IMMUNOLOGICAL SCIENCES Product Data Sheet

CO-K298-M Thiobarbituric Acid Reactants (TBARS) Colorimetric Assay Kit

Method: Colorimetric method

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

Instrument: Microplate reader

Sensitivity: 0.85 μmol/L

Detection range: 2.6-100 μmol/L

Average intra-assay CV(%): 4.3

Average inter-assay CV(%): 7.1

Average recovery rate(%): 100.9

- 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**

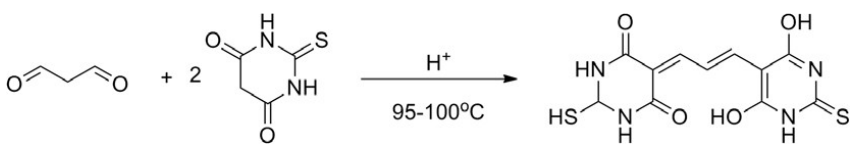
This kit can be used to measure TBARS concentration in serum (plasma), animai tissue samples.

.I 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 clinica! science, MDA is a biomarker of lipid peroxidation, which can reflect the degree of lipid peroxidation in organism and indirectly reflect the degree of celi 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 relateci lo the concentration of TBARS in the sample. The TBARS concentration can be calculated by measuring the OD values at 530-540 nm.



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Kit components & storage

Item	Component	Specification	Storage
Reagent 1	Clarificant	12 mlx 1 vial	2-8°C, 12months
Reagent2	Acid Reagent	12 mlx 1 vial	2-8°C, 12months
Reagent3	TBAReagent	Powder x 1 vial	2-8°C, 12 months, shading light
Reagent4	200 μmo/ll Standard	5 mlx 1 vial	2-8°C, 12months
	Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	
Note: The reagents musi be stored strictly according lo the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other.			

Materials prepared by users

Instruments

Microplate reader (530-540 nm), Vortex mixer, Micropipettor, Water bath

Reagents

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

A Safety data

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

A 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°C , 60 min)
2. In the incubation ol 100°c 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 lor colorimetric measurement should not contain sediment, otherwise it will affect the OD values. It is recommended lo use a pipette to take the supernatant.

Pre-Assay preparation

• Reagent preparation

1. Reagent 1 will solidify when stored at 2-8"C . Incubate the Reagent 1 at 37°C until transparent, liquid can be used.
2. Preparation of Reagent 2 application solution:
   Mix 1.2 ml of Reagent 2 and 34 ml of double-distilled water fully. Prepare the fresh solution before use and it can be stored al 2-8"C lor a day.
3. Preparation of Reagent 3 application solution:
   Dissolve a vial of reagent 3 powder with 60 ml of double distilled water (90-100℃) and mix fully. Then add 60 ml of glacial acetic acid (self- prepared), mix fully and cool to room temperature. The prepared reagent 3 application solution can be stored at 2- 8"C in the dark lor 1 month.
4. Preparation of chromogenic agent:
   Prepare the chromogenic agent according lo 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 referto appendix II.

.i. Dilution of sample

II is recommended lo lake 2-3 samples wilh expecled large difference lo do pre-experiment before formai experiment and dilute the sample according to the result of the formai experiment and the detection range (2.6-100 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 serum	1
Rat serum	1
10% Rat liver tissue homogenizalion	1

Note: The diluent is nornal saline (0.9% NaCI) 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 200 μmol/L Standard with double distilled water lo a serial concentration. The recommended dilution gradient is as follows: O, 5, 1O, 20, 40, 60, 80, 100 μmol/L.

Reference is as follows

Number	Standard concentrations (μmol/L)	200 μmol/L Standard(μL)	Double distilled water (μL)
A	0	0	1000
B	5	25	975
C	10	50	950
D	20	100	900
E	40	200	800
F	60	300	700
G	80	400	600
H	100	500	500

The measurement of samples

(1) **Standard tube**: Take 0.1 ml of standardsolution with different concentrations into numbered 10 ml glass tubes.
Sample tube: Take 0.1 ml of tested sample inia numbered 1O 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°C far 60 min.

(5) Cool the tubes to room temperature with running water, centrifuge the tubes at 1600 g far 10 min.

(6)Take 0.25 ml of the supernatant lo the microplate with a micropipette (the precipitation cannot be added lo the microplate).

(7) Measure the OD value at 532 nm with microplate reader.

Summary Operation table

	Standard	Sample
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 al 100° C far 60 min. Cool and centrifuge the tubes. Take 0.25 ml the supernatant lo the microplate with a micropipette. Determine the OD value at 532 nm.		

Calculation

Plot the standard curve by using OD value 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 OD value of sample.

The standard curve is: y= ax + b.

1. **Serum (plasma)**: TBARS (μmol/L)=(M -b)÷axf
2. **Tissue sample:** TBARS (μmol/gprot)= **(ΔA-b)** ÷a^{x}f ÷C_{pr}

Note:

y: The absolute OD value of standard ($OD_{standard} - OD_{blank}$)

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)

ΔA: Absolute OD ($OD_{Sample} - OD_{blank}$)

Appendix I Data

Example analysis

Take 0.1 ml of 10% mouse tissue homogenate, carry the assay according lo the operation table.

The results are as follows:

standard curve: y = 0.0038 x - 0.0013, the average OD value of the sample well is 0.106, the average OD value ofthe blank well is 0.047, the concentration of protein in sample is 15.18 gprot/L, and the calculation result is:

TBARS conteni (μmo/lgprot) = (0.106-0.047+00013)+0.0038+15.15

=1.05 μmol/gprot

Appendix II Sample Preparation

The tollowing sample pretreatment methods are tor reterence only.

- Serum

Collect fresh blood and stand at 25°C tor 30 min to clot the blood. Then centrituge at 2000 g for 15 min at 4"C . Take the serum (which is the upper light yellow clarified liquid layer) lo preserve il on ice for detection. It noi detected on the same day, the serum can be stored at -80"C tor a month.

- Plasma

Take fresh blood into the tube which has anticoagulant , centrituge al 700-1000 g *tor* 10 min al 4"C . Take the plasma (which is the upper light yellow clarified liquid layer, don't take whi te blood cells and platelets in the middle layer) lo preserve il on ice for detection. It not detected on the same day, the plasma can be stored at -80"C tor a month.

- Tissue sample

Take 0.02-1g fresh tissue lo wash with homogenization medium al 2-8"C . Absorb the wate r with filter paper and weigh. Homogenize al the ratio *ot* the volume*ot* homogenized medium (2-8"C) (ml): the weight *ot* the tissue (g) =9:1, then centrituge the tissue homogenate for 10 min al 1500 gal 4°C . Take the supernatant lo preserve il on ice tor detection. Meanwhile, determine the protein concentration *ot* supernatant. It noi detected on the same day, the tissue sample (withouthomogenizatio)ncan be stored al -80"C tor a month.

Note:

1. Homogenized medium: PBS (0.01 M, pH7.4).
2. Homogenized method:

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

Or put the tissue into the mortar, and add liquid nitrogen to grind lully. Then add the homogenized medium lo 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 ol skin, muscle and plant tissue, the time ol homogenization can be properly prolonged.)

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