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 2-8℃.

Reagent I: Liquid 42 mL×1. Storage at 2-8℃.

Reagent II: Powder×2. Storage at 2-8℃.

MDA working reagent: Add 20 mL of Reagent I to each Reagent II, dissolve and mix thoroughly. Storage at 2-8℃ for one month. 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.

Reagent III: Liquid 12 mL×1. Storage at 2-8℃.

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 brownish red 3,5,5- three methyl sulfamethoxazole -2,4-two ketone is synthesized with MDA and thiobarbituric acid (TBA) taking place condensation reaction, and the largest absorption wavelength is 532 nm. After colorimetry, the MDA content in the sample can be estimated.

Reagents and Equipment Required but Not Provided:

Spectrophotometer/microplate reader, water bath, centrifuge, transferpettor, micro glass cuvette/96 well flat-bottom plate, mortar/homogenizer/cell ultrasonic crusher, 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.

4. Samples with high fat content:

Add 40 μL sample to 80 μL ethanol (dilute sample three times with ethanol), mix for 5 minutes. The appropriate dilution ratio of the sample was determined by pre-experiment. And replace 100μL distilled water of blank tube with 33μL distilled water and 67μL ethanol.

Note: The sample homogenate supernatants of the above 1, 2 and 3 numbers of the kit are also available for BC0090/BC0095 (Peroxidase), BC0170/BC0175 (Superoxide Dismutase), BC5160/BC5165 (Superoxide Dismutase), BC0200/BC0205 (Catalase), BC0680/BC0685 (L-Lactate Dehydrogenase) determinations.

II. Determination procedure:

1. Preheat the spectrophotometer/microplate reader for 30 minutes, set spectrophotometer to zero with distilled water.

2. Add reagents with the following list: (read the following notes firstly)

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 532 nm and 600 nm, ΔA532=A532(T)-A532(B), ΔA600 =A600(T)-A600(B), ΔA=ΔA532- ΔA600. Blank tube needs to test once or twice.

Note1: Replace 100μL distilled water of blank tube with 33μL distilled water and 67μL ethanol for Samples with high fat content;

Note2: Be careful during the reaction in a boiling water bath. It is recommended to use EP tubes with screw caps. Or use a needle to pierce a small hole on the lid of tube with snap cap to prevent the lid from bursting. The tube cap could be pressed by heavy objects if heated by using metal bath.

III. Calculation:

A. Micro glass cuvette

1) Protein concentration:

MDA content (nmol/mg prot)= [ΔA×Vrv÷（ε×d）×10^9]÷(Cpr×Vs) ×F =32.258×ΔA÷Cpr ×F

2) Sample weight:

MDA content (nmol/g weight)= [ΔA×Vrv÷（ε×d）×10^9]÷(W×Vs÷Vsv) ×F = 32.258×ΔA÷W×F

3) Cell amount:

MDA content (nmol/10^4cell)= [ΔA×Vrv÷（ε×d）×10^9] ÷(500×Vs÷Vsv) ×F =0.0645×ΔA×F

4) Serum or other liquid volume:

MDA content (nmol/mL)= [ΔA×Vrv÷（ε×d）×10^9]÷Vs×F =32.258×ΔA×F

Vrv: Total reaction volume, 5×10^{-4}L;

ε: Molar extinction coefficient, 1.55×10^5 L/mol/cm

d: light path of cuvette, 1cm

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.

F：dilution factor

B. 96 well plate

1) Protein concentration:

MDA content (nmol/ mg prot) =[ΔA×Vrv÷（ε×d）×10^9]÷(Cpr×Vs)×F= 53.763×ΔA÷Cpr×F

2) Sample weight:

MDA content (nmol/g weight)=[ΔA×Vrv÷（ε×d）×10^9]÷(W×Vs÷Vsv) ×F = 53.763×ΔA÷W×F

3) Cell amount:

MDA content (nmol/10^4cell)= [ΔA×Vrv÷（ε×d）×10^9] ÷(500×Vs÷Vsv) ×F =0.1075×ΔA×F

4) Serum or other liquid volume:

MDA content (nmol/mL)=[ΔA×Vrv÷（ε×d）×10^9]÷Vs×F =53.763×ΔA×F

Vrv: Total reaction volume, 5×10^{-4}L;

ε: Molar extinction coefficient, 1.55×10^5 L/mol/cm

d: light path of 96-well plate, 0.6cm

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.

F：dilution factor

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 horse plasma is taken and operated according to the determination steps. The absorbances are measured by 96 well plate. ΔA532=A532(T)-A532(B)=0.078-0.047=0.031, ΔA600 =A600(T)-A600(B) =0.053-0.043=0.01， ΔA=ΔA532-ΔA600=0.021. The result is calculated according to sample volume:
   MDA content (nmol/mL) =53.763×ΔA = 1.129nmol/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. ΔA532=A532(T)-A532(B)=0.101-0.045=0.055, ΔA600=A600(T)-A600(B) =0.043- 0.043=0 ， ΔA=ΔA532-ΔA600=0.055. The result is calculated according to cells number:
   MDA content (nmol/10^4 cell) =0.1075×ΔA =0.006 nmol/10^4 cell
3. 0.1g mouse liver is taken 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. ΔA532=A532(T)-A532(B)=0.196-0.047=0.149, ΔA600 =A600(T)-A600(B) =0.125-0.043 =0.082，ΔA=ΔA532-ΔA600=0.067. The result is calculated according to sample weight:
   MDA content (nmol/g weight) =53.763×ΔA ÷W =36.02 nmol/g weight

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)

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

BC1280/BC1285	Diamine Oxidase (DAO) Activity Assay Kit
BC1290/BC1295	Superoxide Anion Content Assay Kit