Malondialdehyde (MDA) Content Assay Kit

Note: It is necessary to predict 2-3 large difference samples before the formal determination.

Operation Equipment: Spectrophotometer/Microplate reader

Cat No: BC0025

Size: 100T/96S

Components:

Extraction reagent: Liquid 110 mL×1. Storage at 4℃.

Reagent I: Liquid 42 mL×1. Storage at 4℃. 你

Reagent II: Powder×2. Storage at 4℃.

MDA working reagent: add 20 mL Reagent I to Reagent II, dissolve (heat at 70℃ or with ultrasonic) and mix thoroughly. Storage at 4℃.

Reagent III: Liquid 12 mL×1. Storage at 4℃.

Note: The working solution for MDA detection is difficult to dissolve, which can be heated at 70℃ and vibrated violently to promote dissolution. Or by ultrasonic treatment to promote dissolution.

Product Description:

Lipid peroxide is produced by the action of oxygen free radicals on unsaturated fatty acid, then resolves to compounds, including malondialdehyde (MDA), the level of lipid peroxidation can be showed by detecting the level of MDA.

Under acidic and high temperature conditions, the brown red 3,5,5- three methyl sulfamethoxazole -2,4- two ketone was synthesized with MDA and thiobarbituric acid (TBA) taking place condensation reaction, and the largest absorption wavelength is 532 nm. The content of lipid peroxidation can be estimated after colorimetric. But the soluble sugar will disturb the detection, the production (color reaction of soluble sugar with TBA) have absorption wavelength in 450 nm and 532 nm. In this kit, the MDA content is calculated by the difference between the absorbance at 532 nm, 450 nm and 600 nm.

Because of sucrose in plant tissues and glucose in animal tissues, this kit have two computational formulas for sucrose and glucose. The two formulas are suit for fat.

Reagents and Equipment Required but Not Provided:

Spectrophotometer/microplate reader, water bath, centrifuge, transferpettor, micro glass cuvette/96 well flat-bottom plate, mortar/homogenizer, ice and distilled water.

Procedure:

I. Sample preparation

1. Bacteria or cells:

Collect bacteria or cells into the centrifuge tube. 5 million bacteria or cells could be mixed with 1 mL of Extraction reagent. Use ultrasonication to split bacteria and cells (placed on ice, ultrasonic power 200W,

ultrasonic time 3 seconds, interval 10 seconds, repeat for 30 times). Centrifuge at 8000 ×g for 10 minutes at 4℃ to remove insoluble materials, and take the supernatant on ice before testing.

2. Tissue sample:

0.1 g of tissue could be mixed with 1 mL of Extraction reagent and fully homogenized on ice bath. Then centrifuge at 8000 ×g for 10 minutes at 4℃ to remove insoluble materials, and take the supernatant on ice before testing.

3. Serum: Detect directly.

II. Determination procedure:

1. Preheat the spectrophotometer for 30 minutes, set zero with distilled water.
2. Add reagents with the following list:

Reagent (μL)	Test tube (T)	Blank tube(B)
MDA working reagent	300	300
Sample	100	-
Distilled water	-	100
Reagent III	100	100

The mixture would be incubated at 100℃ for 60 minutes (tightly close to prevent moisture loss), cooled on ice, and centrifuged at 10000 ×g for 10 minutes at room temperature to remove insoluble materials. Take 200μL of supernatant in micro glass cuvette/96 well flat-bottom plate, and measure the absorbance at 450 nm, 532 nm and 600 nm. ΔA450=A450(T)-A450(B), ΔA532=A532(T)-A532(B), ΔA600 =A600(T)-A600(B). Blank tube needs to test once or twice.

III. Calculation:

A 96 well plate

1. Tissue, bacteria or cultured cells

1) Protein concentration:

MDA (nmol/ mg prot)=(12.9×(ΔA532-ΔA600)-2.58×ΔA450)×Vrv÷(Cpr×Vs)

=5×(12.9 ×(ΔA532-ΔA600)-2.58×ΔA450)÷Cpr

2) Sample weight:

MDA (nmol/g weight)=(12.9 ×(ΔA532-ΔA600)-2.58×ΔA450)×Vrv÷(W×Vs÷Vsv)

=5×(12.9 ×(ΔA532-ΔA600)-2.58×ΔA450)÷W

3) Cell amount:

MDA (nmol/10^4cell)=(12.9 ×(ΔA532-ΔA600)-2.58×AΔ450)×Vrv÷(400×Vs÷Vsv)

=0.01×(12.9 ×(ΔA532-ΔA600)-2.58×ΔA450)

4) Serum (plasma) sample

MDA (nmol/ mL)=(12.9 ×(ΔA532-ΔA600)-2.58×ΔA450)×V÷Vs

=5×(12.9 ×(ΔA532-ΔA600)-2.58×ΔA450)

2. Plants tissue

1) Sample weight

MDA (nmol/ g weight)=(12.9 ×(ΔA532-ΔA600)-1.12×ΔA450)×Vrv÷(W×Vs÷Vsv)

=5×(12.9 ×(ΔA532-ΔA600)-1.12×ΔA450)÷W

2) Protein concentration:

MDA (nmol/ mg prot)=(12.9 ×(ΔA532-ΔA600)-1.12×ΔA450)×Vrv÷(Cpr×Vs)

=5×(12.9 ×(ΔA532-ΔA600)-1.12×ΔA450)÷Cpr

Vrv: Total reaction volume, 0.5 mL;

Vs: Sample volume, 0.1 mL;

Vsv: The volume of Extraction reagent, 1 mL;

Cpr: Sample protein concentration, mg/mL;

W: Sample weight, g;

500: Total number of bacteria and cells, 5 million.

B．Micro glass cuvette

1. Tissue, bacteria or cultured cells

1) Protein concentration:

MDA (nmol/ mg prot)=(6.45×(ΔA532-ΔA600)-1.29×ΔA450)×Vrv÷(Cpr×Vs)

=5×(6.45 ×(ΔA532-ΔA600)-1.29×ΔA450)÷Cpr

2) Sample weight:

MDA (nmol/g weight)=(6.45 ×(ΔA532-ΔA600)-1.29×ΔA450)×Vrv÷(W×Vs÷Vsv)

=5×(6.45 ×(ΔA532-ΔA600)-1.29×ΔA450)÷W

3) Cell amount:

MDA (nmol/10^4cell)=(6.45 ×(ΔA532-ΔA600)-1.29×ΔA450)×Vrv÷(400×Vs÷Vsv)

=0.01×(6.45 ×(ΔA532-ΔA600)-1.29×ΔA450)

4) Serum (plasma) sample:

MDA (nmol/ mL)=(6.45 ×(ΔA532-ΔA600)-1.29×ΔA450)×V÷Vs

=5×(6.45 ×(ΔA532-ΔA600)-1.29×ΔA450)

2. Plants tissue

A. Sample weight

MDA (nmol/ g weight)=(6.45 ×(ΔA532-ΔA600)-0.56×ΔA450)×Vrv÷(W×Vs÷Vsv)

=5×(6.45 ×(ΔA532-ΔA600)-0.56×ΔA450)÷W

B. Protein concentration

MDA (nmol/ mg prot)=(6.45×(ΔA532-ΔA600)-0.56×ΔA450)×Vrv÷(Cpr×Vs)

=5×(6.45 ×(ΔA532-ΔA600)-0.56×ΔA450)÷Cpr

Vrv: Total reaction volume, 0.5 mL;

Vs: Sample volume, 0.1 mL;

Vsv: Extraction volume, 1 mL;

Cpr: Sample protein concentration, mg/mL;

W: Sample weight, g;
500: Total number of bacteria and cells, 5 million.

Note:

If it is found that the absorbance value of the sample is too low, the boiling water bath time can be adjusted from 60 minutes to 90 minutes or longer. The detection of MDA in the same experiment needs to be extended to the same time to avoid errors.

Experimental example:

1.The plasma is taken and operated according to the determination steps. The absorbances are measured by 96 well plate. $\Delta A450 = A450_T - A450_B = 0.320-0.052 = 0.268$, $\Delta A532 = A532_T - A532_B = 0.168-0.046 = 0.122$, $\Delta A600 = A600_T - A600_B = 0.093-0.043 = 0.05$

MDA content (nmol/mL) = $5 \times (12.9 \times (\Delta A532 - \Delta A600) - 2.58 \times \Delta A450) = 1.1868$ nmol/mL.

2. 5 million HeLa cells are collected and treated with 1 mL of Extraction reagent. After centrifugation, the supernatant is determined according to the determination steps. The absorbances are measured by 96 well plate. $\Delta A450 = A450_T - A450_B = 0.097-0.052 = 0.045$, $\Delta A532 = A532_T - A532_B = 0.101-0.046 = 0.055$, $\Delta A600 = A600_T - A600_B = 0.043-0.043 = 0.0$

MDA content (nmol/10^4 cell) = $0.01 \times (12.9 \times (\Delta A532 - \Delta A600) - 2.58 \times \Delta A450) = 0.01 \times (12.9 \times (0.055-0) - 2.58 \times 0.045) = 0.0059$ nmol/10^4 cell

Recent product citations：

[1] QianYi Peng, YiMin Wang, CaiXia Chen, et al. Inhibiting the CD38/cADPR pathway protected rats against sepsis associated brain injury. Brain Research. January 2018;(IF2.929）

[2] Zhigang Chen, Qiaoling Yuan, Guangren Xu, et al. Effects of Quercetin on Proliferation and H2O2-Induced Apoptosis of Intestinal Porcine Enterocyte Cells. Molecules. 2018; (IF3.06)

[3] Huiwen Xiao, Yuan Li, Dan Luo, et al. Hydrogen-water ameliorates radiation-induced gastrointestinal toxicity via MyD88's effects on the gut microbiota. experimental and molecular medicine. January 2018;(IF4.743)

[4] Xuejuan Xia, Yuxiao Xing, Guannan Li, et al. Antioxidant activity of whole grain Qingke (Tibetan Hordeum vulgare L.) toward oxidative stress in d-galactose induced mouse model. Journal of Functional Foods. June 2018;(IF3.197)

[5] Qilong Wang, Guosheng Xiao, Guoliang Chen, et al. Toxic effect of microcystin-LR on blood vessel development. Toxicological & Environmental Chemistry. Feb 2019;(IF3.547)

[6] Zeyong Zhang, Huanhuan Liu, Ce Sun, et al. A C_2H_2 zinc-finger protein OsZFP213 interacts with OsMAPK3 to enhance salt tolerance in rice. Journal of Plant Physiology. October 2018;(IF2.825)

[7] Lijiao Gu, Hantao Wang, Hengling Wei, et al. Identification, Expression, and Functional Analysis of the Group IId WRKY Subfamily in Upland Cotton (Gossypium hirsutum L). Frontier in Immunology. November 2018;(IF4.259)

[8] Ping Shao, Pei Wang, Ben Niu, et al. Environmental stress stability of pectin-stabilized resveratrol liposomes with different degree of esterification. International Journal of Biological Macromolecules. November 2018;(IF4.784)

References：

[1] Spitz D R, Oberley L W. An assay for superoxide dismutase activity in mammalian tissue homogenates[J]. Analytical Biochemistry,1989

[2] Masayasu M, Hiroshi Y. A simplified assay method of superoxide dismutase activity for clinical use[J]. Clinica Chimica Acta.

Related products:

BC3590/BC3595	Hydrogen Peroxide (H_2O_2) Content Assay Kit
BC1090/BC1095	Xanthine Oxidase (XOD) Activity Assay Kit
BC0690/BC0695	Glucose Oxidase (GOD) Activity Assay Kit
BC1270/BC1275	Protein Carbonyl Content Assay Kit
BC1280/BC1285	Diamine Oxidase (DAO) Activity Assay Kit
BC1290/BC1295	Superoxide Anion Content Assay Kit