplate	48 wells	96 wells	No requirement
	Plate Sealer	2 pieces	2 pieces	

4. Materials prepared by users

Instruments:

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

Reagents:

Double distilled water, Absolute ethanol

5. Reagent preparation

1. Equilibrate all reagents to room temperature before use.
2. Clarificant will solidify in cold weather. Please warm it in a 37°C water bath until the liquid turns transparent before the experiment.
3. **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 immediately before use.
4. **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. **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.

6. Sample preparation

1. **Tissue sample preparation:** 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°C.
4. Centrifuge at 10,000×g for 15 min at 4°C to remove insoluble material. Collect supernatant and keep it on ice for detection.

7. 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°C, 40 min). Cool the tubes with running water immediately once the incubation is 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 without bubbles.

8. Operating steps

1. **Sample addition:** 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°C water bath for 40 min.
4. Cool the tubes to room temperature with running water. Centrifuge at 2,000×g for 10 min.
5. Take 250 µL 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.

9. 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 absolute OD value.

3. Plot the standard curve by using absolute 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:

MDA (nmol/g) = (ΔA_532 - b) ÷ a × f ÷ m × V

[Note]

ΔA_532: Absolute 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.

10. Appendix I Performance Characteristics

Parameter:

Intra-assay Precision

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

Intra-assay Precision

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

Inter-assay Precision

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

Inter-assay Precision

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 in parallel to get the average recovery rate of 100%.

Recovery

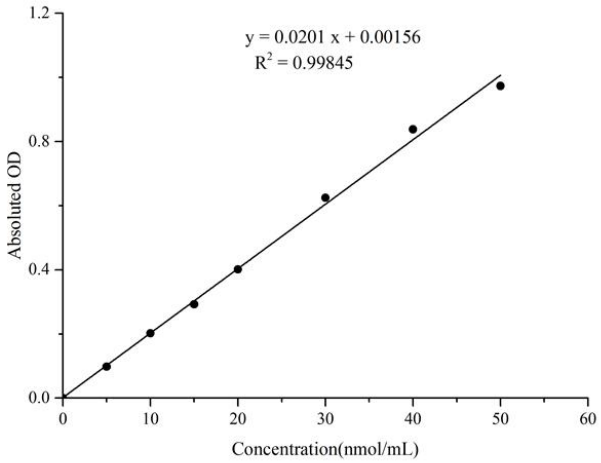
	Standard 1	Standard 2	Standard 3
Expected Conc. (nmol/mL)	8.5	18.3	33.5
Observed Conc. (nmol/mL)	8.6	18.1	33.5
Recovery rate (%)	101	99	100

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.

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), the standard curve and data are provided below for reference only:



Standard curve data

Concentration (nmol/mL)	Average OD	Absolute OD
0	0.040	0
5	0.138	0.098
10	0.242	0.202
15	0.333	0.293
20	0.442	0.402
30	0.665	0.625
40	0.878	0.838
50	1.014	0.974

11. Appendix II Example Analysis

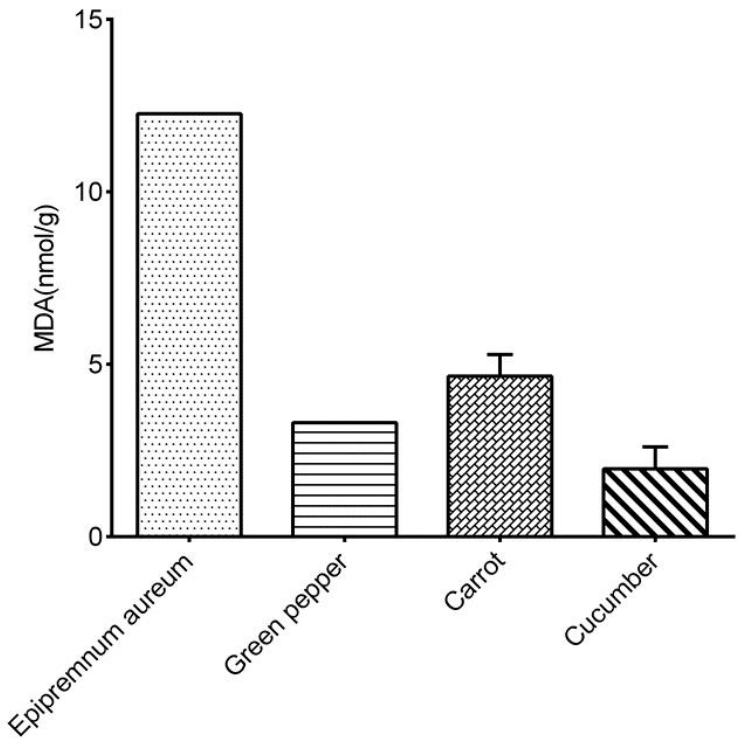
Example analysis:

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 1,000×g for 15 min, then take 100 μL of the supernatant and carry out the assay according to the operation table. The results are as follows:

Standard curve: y = 0.0201x + 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:

MDA (nmol/g) = (0.052 - 0.040 - 0.00156) ÷ 0.0201 × 1 ÷ 0.1 × 0.9 = 4.67 nmol/g

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



12. Statement

1. This assay kit is for Research Use Only. Assay Genie assumes no responsibility for any problems or legal liabilities arising from the use of this kit for clinical diagnosis or any other purpose.

2. Please read the instructions carefully and calibrate the instruments before performing the experiments. Follow the instructions strictly throughout the procedure.
3. Appropriate protective measures must be taken, including wearing a lab coat and latex gloves.
4. If the concentration of the substance falls outside the detection range, perform an additional dilution or concentration step on the sample.
5. It is recommended to perform a pre-test if your sample type is not listed in the instruction manual.
6. The experimental results are closely related to reagent quality, operator technique, environmental conditions, and other factors. Assay Genie guarantees the quality of the kits only and is not responsible for sample consumption resulting from use of the assay kits. It is advisable to estimate the expected sample usage and reserve sufficient samples before starting the experiment.

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.