Malondialdehyde (MDA) Content Assay Kit

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

Operation Equipment: Spectrophotometer

Cat No: BC0020

Size: 50T/48S

Components:

Extraction reagent: Liquid 60 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, water bath, desk centrifuge, transferpettor, mortar/homogenizer/cell ultrasonic crusher, 1 mL glass cuvette, 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 supernatant on ice before testing.

2. Tissue:

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 200μL distilled water of blank tube with 66μL distilled water and 134μL 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 spectrophotometer for more than 30 minutes, set 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	600	600
Sample	200	-
Distilled water	-	200
Reagent III	200	200

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 supernatant in 1 mL glass cuvette 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 200μL distilled water of blank tube with 66μL distilled water and 134μL 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:

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 volume:

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

Vrv: Total reaction volume, 0.001L;
ε: Molar extinction coefficient, 1.55×10^5 L/mol/cm
d: light path of cuvette, 1cm
Vs: Sample volume, 0.2 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;
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.

Recent product citations:

[1] Lian Q, Liu W, Ma D, Liang Z, Tang Z, Cao J, He C, Xia D. Precisely Orientating Atomic Array in One-Dimension Tellurium Microneedles Enhances Intrinsic Piezoelectricity for an Efficient Piezo-Catalytic Sterilization. ACS Nano. 2023 May 9;17(9):8755-8766. doi: 10.1021/acsnano.3c02044. Epub 2023 Apr 18. PMID: 37070712.

[2] Jiang F, Jia K, Chen Y, Ji C, Chong X, Li Z, Zhao F, Bai Y, Ge S, Gao J, Zhang X, Li J, Shen L, Zhang C. ANO1-Mediated Inhibition of Cancer Ferroptosis Confers Immunotherapeutic Resistance through Recruiting Cancer-Associated Fibroblasts. Adv Sci (Weinh). 2023 Aug;10(24): e2300881. doi: 10.1002/advs.202300881. Epub 2023 Jun 21. PMID: 37341301; PMCID: PMC10460848.

[3] Zhang D, Liu J, Zhang Y, Wang H, Wei S, Zhang X, Zhang D, Ma H, Ding Q, Ma L. Morphophysiological, proteomic and metabolomic analyses reveal cadmium tolerance mechanism in common wheat (Triticum aestivum L.). J Hazard Mater. 2023 Mar 5; 445:130499. doi: 10.1016/j.jhazmat.2022.130499. Epub 2022 Nov 25. PMID: 36455318.

[4] Liu X, Cheng C, Min Y, Xie X, Muzahid ANM, Lv H, Tian H, Zhang C, Ye C, Cao S, Chen P, Zhong C, Li D. Increased ascorbic acid synthesis by overexpression of AcGGP3 ameliorates copper toxicity in kiwifruit. J Hazard Mater. 2023 Oct 15; 460:132393. doi: 10.1016/j.jhazmat.2023.132393.

[5] Tian D, Yu Y, Yu Y, Lu L, Tong D, Zhang W, Zhang X, Shi W, Liu G. Tris(2-chloroethyl) Phosphate Exerts Hepatotoxic Impacts on Zebrafish by Disrupting Hypothalamic-Pituitary-Thyroid and Gut-Liver Axes. Environ Sci Technol. 2023 Jun 20;57(24):9043-9054. doi: 10.1021/acs.est.3c01631. Epub 2023 Jun 5. PMID: 37276532.

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