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Malondialdehyde (MDA) Content Assay Kit

50T/48S

Catalogue Number:AK0662-50T-48S

Store all reagents at 4℃

Validity Period: six months

Operation Equipment: Spectrophotometer/Microplate reader

For samples:

Serum, plasma, cell, Bacteria, fungj, Tissue homogenate.

FOR RESEARCH USE ONLY！

NOT FOR THERAPEUTIC OR DIAGNOSTIC APPLICATIONS！

PLEASE READ THROUGH ENTIRE PROCEDURE BEFORE BEGINNING!

Malondialdehyde (MDA) Content Assay Kit

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

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 ofMDA.

Under acidic and high temperature conditions, the brown 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. 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 has two computational formulas for sucrose and glucose. The two formulas are suit for fat.

Components:

Extraction reagent: Liquid 60 mL×1. Storage at 4℃.

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

Reagent Ⅱ: Powder ×2. Storage at 4℃.

MDA working reagent: Add 20 mL of 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.

Reagents and Equipment Required but Not Provided:

Spectrophotometer, water bath, desk centrifuge, transferpettor,1 mL glass cuvette, 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 supernatant on ice before testing.

2. Tissue:

0.1 g oftissue 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 spectrophotometer for more than 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	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 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:

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×AΔ450)×Vrv÷(400×Vs÷Vsv)

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

4) Serum:

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

=5×(6.45×(ΔA532 -ΔA600)-1.29×ΔA450)

2. Plants tissue:

1) 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

2) 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, 1 mL;

Vs: Sample volume, 0.2 mL;

Vsv: Extraction volume, 1 mL;

Cpr: Sample protein concentration, mg/mL;

W: Sample weight, g;

400: 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.

Recent product citations：

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[8] Ying Zhao, Wengang Yu, Xiangyu Hu, et al. Physiological and transcriptomic analysis revealed the involvement of crucial factors in heat stress response of Rhododendron hainanense. Gene. June 2018;(IF2.638)

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of the Group IId WRKY Subfamily in Upland Cotton (Gossypium hirsutum L.). Frontier in Immunology. November 2018;(IF4.259)

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