Lipid Peroxidation (MDA) Assay Kit (Plant Sample)

Introduction

Malondialdehyde (MDA) is one of the products of lipid peroxidation in organisms, and it is widely used as a detection indicator for lipid peroxidation.

Lipid Peroxidation (MDA) Assay Kit (Plant Sample) is a kit for the detection of MDA in plant samples by thiobarbituric acid (TBA). The detection principle is that MDA reacts with TBA to produce a red product that is specifically absorbed at 535 nm and can therefore be determined by colorimetry. At the same time, the reaction products can also be excited at 535 nm, resulting in a maximum emission wavelength of 553 nm, so fluorescence detection can also be performed.

This product is specifically optimized for plant tissue samples, making it highly suitable for detecting the level of lipid peroxidation in plants.

Components and Storage

Components \ Size	48 Assays	96 Assays	Storage
Acid Reagent	45 mL	2 x 45 mL	4°C
Chromogenic Reagent	15 mL	30 mL	4°C away from light
200 nmol/mL Standard	5 mL	5 mL	4°C
10X Extracting Solution	40 mL	40 mL	4°C
Shipping: Blue ice	Shelf life: 1 year		

Protocol

1. Preparation before the experiment

1) Equilibrate all reagents to room temperature before use.

2) Preparation of MDA working solution: Mixing Acid Reagent and Chromogenic Reagent at a 3:1 ratio to prepare the MDA working solution. The MDA working solution is stable for one week at 4°C away from light. But it is still recommended to prepare a fresh MDA working solution every time.

3) Preparation of 1X Extracting Solution: Dilute an appropriate amount of 10X Extracting Solution with ddH_2O to make 1X Extracting Solution as needed for the experiment.

2. **Plant Sample Preparation:** For plant tissues, make 10% homogenization according to the conventional operation. For example, homogenize 0.1 g of tissue with 1 mL 1X Extracting Solution on ice. Then centrifuge at 4°C, 10000-12000 g for 10 min to obtain the supernatant for detection.

3. **Dilution of MDA Standard:** Gradient dilution of 200 nmol/mL Standard with ddH_2O for subsequent preparation of the standard curve. Always prepare a fresh set of standards for every use.

#	200 nmol/mL Standard (μL)	ddH_2O	Final concentration (nmol/mL)
1	0	1000	0
2	25	975	5
3	50	950	10
4	75	925	15
5	100	900	20
6	150	850	30
7	200	800	40
8	250	750	50

4. **Detection:**

1) Prepare the detection system in centrifuge tubes or other suitable containers referring to the following table:

	Blank	**Set of standards**	**Sample**
1X Extracting Solution	0.1 mL	-	-
Serial of MDA standard	-	0.1 mL	-
Sample	-	-	0.1 mL
MDA working solution	0.6 mL	0.6 mL	0.6 mL

2) After mixing the reaction system, heat tubes in a 95-100°C metal bath or water bath for 40 min. The most accurate way to heat is to use a thermal cycler with a heated lid that can accommodate 0.5 mL PCR tubes.

***Note:** When using a metal bath or water bath, be sure to prevent the liquid from spilling out. When using a metal bath, you can press down on the cap with a weight. When using a water bath, you can seal the opening tube with parafilm and prick small holes in the parafilm.

3) Then cool tubes to room temperature under running water.

4) Centrifuge at 2000 g for 10 min at room temperature. Take 250 μL of supernatant from each sample into a

96-well plate. Measure the absorbance at 532 nm by a microplate reader.

5. Results Analysis

1) First, subtract the blank reading from all readings. Use the corrected readings to generate the standard curve: y = ax + b.

2) Calculate the MDA content in the sample using the following formula:

$$MDA\ (nmol/g) = (A_{532} - b) \times d \times V / (a \times m)$$

A_{532}: Corrected reading of the sample.

d: sample dilution factor before addition to the reaction system.

V: Volume of 1X Extracting Solution added during sample processing, mL.

m: Weight of the tissue sample, g.

Note

1. For your safety and health, please wear lab coats and gloves during the experiment.
2. For research use only. Not to be used in clinical diagnostic or clinical trials.

APExBIO Technology
www.apexbt.com
7505 Fannin street, Suite 410, Houston, TX 77054.
Tel: +1-832-696-8203 | Fax: +1-832-641-3177 | Email: info@apexbt.com