PRODUCT INFORMATION & MANUAL

TBARS/Thiobarbituric Acid Reactive Substances Assay Kit (Fluorometric)

NBP3-24538

For research use only.

Not for diagnostic or therapeutic procedures.

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TBARS/Thiobarbituric Acid Reactive Substances Assay Kit (Fluorometric)

Catalog No: NBP3-24538

Method: Fluorimetric method

Specification: 96T (Can detect 80 samples without duplication)

Instrument: Fluorescence Microplate reader

Sensitivity: 0.09 μmol/L

Detection range: 0.09-10 μmol/L

Average intra-assay CV (%): 1.7

Average inter-assay CV (%): 2.8

Average recovery rate (%): 95.7

- ▲ This kit is for research use only.
- ▲ Instructions should be followed strictly, changes of operation may result in unreliable results.
- ▲ Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

General information

▲Intended use

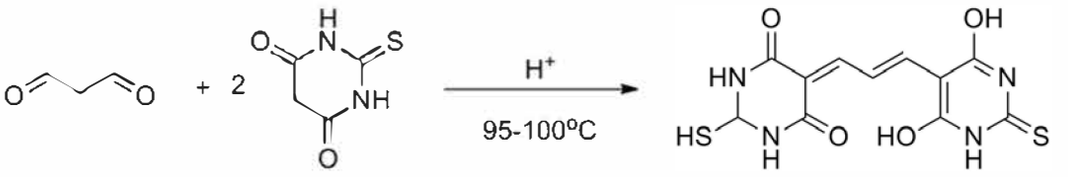
This kit can be used to measure TBARS concentration in serum (plasma), animal tissue, culture cells and other samples.

▲Background

The organism produces oxygen free radicals through enzyme system and non-enzyme system, attacks polyunsaturated fatty acids in biofilm, induces lipid peroxidation, and thus forms lipid peroxide. Malondialdehyde (MDA) is one of the common products of lipid peroxidation in organisms. In clinical science, MDA is a biomarker of lipid peroxidation, which can reflect the degree of lipid peroxidation in organism and indirectly reflect the degree of cell injury.

▲Detection principle

TBARS and TBA can react under high temperature and acid conditions and then form a pink compound, the concentration of which is linearly related to the concentration of TBARS in the sample. The TBARS concentration can be calculated by measuring the fluorescence values at the excitation wavelength of 520 nm and the emission wavelength of 550 nm.



▲Kit components & storage

Item	Component	Specification	Storage
Reagent 1	Clarificant	12 mL × 1 vial	2-8℃ , 12 months
Reagent 2	Acid Reagent	12 mL × 1 vial	2-8℃ , 12 months
Reagent 3	TBA Reagent	Powder × 1 vial	2-8℃ , 12 months, shading light
Reagent 4	20 μmol/L Standard	5 mL × 1 vial	2-8℃ , 12 months
	Black Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	
Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other.			

▲Materials prepared by users

Instruments

Fluorescent Microplate reader(λex/em = 520 nm/550 nm), Vortex mixer, Water bath, Micropipettor

Reagents

Double distilled water, Acetic acid, Normal saline (0.9% NaCl), PBS (0.01 M, pH 7.4)

⚠ Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the skin and clothing. Wash immediately with plenty of water if touching it carelessly. All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

⚠ Precautions

Before the experiment, please read the instructions carefully, and wear gloves and work clothes.

▲ The key points of the assay

1. The temperature of water-bath and the time of incubation should be stabilized (95-100℃ , 60 min)
2. In the incubation of 100℃ water bath, the EP tube should not be closed directly. It is recommended to fasten the tube mouth with fresh-keeping film and make a small hole in the film.
3. The supernatant for fluormetric measurement should not contain sediment, otherwise it will affect the fluorescence values. It is recommended to use a pipette to take the supernatant.

Pre-assay preparation

▲Reagent preparation

1. Reagent 1 will solidify when stored at 2-8℃ . Incubate the reagent 1 at 37℃ until transparent liquid can be used.
2. Preparation of reagent 2 application solution:
   Mix 1.2 mL reagent 2 and 34 mL double-distilled water fully. Prepare the fresh solution before use and it can be stored at 2-8℃ for 24 hour.
3. Preparation of reagent 3 application solution:
   Dissolve a vial of reagent 3 powder with 60 mL double distilled water (90-100℃) and mix fully. Then add 60 mL glacial acetic acid (self-prepared), mix fully and cool to room temperature. The prepared reagent 3 application solution can be stored at 2~8℃ in the dark for 1 month.
4. Preparation of chromogenic agent:
   Prepare the chromogenic agent according to the ratio of reagent 2 application solution: reagent 3 application solution =3: 1 (mix fully). Prepare the fresh solution before use and it must be use out in 24 hours.

▲Sample preparation

The samples should be prepared as conventional methods. Also please refer to appendix II.

▲Dilution of sample

It is recommended to take 2~3 samples with expected large difference to do pre-experiment before formal experiment and dilute the sample according to the result of the pre-experiment and the detection range (0.09-10 μmol/L).

The recommended dilution factor for different samples is as follows (for reference only)

Sample type	Dilution factor
Human serum	1
Rat serum	1
10% Mouse brain tissue homogenization	1
Human plasma	1
Mouse serum	1
10% Rat liver tissue homogenization	1

Note: The diluent is normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4).

Assay protocol

▲Plate set up

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S1	S9	S17	S25	S33	S41	S49	S57	S65	S73
B	B	B	S2	S10	S18	S26	S34	S42	S50'	S58	S66	S74
C	C	C	S3	S11	S19	S27	S35	S43	S51	S59	S67	S75
D	D	D	S4	S12	S20	S28	S36	S44	S52	S60	S68	S76
E	E	E	S5	S13	S21	S29	S37	S45	S53	S61	S69	S77
F	F	F	S6	S14	S22	S30	S38	S46	S54	S62	S70	S78
G	G	G	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79
H	H	H	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80

Note: A-H, standard wells; S1-S80, sample wells.

▲Detailed operating steps

The preparation of standard curve

Dilute 20 μmol/L Standard with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 0.5, 1, 2, 4, 6, 8, 10 μmol/L.

Reference is as follows:

Number	Standard concentrations (μmol/L)	20 μmol/L Standard(μL)	Double distilled water (μL)
A	0	0	1000
B	0.5	25	975
C	1	50	950
D	2	100	900
E	4	200	800
F	6	300	700
G	8	400	600
H	10	500	500

The measurement of samples

(1) Standard tube: Take 0.1 mL of standard solution with different concentrations into numbered 10 mL glass tubes.
Sample tube: Take 0.1 mL of sample into numbered 10 mL glass tubes.

(2) Add 0.1 mL of reagent 1 into each tube of Step 1.

(3) Add 4 mL of chromogenic agent into each tube of Step 2.

(4) Fasten the tube mouth with fresh-keeping film, mix fully, and make a small hole in the film. Then incubate the tubes at 100℃ for 60 min.

(5) Take the tubes out and put them in an ice bath to stop the reaction. After cooling to room temperature with running water, centrifuge the tubes at 1600 g for 10 min.

(6) Take 0.25 mL the supernatant to the microplate with a micropipette (the precipitation cannot be added to the microplate).

(7) Measure the fluorescence values at the excitation wavelength of 520 nm and the emission wavelength of 550 nm.

▲Detailed operating steps

	Standard tube	Sample tube
Standard solution of different concentrations (mL)	0.1	
Sample (mL)		0.1
Reagent 1 (mL)	0.1	0.1
Chromogenic agent (mL)	4	4
Fasten the tube mouth with fresh-keeping film, mix fully, and make a small hole in the film. Then incubate the tubes at 100℃ for 60 min. Cool and centrifuge the tubes. Take 0.25 mL the supernatant to the microplate with a micropipette. Measure the fluorescence values at the excitation wavelength of 520 nm and the emission wavelength of 550 nm.		

▲Calculation

Plot the standard curve by using fluorescence value (F) of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve with graph software (or EXCEL). The concentration of the sample can be calculated according to the formula based on the F value of sample.

The standard curve is: y= ax + b.

1. Serum (plasma):

TBARS (μmol/L)=(ΔF-b)÷a×f

2. Tissue sample and cells:

TBARS (μmol/gprot)=(ΔF-b)÷a×f÷C_{pr}

Note:

y: The absolute F value ($F_{Standard}$ – F_{Blank}) of standard, (F_{Blank} is the F value when the standard concentration is 0).

x: The concentration of standard

a: The slope of standard curve

b: The intercept of standard curve

f: Dilution factor of sample before test

C_{pr}: Concentration of protein in sample (gprot/L)

ΔF: Absolute fluorescence value of sample (F_{Sample} – F_{Blank})

Appendix I Data

▲Example analysis

Dilute 10% mouse liver homogenate for 10 times, take 0.1 mL of diluted sample, carry the assay according to the operation table.

The results are as follows:

standard curve: y = 63.104 x + 13.471, the average fluorescence value of the sample well is 70.527, the average fluorescence value of the blank well is 33.423, the concentration of protein in sample is 16.56 gprot/L, and the calculation result is:

TBARS content (μmol/gprot) = (70.527-33.423-13.471)÷63.104×10÷16.56

=0.23 μmol/gprot

Appendix II Sample preparation

The following sample pretreatment methods are for reference only.

▲Serum

Collect fresh blood and stand at 25℃ for 30 min to clot the blood. Then centrifuge at 2000 g for 15 min at 4℃ . Take the serum (which is the upper light yellow clarified liquid layer) to preserve it on ice for detection. If not detected on the same day, the serum can be stored at -80℃ for a month.

▲Plasma

Take fresh blood into the tube which has anticoagulant, centrifuge at 700-1000 g for 10 min at 4 ℃ . Take the plasma (which is the upper light yellow clarified liquid layer, don't take white blood cells and platelets in the middle layer) to preserve it on ice for detection. If not detected on the same day, the plasma can be stored at -80℃ for a month.

▲Tissue sample

Take 0.02-1g fresh tissue to wash with homogenization medium at 2-8℃ · Absorb the water with filter paper and weigh. Homogenize at the ratio of the volume of homogenized medium (2-8℃) (mL): the weight of the tissue (g) =9:1, then centrifuge the tissue homogenate for 10 min at 1500 g at 4℃ . Take the supernatant to preserve it on ice for detection. Meanwhile, determine the protein concentration of supernatant. If not detected on the same day, the tissue sample (without homogenization) can be stored at-80℃ for a month.

▲Cells

Collect the cells and wash the cells with homogenization medium for 1~2 times. Centrifuge at 1000 g for 10 min and then discard the supernatant and keep the cell sediment. Add homogenization medium at a ratio of cell number (10^6): homogenization medium (μL) =1: 300-500. Sonicate or grind with hand-operated in ice water bath. Centrifuge at 10000 g for 10 min, then take the supernatant and preserve it on ice for detection. Meanwhile, determine the protein concentration of supernatant. If not detected on the same day, the cells sample (without homogenization) can be stored at -80℃ for a month.

Note:

1. Homogenized medium: PBS (0.01 M, pH 7.4) or 50 mM Tris-HCl (pH 7.4) including 150 mM NaCl, 1% NP-40, 1 mM EDTA.

2. Homogenized method:

(1) Hand-operated: Weigh the tissue and mince to small pieces (1 mm^3), then put the tissues pieces to glass homogenized tube. Add homogenized medium into homogenized tube, place the tube into the ice bath with left hand, and insert the glass tamping rod vertically into the homogenized tube with the right hand to grind up and down for 6-8 min.

Or put the tissue into the mortar, and add liquid nitrogen to grind fully. Then add the homogenized medium to homogenize.

(2) Mechanical homogenate: Weigh the tissue to EP tube, add the homogenized medium to homogenize the tissue with homogenizer instrument (60 Hz, 90s) in the ice bath. (For samples of skin, muscle and plant tissue, the time of homogenization can be properly prolonged.)

(3) Ultrasonication: Treat the cells with ultrasonic cell disruptor (200 W, 2 s/ time, interval for 3 s, the total time is 5 min).

Appendix III References

1. Del R D, Stewart A J, Pellegrini N. A review of recent studies on malondialdehyde as toxic molecule and biological marker of oxidative stress. Nutrition Metabolism & Cardiovascular Diseas, 2005, 15(4): 316-328.
2. Gaschler M M, Stockwell B R. Lipid peroxidation in cell death. Biochemical & Biophysical Research Communications, 2017, 482(3): 419-425.
3. Niki E. Biomarkers of lipid peroxidation in clinical material. Biochim Biophys Acta, 2014, 1840(2): 809-817.
4. Draper H H, Hadley M. Malondialdehyde Determination as Index of Lipid Peroxidation. Methods Enzymol, 1990, 186(186): 421-431.
5. Nielsen F, BO B M, Nielsen J B, et al. Plasma malondialdehyde as biomarker for oxidative stress: reference interval and effects of life-style factors. Clinical Chemistry, 1997, 43(7): 1209-1214.