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CheKine™ Micro Lipid Peroxidation (MDA) Assay kit

Cat #: KTB1050-EN Size: 48 T/96 T

	Micro Lipid Peroxidation (MDA) Assay kit		
REF	**Cat #:** KTB1050-EN	LOT	**Lot #:** Refer to product label
	Applicable samples: Serum, Plasma, Urine, Animal and Plant Tissues, Cells, Bacteria		
	Storage: Stored at 4℃ for 6 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. Lipid peroxidation may contribute to many diseases, including atherosclerosis, diabetes, and Alzheimer's disease. CheKine™ Micro Lipid Peroxidation (MDA) Assay kit provides a convenient tool for detection of the malondialdehyde (MDA) present in a variety of samples. Malondialdehyde (MDA) can be condensed with thiobarbituric acid (TBA) under acidic and high temperature conditions to produce the brown-red trimethyltran (3,5, 5-trimethyloxazol-2, 4-dione) with a maximum absorption wavelength of 532 nm. Meanwhile, the absorbance at 600 nm was measured to eliminate the interference of sucrose, and MDA was calculated by the difference between the absorbance at 532 nm and 600 nm.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	50 mL	100 mL	4℃
Reagent Ⅰ	1	1	4℃, protected from light
Reagent Ⅱ	15 mL	30 mL	4℃

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at OD532 nm and OD600 nm
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips
- Incubator, centrifuge
- Deionized water
- Homogenizer (for tissue samples)

Reagent Preparation

Extraction Buffer: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Reaction Mix: Prepared before use. Reagent Ⅰ was all transferred to Reagent Ⅱ bottle for full dissolution. Reaction Mix is difficult to dissolve and can be heated at 70℃ and violently oscillated to promote dissolution, or treated by ultrasound to promote dissolution. Stored at 4℃ for 6 months.

Sample Preparation

1. Animal or Plant Tissues: Weigh about 0.1 g Tissue and add 1 mL ice-cold Extraction Buffer, homogenize on ice. Centrifuge at 13,000 g for 10 min at 4℃, take the supernatant for further analysis.
2. Cells or Bacteria: Collect 5×10^6 cells or bacteria. Wash cells or bacteria with cold PBS, discard the supernatant after centrifugation, add 1 mL ice-cold Extraction Buffer to ultrasonically disrupt the cells or bacteria in ice bath 5 min (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times). Centrifuge at 13,000 g for 10 min at 4℃, take the supernatant for further analysis.
3. Serum, Plasma and Urine: Tested directly by adding samples to the microcentrifuge tubes. However, to find the optimal values and ensure your readings will fall within the standard values, we recommend performing several dilutions of the samples.

Note: If you cannot perform the assay at the same time, we suggest that you freeze the sample at -80℃. The sample will be stable for at least one month. 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 EP Tube:

Reagent	Blank Tube (μL)	Test Tube (μL)
Reaction Mix	300	300
Deionized Water	100	0
Sample	0	100

3. Mix well and incubate in water bath 30 min at 95℃ (cover tightly to prevent moisture loss). Then cool to room temperature in an ice bath. Centrifuge for 10 min at 25℃ at 10,000 g.
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: In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If $\Delta\Delta A_{Test}$ is greater than 1.0, 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 0.001, increase the sample quantity appropriately.

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)=$[\Delta\Delta A_{Test}\times V_{Reaction\ Total}\div(\varepsilon\times d)\times10^9]\div(Cpr\times V_{Sample})$=**$51.6\times\Delta\Delta A_{Test}\div Cpr$**

2. Calculated by fresh weight of samples

MDA (nmol/g)=$[\Delta\Delta A_{Test}\times V_{Reaction\ Total}\div(\varepsilon\times d)\times10^9]\div(W\times V_{Sample}\div V_{Sample\ Total})$=**$51.6\times\Delta\Delta A_{Test}\div W$**

3. Calculated by number of cells or bacteria

MDA (nmol/10^4)=$[\Delta\Delta A_{Test}\times V_{Reaction\ Total}\div(\varepsilon\times d)\times10^9]\div(500\times V_{Sample}\div V_{Sample\ Total})$=**$0.1032\times\Delta\Delta A_{Test}$**

4. Calculate by liquid sample(serum, plasma and urine)

MDA (nmol/mL)=[$\Delta\Delta A_{Test} \times V_{Reaction\ Total} \div (\varepsilon \times d) \times 10^9$]÷V_{Sample}=**$51.6 \times \Delta\Delta A_{Test}$**

Where: $V_{Reaction\ Total}$: total reaction volume, 4×10^{-4} L; ε: MDA molar extinction coefficient, 155×10^3 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; $V_{Sample\ Total}$: Extraction Buffer volume added, 1 mL; 500: Total number of cells or bacteria, 5×10^6.

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.