Malondialdehyde Microplate Assay Kit

Catalog # AS0011

Detection and Quantification of Lipid Peroxidation (MDA) Content in Urine, Serum, Plasma, Tissue extracts, Cell lysate, Cell culture media and Other biological fluids Samples.

This instruction must be read in its entirety before using this product.

For research use only, Not for use in diagnostic procedures.

Contact information:

Tel:+1 (301) 446-2499 Fax:+1 (301) 446-2413

Email:techcn@signalwayantibody.com Web:www.sabbiotech.com

I. INTRODUCTION

Quantification of lipid peroxidation is essential to assess oxidative stress in pathophysiological processes. Lipid peroxidation forms Malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE), as natural bi-products. Measuring the end products of lipid peroxidation is one of the most widely accepted assays for oxidative damage. MDAMicroplate Assay Kit provides a convenient tool for sensitive detection of the MDA in a variety of samples. The MDA in the sample is reacted with Thiobarbituric Acid (TBA) to generate the MDA-TBA adduct. The MDA-TBA adduct can be easily quantified colorimetrically (λ = 532 nm).

II.KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Dye Reagent	20 ml x 1	4 °C
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

Note:

Make sure Dye Reagent is completely dissolved, if not, you can put it in water bath of 70 °C and shake it occasionally.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 532 nm and 600 nm.
2. Distilled water
3. Pipettor
4. Pipette tips
5. Mortar
6. Ice
7. Centrifuge
8. Timer

IV. SAMPLE PREPARATION

1.For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 mlAssay buffer for 5 $\times 10^6$ cell or bacteria, sonicate (with power 20%, sonication 3s, intervation 10s,repeat 30 times); centrifuged at 8000g 4°C for 10minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

2.For tissue samples

Weigh 0.1 g tissue, homogenize with 1 mlAssay buffer on ice, centrifuged at 8000g 4°C for 10minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

3.For serum or plasma samples

Detect directly.

V. ASSAY PROCEDURE

Add following reagents in the microcentrifuge tubes:

Reagent	Sample
Sample	100 µl
Dye Reagent	200 µl
Mix,put it in the oven,90 °C for 30 minutes, then put it on ice, centrifuged at 10000g, 25°C for 10 minutes. Add the supernatant into the microplate.	
The supernatant	200 µl
Record absorbance measured at 532nm, 600nm.	

VI. CALCULATION

1. According to the volume of serum or plasma

$MDA\ (nmol/ml) = [(OD_{532}-OD_{600})\times V_{Total} / (\varepsilon\times d)\times 10^9] / V_{Sample}$

$=32.25\times(OD_{532}-OD_{600})$

2. According to the protein concentration of sample

$MDA\ (nmol/mg) =[(OD_{532}- OD_{600})\times V_{Total} / (\varepsilon\times d) \times 10^9] / (C_{Protein}\times V_{Sample})$

$=32.25\times(OD_{532}- OD_{600})/ C_{Protein}$

3. According to the weight of sample

$MDA\ (nmol/g) = [(OD_{532}- OD_{600})\times V_{Total} / (\varepsilon\times d) \times 10^9] / (W \times V_{Sample}/ V_{Assay})$

$=32.25\times(OD_{532}- OD_{600}) /W$

4. According to the quantity of cells or bacteria

$MDA\ (nmol/10^4) = [(OD_{532}- OD_{600})\times V_{Total} / (\varepsilon\times d) \times 10^9] / (N\times V_{Sample}/ V_{Assay})$

$=32.25\times(OD_{532}- OD_{600}) / N$

ε: molar extinction coefficient of MDA,155×10^3L/mol/cm;

d: the well diameter of 96-Well microplate,0.6 cm;

$C_{Protein}$: the protein concentration, mg/ml;

W: theweight of sample, g;

V_{Total}: the total volume of the enzymatic reaction, 0.3 ml;

V_{Sample}: the volume of sample, 0.1 ml;

V_{Assay}: the volume of Assay buffer, 1 ml;

N: the quantity of cell or bacteria, $N\times10^4$.

VII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.sabbiotech.cn or contact us at techcn@signalwayantibody.com

VIII. NOTES