Technical Manual

Thiobarbituric Acid Reactants (TBARS) Fluorometric Assay Kit

- Catalogue Code: MAES0173
- Size: 96T
- Research Use Only

1. Key Features and Sample Types

Detection method:

Fluorimetric method

Specification:

96T

Range:

0.09-10 µmol/L

Sensitivity:

0.09 µmol/L

Storage:

2-8°C for 6 months

Expiry:

See Kit Label

Experiment Notes:

This kit is for **research use only.**

Instructions should be strictly followed. Changes of operation may result in unreliable results.

The validity of kit is 6 months.

Do not use components from different batches of kit.

2. 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.

3. Intended Use

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

4. 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.

5. Kit Components & Storage

Item	Specification	Storage
Clarificant	12 mL × 1 vial	2-8°C, 6 months
Acid reagent	12 mL × 1 vial	2-8°C, 6 months
TBA Reagent	Lyophilized × 1 vial	2-8°C, 6 months, avoid direct sunlight
Standard Solution (20 μmol/L)	5 mL × 1 vial	2-8°C, 6 months
Black Microplate	96 wells	No requirement
Plate Sealer	2 pieces	

Materials required but not supplied

- Micropipettor
- Incubator
- Centrifuge
- Fluorescent Microplate reader(λex/em = 520 nm/550 nm)
- Tips (10 μL, 200 μL, 1000 μL)
- EP tubes (1.5 mL, 2 mL)
- Double distilled water
- Normal Saline (0.9% NaCl)
- PBS (0.01 M, pH 7.4)
- Acetic acid

6. Assay Notes:

1. The temperature of the water-bath and the time of incubation should be stabilized (95-100°C, 60 min)

2. In the incubation of 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 for colorimetric measurement should not contain sediment, otherwise it will affect the OD values. It is recommended to use a pipette to take the supernatant.

7. Reagent Preparation:

1. Bring all reagents to room temperature before use.

2. Clarificant will solidify when stored at 2-8°C. Incubate the clarificant at 37°C until transparent, liquid can be used

3. Preparation of **acid application solution:** Mix 1.2 mL acid reagent and 34 mL double-distilled water fully. Prepare the fresh solution before use and it can be stored at 2-8°C for 24 hours.

4. Preparation of **TBA application solution:** Dissolve a vial of TBA reagent powder with 60 mL double distilled water (90-100°C) and mix fully. Then add 60 mL glacial acetic acid (self-prepared), mix fully and cool to room temperature. The prepared TBA application solution can be stored at 2~8°C and avoid direct sunlight for 1 month.

5. Preparation of **chromogenic agent:** Prepare the chromogenic agent according to the ratio of acid application solution: TBA application solution =3: 1 (mix fully). Prepare the fresh solution before use and it must be use out in 24 hours.

8. Sample Preparation

1. Serum sample:

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

2. Plasma sample:

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

3. Cell sample:

Collect the cells and wash the cells with PBS (0.01 M, pH 7.4) 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 (2×10^6): 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 (μL) =1: 200. Sonicate the sample on an ice water bath. Centrifuge at 10000 g for 10 min, then take the supernatant and preserve on ice before detection. If not detected on the same day, the cells sample (without homogenization) can be stored at -80°C for a month.

4. Tissue sample:

Take 0.02-1g fresh tissue to wash with PBS (0.01 M, pH 7.4) at 2-8°C. Use filter paper to absorb excess water and weigh. Homogenize at the ratio of the volume of 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 (2-8°C) (mL): the weight of the tissue (g) =9:1, then centrifuge the tissue homogenate for 10 min at 10000 g at 4°C. Take the supernatant and preserve on ice before detection. If not detected on the same day, the tissue sample (without homogenization) can be stored at -80°C for a month.

Sample Notes:

The concentration should be determined before preforming the assay. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.

Dilution of Samples:

Large variances in results may be seen when performing pre-experiments. 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);

9. Assay Protocol

Ambient Temperature: 25-30°C

Optimum detection wavelength: Ex/Em = 520 nm/550 nm

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.

10. Operation 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.

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 tested **sample** into numbered 10 mL glass tubes.

2. Add 0.1 mL of clarificant 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 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.

Operation Table

	Standard well	Sample well
Standard solution of different concentrations (mL)	0.1	
Sample (mL)		0.1
Clarificant (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°C for 60 min. After cooling to room temperature with running water, centrifuge the tubes at 1600 × g for 10 min. 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.		

11. Calculations

Plot the standard curve by using fluorescence 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) sample:

$$\text{TABRS (μmol/L)} = (\Delta F\text{-}b) \div a \times f$$

2. Tissue and cell sample:

$$\text{TABRS (μmol/gprot)} = (\Delta F\text{-}b) \div a \times f \div C_{pr}$$

y: The absolute F value ($F_{Standard} - F_{Blank}$) of standard

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 ($F_{Sample} - F_{Blank}$).

12. Performance Characteristics

Detection Range	0.09-10 μmol/L
Sensitivity	0.09 μmol/L
Average recovery rate (%)	95.7
Average inter-assay CV (%)	2.8
Average intra-assay CV (%)	1.7

Analysis

For rat liver tissue, dilute 10% rat 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:

$$\text{TBARS content (μmol/gprot)} = (70.527-33.423-13.471)\div 63.104\times 10\div 16.56$$

$$=0.23\ \mu mol/gprot$$

Email: info@assaygenie.com **Web:** www.assaygenie.com

Safety Notes

Some of the reagents in the kit contain dangerous substances. Prevent touching 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.

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

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Notes:

Notes:

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