# Lipid Peroxidation (MDA) Assay Kit

## 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.

The Lipid Peroxidation (MDA) Assay Kit is a kit for the detection of MDA in samples such as tissues, cell lysates, plasma, serum, and urine 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.

The kit provides antioxidants that can inhibit the production of new MDA in the sample during the detection process, making the detection more accurate. At the same time, the kit can detect MDA as low as 1 μM and has a good linear relationship in 1-200 μM.

## Components and Storage

| Components | K2167-100 T | K2167-500 T |
|---|---|---|
| TBA | 25 mg | 125 mg |
| TBA Preparation Buffer | 7 mL | 35 mL |
| TBA Dilution Buffer | 15 mL | 75 mL |
| Antioxidant | 300 μL | 1.5 mL |
| MDA Standard (1 mM) | 200 μL | 1 mL |
| Store the kit at -20°C, stable for 1 year. TBA and the antioxidant should be stored away from light. | | |

## Protocol

1. **Sample Preparation:**

   1) Plasma, serum, or urine samples can be used directly for detection.

   2) For cells, after harvesting cells, add 100 μL of WB and IP cell lysis buffer (Cat. No. K1123) per $10^6$ cells and lyse cells on ice. Then 4°C, 10000-12000 g centrifuge for 10 min to obtain the supernatant for detection.

   3) For tissues, make 10% homogenization according to the conventional operation and take the

supernatant by centrifugation for detection. For example, 0.1 g of tissue is taken and homogenized with 1 mL PBS or lysis buffer on ice. Then 4°C, 10000-12000 g centrifuge for 10 min to obtain the supernatant for detection.

***Note:**

a) Cell lysis or tissue homogenization needs to be performed on ice.

b) For some samples, where centrifugation does not yield a clarified supernatant, the supernatant can be filtered through a 0.22 μm filter to obtain a clarified supernatant.

c) For cell or tissue samples, the protein concentration can be detected with a BCA concentration determination kit after preparation, so as to facilitate the subsequent calculation of the MDA content in cells or tissues per unit weight of protein.

2. **Preparation of the TBA stock solution:** Weigh an appropriate amount of TBA and prepare a 0.37% concentration of TBA stock solution with TBA Preparation Buffer. For example, dissolve 25 mg of TBA in 6.76 mL of TBA Preparation Buffer. TBA is difficult to dissolve and needs to be heated to 70°C and vigorously vortex to facilitate dissolution. The prepared stock solution can be stable for at least 3 months when stored at room temperature away from light.

3. **Preparation of the MDA working solution:** Refer to the following table to prepare the MDA working solution. The antioxidant needs to be warmed to room temperature before use and observed for crystallization. If there are crystals, it can be heated at 70°C until completely dissolved. Meanwhile, after the MDA working solution is configured, it also needs to be heated at 70°C and vortex vigorously to promote dissolution. if necessary, appropriate ultrasonication can be carried out. The MDA working solution should be completely dissolved before use. Always prepare a fresh MDA working solution for every use.

| **Samples** | **1** | **10** | **20** | **50** |
|---|---|---|---|---|
| TBA stock solution | 50 μL | 500 μL | 1 mL | 2.5 mL |
| TBA Dilution Buffer | 150 μL | 1.5 mL | 3 mL | 7.5 mL |
| Antioxidant | 3 μL | 30 μL | 60 μL | 150 μL |

4. **Dilution of MDA Standard:** Gradient dilution of MDA Standard (1 mM) with sterilized water for subsequent preparation of the standard curve. The concentration of the MDA Standard can be set to 1, 2, 5, 10, 20, 50, 100, 150, 200 μM. Always prepare a fresh set of standards for every use.

5. **Detection:**

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

| | Control | Set of standards | Sample |
|---|---|---|---|
| PBS or lysis buffer | 0.1 mL | - | - |
| Serial of MDA standard | - | 0.1 mL | - |
| Sample | - | - | 0.1 mL |
| MDA working solution | 0.2 mL | 0.2 mL | 0.2 mL |

2) After mixing the reaction system, heat it in a 100°C metal bath or boiling water bath for 15 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 a boiling 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 boiling water bath, you can seal the nozzle with parafilm and prick a small hole in the cap with a needle.

3) Then cool to room temperature in a water bath and centrifuge at 1000 g for 10 minutes at room temperature. Take 200 µL of supernatant from each sample into a 96-well plate. Measure the absorbance at 532 nm by a microplate reader.

4) For samples such as plasma, serum, or urine, the concentration of MDA in the sample can be calculated directly from the standard curve. For cell or tissue samples, the concentration of MDA in the test sample can be expressed in µmol/mg of protein if a protein quantification has been performed.

## Note

1. The kit has a good linearity between 1-200 µM, and if the concentration of MDA in the sample is too high or too low, dilute or concentrate the sample appropriately.
2. Aldehydes or high concentrations of soluble sugars (e.g., sucrose) can interfere with the reaction, and dual wavelength detection can be performed by setting 450 nm as the reference wavelength to eliminate the influence of such interferences on the reaction.
3. For your safety and health, please wear lab coats and gloves during the experiment.
4. For research use only. Not to be used in clinical diagnostic or clinical trials.

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