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CheKine™ Plus Micro Malondialdehyde (MDA) Content Assay Kit

Cat #: KTB1052 Size: 48 T/48 S 96 T/96 S

	Plus Micro Malondialdehyde (MDA) Content Assay Kit		
REF	**Cat #:** KTB1052	LOT	**Lot #:** Refer to product label
	Applicable samples: Animal and Plant Tissues, Cells, Bacteria, Serum, Plasma, Urine, Hyperlipidemia Samples, and Lipid-containing Samples		
	Storage: Stored at 4℃ for 24 months, protected from light		

Assay Principle

Oxygen free radicals react on lipid unsaturated fatty acids to produce lipid peroxidation. The latter gradually breaks down into a series of complex compounds, including malondialdehyde (MDA). Lipid oxidation levels can be measured by detecting MDA levels. CheKine™ Plus Micro Malondialdehyde (MDA) Content Assay Kit provides a convenient tool for detection of the MDA present in a variety of samples.MDA can be condensed with thiobarbituric acid (TBA) under acidic and high temperature conditions to produce the Red MDA-TBA adduct with a maximum absorption wavelength of 532 nm. Meanwhile, the absorbance at 600 nm was measured to eliminate non-specific interference, and MDA was calculated by the difference between the absorbance at 532 nm and 600 nm. This kit incorporates novel stabilizers to enhance sensitivity and color development efficiency, making it more compatible with complex sample matrices.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	60 mL	120 mL	4℃
Reagent Ⅰ	12 mL	24 mL	4℃, protected from light
Reagent Ⅱ	6 mL	12 mL	4℃, protected from light
Reagent Ⅲ	3 mL	6 mL	4℃
Reagent Ⅳ	3 mL	6 mL	4℃, protected from light

Note: Before formal testing, it is recommended to select 2-3 samples with large expected differences for pre-experiment.

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 532 nm and 600 nm
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips
- Incubator, centrifuge

• Deionized water
• Homogenizer (for tissue samples), Ultrasonic disruptor

Reagent Preparation

Extraction Buffer: Ready to use as supplied. Keep on ice during the assay. Store at 4℃.

Reagent Ⅰ: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃, protected from light.

Reagent Ⅱ: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃, protected from light.

Reagent Ⅲ: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃. **Note: Reagent Ⅲ can solidify at 4°C, and must be allowed to equilibrate to room temperature and thoroughly mixed before use.**

Reagent Ⅳ: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃, protected from light.

Sample Preparation

1. Animal or Plant Tissues: Weigh about 0.1 g Tissue and add 1 mL Extraction Buffer, homogenize on ice. Centrifuge at 8,000 g for 10 min at 4℃, take the supernatant for further analysis.
2. Cells or Bacteria: Collect 5×10^6 cells (bacteria), and wash with cold PBS, discard the supernatant after centrifugation at 800 g for 2 min at 4℃. Add 0.5 mL Extraction Buffer to ultrasonically disrupt the cells or bacteria 5 min (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times). Centrifuge at 8,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.
3. Serum, Plasma and Urine: Tested directly. If necessary, it is recommended to dilute the sample at various concentrations before proceeding with the assay.
4. Hyperlipidemia or lipid-containing samples: Mix 40 μL of sample with 80 μL of anhydrous ethanol (i.e., dilute the sample 3-fold), vortex-shake for 5 minutes, and then proceed with the assay. Different high-fat samples may require different dilution ratios, a preliminary experiment can be conducted using various dilution factors to determine the optimal dilution ratio. Additionally, when performing the procedure, the deionized water in the blank tube should be replaced with a solution prepared from deionized water and anhydrous ethanol at a volume ratio of 66:134.

Note: We recommend that you use fresh samples. If not assayed immediately, samples can be stored at -80℃ for one month. When it is time to conduct this experiment, remove the sample from -80℃ and thaw it on ice. However, please note that this process may affect the stability of the sample, and the experimental results may be lower than expected. If the protein concentration of the sample is need to determined, it is recommended to use Abbkine Cat #: KTD3001 Protein Quantification Kit (BCA Assay) to measure the protein concentration of the sample.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength. Visible spectrophotometer was returned to zero with deionized water.
2. Add the following reagents to threaded EP tubes (secure the threaded cap to prevent water loss and avoid the cap bursting during heating):

Reagent	Blank Tube (μL)	Test Tube (μL)
Sample	0	100
Deionized Water	100	0
Reagent Ⅰ	200	200
Reagent Ⅱ	100	100
Reagent Ⅲ	50	50
Reagent Ⅳ	50	50

3. Mix well and incubate in water bath 30 min at 95℃. Then cool to room temperature in an ice bath. Centrifuge at 10,000 g for 10

min at room temperature (25℃).

4. Transfer 200 μL of the supernatant into a 96-well plate or microglass cuvette. Measure the absorbance at 532 nm and 600 nm. Calculate $\Delta A=A_{532}-A_{600}$. Calculate $\Delta\Delta A_{Test}=\Delta A_{Test}-\Delta A_{Blank}$ (Only one blank well needs to be detected).

Note: 1. In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples.

2. If $\Delta\Delta A_{Test}$ is greater than 1.6, the sample can be appropriately diluted with Extraction Buffer, the calculated result multiplied by the dilution factor. If $\Delta\Delta A_{Test}$ is less than 0.01, increase the sample quantity or reduce the volume of the Extraction Buffer appropriately.

3. When dispensing samples into the 96-well plate, bubbles may form; avoiding contact between the pipette tip's dispensing port and the well walls of the plate can significantly reduce bubble formation. The presence of a single small bubble does not affect the assay results; however, if multiple or larger bubbles are present, it is advisable to puncture them before proceeding with the assay to prevent any potential interference with the results.

Data Analysis

Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

A. 96-well plate calculation formula

1. Calculated by protein concentration

MDA (nmol/mg prot)=$[\Delta\Delta A_{Test}\times V_{Reaction\ Total}\div(\varepsilon\times d)\times10^9]\div(Cpr\times V_{Sample})\times n=\mathbf{64.52\times\Delta\Delta A_{Test}\div Cpr\times n}$

2. Calculated by fresh weight of samples

MDA (nmol/g)=$[\Delta\Delta A_{Test}\times V_{Reaction\ Total}\div(\varepsilon\times d)\times10^9]\div(W\times V_{Sample}\div V_{Sample\ Total})\times n=\mathbf{64.52\times\Delta\Delta A_{Test}\div W\times n}$

3. Calculated by number of cells or bacteria

MDA (nmol/10^4)=$[\Delta\Delta A_{Test}\times V_{Reaction\ Total}\div(\varepsilon\times d)\times10^9]\div(N\times V_{Sample}\div V_{Sample\ Total})\times n=\mathbf{32.26\times\Delta\Delta A_{Test}\div N\times n}$

4. Calculated by liquid volume

MDA (nmol/mL)=$[\Delta\Delta A_{Test}\times V_{Reaction\ Total}\div(\varepsilon\times d)\times10^9]\div V_{Sample}\times n=\mathbf{64.52\times\Delta\Delta A_{Test}\times n}$

Where: $V_{Reaction\ Total}$: total reaction volume, 5×10^{-4} L; ε: MDA molar extinction coefficient, 1.55×10^5 L/mol/cm; d: 96-well plate diameter, 0.5 cm; 10^9: 1 mol=1×10^9 nmol; Cpr: sample protein concentration, mg/mL; V_{sample}: sample volume added, 0.1 mL; W: sample weight, g; $V_{Sample\ Total}$: tissues Extraction Buffer volume added, 1 mL, cells or bacteria sample volume added, 0.5 mL; N: total number of cells or bacteria, use 10^4 as the unit (e.g., total number of cells: 5×10^6, N=500); n: the sample dilution factor.

B. Microglass cuvette calculation formula

The optical diameter d:0.5 cm in the above calculation formula can be adjusted to d:1 cm for calculation.

Recommended Products

Catalog No.	Product Name
KTB1200	CheKine™ Micro Protein Carbonyl Assay Kit
KTB1220	CheKine™ Micro Diamine Oxidase (DAO) Activity Assay Kit
KTB1310	CheKine™ Micro Glucose Oxidase Activity (GOD) Assay Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes. For your safety and health, please wear a lab coat and disposable gloves.