Malondialdehyde Assay Kit

Catalog No.: abx294022

Size: 96 tests

Detection Range: 0.17 nmol/ml – 50 nmol/ml

Sensitivity: 0.17 nmol/ml

Storage: Store all components at 4°C. Store the Chromogenic Reagent in the dark.

Application: For detection and quantification of Malondialdehyde content in plant tissue homogenates.

Introduction

Abbexa's Malondialdehyde Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Malondialdehyde content. Malondialdehyde reacts with thiobarbituric acid (TBA) to produce a red-colored compound with an absorbance maximum at 532 nm. The intensity of the color is proportional to the Malondialdehyde content, which can then be calculated.

Kit components

1. 96-well microplate
2. Clarifying Reagent: 2 × 1.5 ml
3. Acid Reagent: 2 × 45 ml
4. Chromogenic Reagent: 30 ml
5. Standard (200 nmol/ml): 5 ml
6. Extraction Solution (10X): 40 ml
7. Plate sealer: 2

Materials required but not provided

1. Microplate reader (532 nm)
2. Double distilled water
3. PBS (0.01 M, pH 7.4)
4. Ethanol (anhydrous)
5. Pipette and pipette tips
6. 1.5 ml microcentrifuge tubes
7. Centrifuge
8. Vortex mixer
9. Dounce homogenizer
10. Incubator

For Reference Only

Protocol

A. Preparation of samples and reagents

1. Samples

Isolate the test samples soon after collecting and analyze immediately or aliquot and store at -20°C or -80°C for long-term storage. Avoid multiple freeze-thaw cycles.

The following sample preparation method is intended as a guide and may be adjusted as required depending on the specific samples used.

- **Tissue Homogenates:** Carefully weigh 20 mg of tissue and wash in cold PBS (0.01 M, pH 7.4). Homogenize the tissue in 180 µl Extraction Working Solution with a Dounce homogenizer at 4°C. Centrifuge at 10,000 × g for 15 minutes at 4°C, then collect the supernatant and store on ice for detection.

It is recommended to carry out a preliminary experiment to determine the optimal dilution factor of samples before carrying out the formal experiment. Dilute samples into different concentrations with Extraction Working Solution, then carry out the assay procedure. The recommended dilution factor for different samples is as follows (for reference only):

Note:

- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.
- Lysis buffers may interfere with the kit. It is therefore recommended to use mechanical lysis methods for tissue homogenates.

2. Reagents

- **Clarifying Reagent:** Preheat to 37°C in a water bath, the liquid should be transparent before use.
- **Reaction Working Solution:** For each sample/standard to be assayed, mix 15 µl Clarifying Reagent, 450 µl Acid Reagent, and 150 µl Chromogenic Reagent (total 615 µl working solution per sample/standard). Prepare fresh before assay and use immediately.
- **Extraction Working Solution:** For each sample/standard to be assayed, mix 18 µl Extraction Solution (10X) with 162 µl double distilled water (10-fold dilution).
- **Standards:** Prepare standard dilutions as summarized in the following table:

Standard concentration (nmol/ml)	0	5	10	15	20	30	40	50
200 nmol/ml Standard (µl)	0	25	50	75	100	150	200	250
Ethanol (anhydrous) (µl)	1000	975	950	925	900	850	800	750

For the blank, or 0 nmol/ml standard, use pure ethanol (anhydrous). The volume of each standard will be 1000 µl.

Note:

- Allow all reagents to equilibrate to room temperature before use.

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B. Assay Procedure

1. Label sterile microcentrifuge tubes for samples and standards.
2. Add 100 µl of sample to sample tubes.
3. Add 100 µl of standard dilutions to standard tubes.
4. Add 600 µl of Reaction Working Solution to all tubes and vortex to mix. It is recommended to seal the tubes with adhesive film and pierce a small hole in the film.
5. Incubate all tubes at 95°C in a water bath for 40 minutes.
6. Cool the tubes to room temperature with running water, then centrifuge at 2,000 × g for 10 minutes.
7. Transfer 250 µl of supernatant from each tube to the microplate for detection. Avoid foaming.
8. Measure the OD of each well with a microplate reader at 532 nm.

C. Calculation of Results

Plot the standard curve, using the OD of the standard dilutions (adjusted for the blank) on the y-axis, and their respective concentrations on the x-axis. The curve should form a straight line, described by the formula $y = ax + b$. Based on this curve, the concentration of Malondialdehyde in each sample well can be derived with the following formulae:

1. **Tissue samples:**

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

where:

ΔA_{532}	$OD_{Sample} - OD_{Blank}$
a	Gradient of the standard curve ($y = \mathbf{a}x + b$)
b	Y-intercept of the standard curve ($y = ax + \mathbf{b}$)
f	Sample dilution factor
m	Tissue sample weight (g)
V	Volume of Extraction Working Solution used (ml)

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