Customer service: service@abbkine.com
Technical support: support@abbkine.com
Website: https://www.abbkine.com

CheKine™ Micro Lipid Peroxidation (LPO) Content Assay Kit

Cat #: KTB1051 Size: 48 T/48 S 96 T/96 S

	Micro Lipid Peroxidation (LPO) Content Assay Kit		
REF	**Cat #:** KTB1051	LOT	**Lot #:** Refer to product label
	Detection range: 0.625-80 nmol/mL		**Sensitivity:** 0.625 nmol/mL
	Applicable samples: Animal and Plant Tissue, Cells, Plasma, Serum or other Liquid samples		
	Storage: Stored at 4℃ for 6 months, protected from light		

Assay Principle

Lipid hydroperoxide (LPO) is a peroxide produced by the action of unsaturated fatty acid chains by free radicals or reactive oxygen species. In pathological conditions, the enhancement of lipid peroxidation can lead to the increase of the originally low level of LPO. The increase of LPO content will cause damage to the structure and function of cells, and LPO content is closely related to the immune system and aging. CheKine™ Micro Lipid Peroxidation (LPO) Content Assay Kit can be used to detect biological samples such as animal and plant tissue, cells, plasma, serum or other liquid samples. In the kit, LPO is heated under acidic conditions to produce malondialdehyde (MDA), which condense with Thiobarbituricacid (TBA) to produce the brown-red substance trimethicine (3,5,5-trimethyloxazol-2,4-dione), whose maximum absorption wavelength is 532 nm. The content of LPO in the sample can be estimated by colorimetry.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer Ⅰ	60 mL	120 mL	4℃
Extraction Buffer Ⅱ	24 mL	48 mL	4℃
Reagent Ⅰ	40 mL	80 mL	4℃
Reagent Ⅱ	Powder×1 vial	Powder×2 vials	4℃, protected from light
Standard	400 μL	400 μL	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 535 nm and 600 nm
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips, 1.5 mL EP tube
- Incubator, ice maker, freezing centrifuge

• Deionized water, ethanol absolute
• Homogenizer or mortar (for tissue samples)

Reagent Preparation

Extraction Buffer Ⅰ : Ready to use as supplied; Equilibrate to room temperature before use; Store at 4℃.
Extraction Buffer Ⅱ: Ready to use as supplied; Store at 4℃.
Reagent Ⅰ: Ready to use as supplied; Equilibrate to room temperature before use; Store at 4℃.
Working Reagent Ⅱ: Prepared before use. After add 12 mL deionized water to each bottle to dissolve thoroughly. The prepared reagent can be stored at 4℃, protected from light for 1 month.
Note: Reagent Ⅱ is difficult to dissolve and can be heated at 70℃ and violently oscillated to promote dissolution, or treated with ultrasound to promote dissolution. Check whether there is powder precipitation before each use. If using Reagent Ⅱ at high temperature, allow it to cool to room temperature before using. Reagent Ⅱ is toxic and has a pungent odor, so it is recommended to experiment in a fume hood.
Standard: Ready to use as supplied; 4.05 mmol/mL TEP; Equilibrate to room temperature before use; Store at 4℃, protected from light.
Standard preparation:
15 μmol/mL Standard: Prepare 15 μmol/mL Standard by diluting 2 μL 4.05 mmol/mL Standard into 538 μL ethanol absolute. Using 15 μmol/mL Standard, prepare standard curve dilution as described in the table:

Num.	Standard Volume (μL)	Ethanol Absolute (μL)	Concentration (nmol/mL)
Std.1	4 μL of 15 μmol/mL Standard	746	80
Std.2	100 μL of Std.1 (80 nmol/mL)	100	40
Std.3	100 μL of Std.2 (40 nmol/mL)	100	20
Std.4	100 μL of Std.3 (20 nmol/mL)	100	10
Std.5	100 μL of Std.4 (10 nmol/mL)	100	5
Std.6	100 μL of Std.5 (5 nmol/mL)	100	2.5
Std.7	100 μL of Std.6 (2.5 nmol/mL)	100	1.25
Std.8	100 μL of Std.7 (1.25 nmol/mL)	100	0.625

Note: Always prepare fresh Standards per use; Diluted Std. solution is unstable and must be used within 4 h.

Sample Preparation

Note: We recommend that you use fresh samples. If not assayed immediately, samples can be stored at -80℃ for one month.When measuring, the temperature and time of thawing should be controlled. When thawing at room temperature, the sample should be thawed within 4 h.
1. Tissues: Weigh 0.1 g tissue, add 1 mL Extraction Buffer Ⅰ and homogenize or mortar on ice. Centrifuge at 8,000 g for 10 min at 4℃. Use supernatant for assay.
2. Cells: Collect 5×10^6 cells into the centrifuge tube, wash cells with cold PBS, discard the supernatant after centrifugation; add 0.4 mL Extraction Buffer Ⅱ to ultrasonically disrupt the cells 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.
3. Plasma, Serum or other Liquid samples: Test directly.
Note: 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 to 535 nm and 600 nm. Visible spectrophotometer was returned to zero with deionized water.

2. Sample measurement. (The following operations are operated in the 1.5 mL EP tube)

Reagent	Blank Well (μL)	Standard Well (μL)	Test Well (μL)	Control Well (μL)
Sample	0	0	100	0
Standard	0	100	0	0
Deionized Water	0	0	0	100
Ethanol Absolute	100	0	0	0
Reagent Ⅰ	300	300	300	300
Working Reagent Ⅱ	100	100	100	100

3. Mix well, water bath at 95℃ for 80 min (cover tightly to prevent water loss), natural cooling to room temperature. Centrifuge at 8,000 g for 10 min at room temperature take 200 μL into a 96-well plate or microglass cuvette. Detect the absorbance at 535 nm and 600 nm. The Blank Well is recorded as A_{Blank}, the Standard Well is marked as $A_{Standard}$, the Test Well is marked as A_{Test}, the Control Well is marked as $A_{Control}$. Finally calculate $\Delta A_{Test}=(A535_{Test}-A535_{Control})-(A600_{Test}-A600_{Control})$, $\Delta A_{Standard}=(A535_{Standard}-A535_{Blank})-(A600_{Standard}-A600_{Blank})$.

Note: (1) The Control Well, the Blank Well and the Standard Well only need to be done 1-2 times. In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If ΔA_{Test} is less than 0.004, increase the sample quantity appropriately. If ΔA_{Test} is greater than 1.5, the sample can be appropriately diluted with corresponding Extraction Buffer, the calculated result multiplied by the dilution factor, or decrease the sample quantity appropriately. (2) In the 95℃ water bath reaction, be sure to avoid liquid splashing and always pay attention to safety. If you use an ordinary buckle cap EP tube, you can wrap the sealing film around the mouth, and use a needle to tie a small hole on the cap to prevent the cap from bursting. It is recommended to use an EP tube with a spiral mouth (also need to wrap the film). If you use a metal bath to heat the tube, you can also press the cap with a heavy weight.

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.

1. Drawing of standard curve

With the concentration of the standard solution as the x-axis and the $\Delta A_{Standard}$ as the y-axis, draw the standard curve and obtain the standard equation. The determination of ΔA_{Test} is brought into the equation to get x (nmol/mL).

2. Calculation of the LPO content

(1) Calculated by protein concentration

LPO (nmol/mg prot)=$(V_{Sample}\times x)\div(V_{Sample}\times Cpr)$**=x÷Cpr**

(2) Calculated by fresh weight of samples

LPO (nmol/g fresh weight)=$(V_{Sample}\times x)\div(W\times V_{Sample}\div V_1)$**=x÷W**

(3) Calculated by cells

LPO (nmol/10^4 cell)=$(V_{Sample}\times x)\div(n\times V_{Sample}\div V_2)$**=0.4x÷n**

(4) Calculated by volume of liquid samples

LPO (nmol/mL)=$(V_{Sample}\times x)\div V_{Sample}$**=x**

V_{Sample}: Added the sample volume, 0.1 mL; V_1: Added the Extraction Buffer Ⅰ volume, 1 mL; V_2: Added the Extraction Buffer Ⅱ volume, 0.4 mL; Cpr: sample protein concentration, mg/mL; W: Sample weight, g; n: Number of cells, calculated in units of ten

thousand.

Typical Data

The following data are for reference only. And the experimenters need to test the samples according to their own experiments.

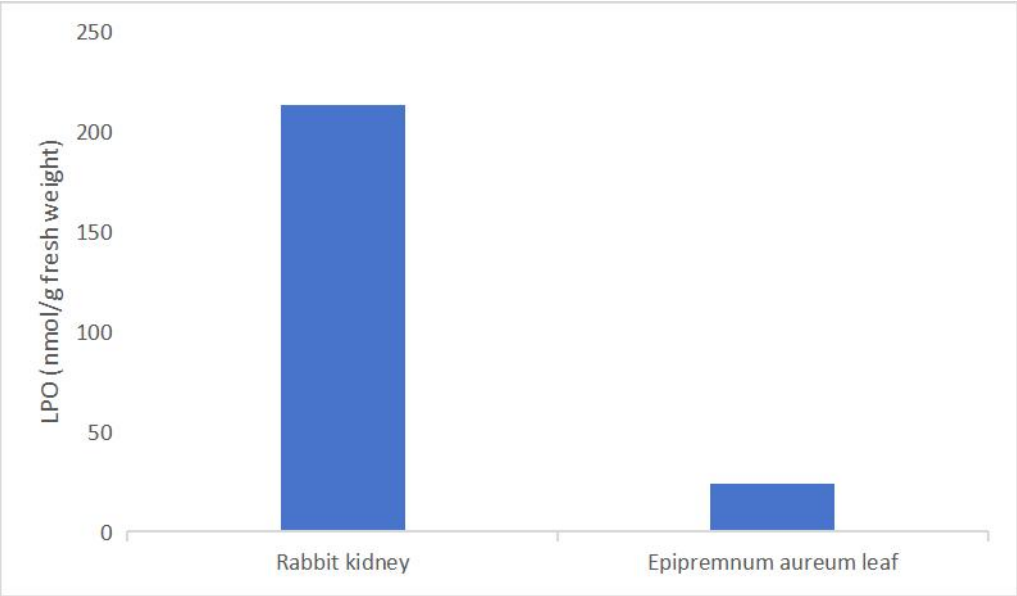

Figure 1. Determination of LPO content in rabbit kidney and *Epipremnum aureum* leaf by this kit.

Recommended Products

Catalog No.	Product Name
KTB1410	CheKine™ Micro Alanine Aminotransferase (ALT/GPT) Activity Assay Kit
KTB1420	CheKine™ Micro Aspartate Aminotransferase (AST/GOT) Activity Assay Kit
KTB1430	CheKine™ Micro Proline (PRO) Content 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.