Lipid Hydroperoxide (LPO) Content Assay Kit

Note: Take two or three different samples for prediction before test.

Operation Equipment: Spectrophotometer / Microplate Reader

Cat No: NA0164

Size:100T/96S

Components:

Extract solution: Liquid 100 mL×1. Store at 2-8℃.

Reagent I: Liquid 11 mL×1. Store at 2-8℃.

Reagent II: Powder×2. Store at 2-8℃. Before use, take one and add 7 ml distilled water to fully dissolve it. This reagent is difficult to dissolve. It can be heated at 70 ℃ and vibrated violently to promote dissolution, or it can be treated by ultrasound to promote dissolution. Before each use, check whether there is powder precipitation. The unused reagent can be stored at 2-8℃ for 1 month

Reagent III: Liquid 4 mL×1. Store at 2-8℃.

Standard Solution: Liquid 1 mL×1. Store at 2-8℃. Standard solution with concentration of 1000nmol/mL.

Diluent: Liquid 20 mL×1. Store at 2-8℃.

Product Description:

Lipid Hydroperoxide (LPO) is a kind of peroxide produced by unsaturated fatty acid chain through the action of free radicals or reactive oxygen species. Under pathological conditions, the enhancement of lipid peroxidation can lead to the increase of LPO with low content. The increase of LPO content will damage the structure and function of cells. LPO content is closely related to the immune system and aging.

LPO is heated under acidic conditions to produce malondialdehyde (MDA). MDA is condensed with thiobarbituric acid (TBA) to produce a brownish red substance named 3,5,5-trimethyloxazole-2,4-dione. Its maximum absorption wavelength is 532nm. The content of LPO in the sample can be estimated after colorimetry.

Reagents and Equipment Required but Not Provided:

Spectrophotometer/microplate reader, desk centrifuge, adjustable transferpettor, water bath, micro quartz cuvette/96 well flat-bottom UV plate, mortar/homogenizer, ice, distilled water.

Procedure:

I. Sample preparation:

1. Tissue:

Add 1 mL of Extract reagent to 0.1 g of tissue, and fully homogenized on ice bath. Centrifuge at 8000×g for 10 minutes at 4℃ to remove insoluble materials, and take the supernatant on ice before testing.

2. Bacteria or cells:

Collecting bacteria or cells into the centrifuge tube. Discard the supernatant after centrifugation.

Suggest add 1 mL of Extract reagent to 5 million of bacteria or cells. Use ultrasonication to splitting bacteria and cells (placed on ice, ultrasonic power 200W, working time 3 seconds, interval 7 seconds, repeat for 18 times). Centrifuge at 8000 ×g for 10 minutes at 4℃ to remove insoluble materials and take the supernatant on ice before testing.

3. Culture medium or other liquid: detect directly. If the solution is turbid, centrifuge and take the supernatant for determination.

II. Determination procedure:

1. Preheat spectrophotometer or microplate reader for 30 minutes, adjust the wavelength to 532 nm and 600nm, set zero with distilled water.

2. Dilute the MDA standard solution with a concentration of 1000nmol/mL with **diluent** to 20, 10, 5, 2.5, 1.25, 0.625, 0.3125, 0.15625nmol/mL for standby.

3. Add the following reagents

Reagent (μL)	Test Tube (T)	Contrast Tube (C)	Standard Tube (S)	Blank Tube (B)
Sample	120	-	-	-
Distilled Water	-	120	-	-
Standard Solution	-	-	120	-
Diluent	-	-	-	120
Reagent I	90	90	90	90
Reagent II	60	60	60	60
Reagent III	30	30	30	30

Mix thoroughly, culture for 60 mintues in 45℃(plant samples)/100℃(other samples) water-bath. After cooling, Centrifuge at 8000 g for 10 minutes at room temperature. Take 200μL of the supernatant to micro glass cuvette/96 well flat-bottom plate. Detect the absorbance at 532 nm and 600 nm. $\Delta A = (A_{T532}-A_{C532})-(A_{T600}-A_{C600})$, $\Delta As = (As_{532}-A_{B532})-(A_{S600}-A_{B600})$. The contrast tube, standard tube and blank tube only need to be measured 1-2 times.

III. Calculation:

1. Make standard curve: Get the standard curve according to concentration of standard solution(x,nmol/mL) and absorbance (y, ΔAs). According to the standard curve, take ΔA(y) into the formula to get the concentration of sample (x,nmol/mL).

2. Protein Concentration:

LPO content (nmol/mg prot) = x×Vs÷（Cpr×Vs）= x÷Cpr

3. Sample Weight:

LPO content (nmol/g weight) = x×Vs÷（W×Vs÷Ve）= x÷W

4. Bacteria or Cultured Cells:

LPO content (nmo/10^4cell) = x×Vs÷（Vs÷Ve ×N）= x÷N

5. Serum(plasma):

LPO content (nmol/mL)= x

Vs: Sample volume, 0.12 mL;

Ve: Extract volume, 1 mL;

N: Number of cells (Unit: 10^4);

Cpr: Sample protein concentration (mg/mL);

W: Sample weight(g);

Note:

1. If there are dense small bubbles in the liquid to be measured, it is recommended to stand for about 20min and wait for the bubbles to disappear before measuring, so as not to affect the measurement results. If there are a small number of bubbles, they can be eliminated by low-speed centrifugation or light knocking.
2. If the solution to be tested has not been clarified, the supernatant can be centrifuged again.
3. In order to prevent water loss during the 60min water bath, it is recommended to use centrifuge tube with screw cap or wrap the EP tube with sealing film.
4. If high temperature is used to help dissolve reagent II, it shall be used after it is cooled to room temperature.
5. If the absorption value is too low or close to blank, the reaction time shall be appropriately prolonged or the sample size shall be increased, and the measurement shall be repeated. If A_{532}>1.5, please dilute the sample to appropriate concentration, multiply dilute times in the formula.

Experimental Example:

1. Take 120 μL sheep serum, according to the determination steps. From the measured data: A_{T532}=0.1, A_{C532}=0.047, A_{T600}=0.076, A_{C600}=0.047, ΔA=0.024, Substituting ΔA determination into standard curve formula:y= 0.0405x-0.0032，x=0.6044.

LPO content (nmol/mL) = x = 0.6044 nmol/mL.

2. Take 0.101 g magnolia leaf, according to the determination steps. From the measured data: A_{T532}=0.17, A_{C532}=0.051, A_{T600}=0.068, A_{C600}=0.045, ΔA=0.096, Substituting ΔA determination into standard curve formula:y= 0.0405x-0.0032，x=2.2044.

LPO content (nmol/g weight) = x÷W=21.826 nmol/g weight

References：

Hiroshi Ohkawa, Nobuko Ohishi, Kunio Yagi, Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction[J], Analytical Biochemistry,1979.

Related Products：

NA0854/NA0386 Catalase (CAT) Activity Assay Kit

NA0864/NA0621 Peroxidase(POD) Activity Assay Kit

NA0855/NA0613 Polyphenol Oxidase (PPO) Activity Assay Kit

NA0853/NA0611 Phenylalanine Ammonia-lyase (PAL) Activity Assay Kit