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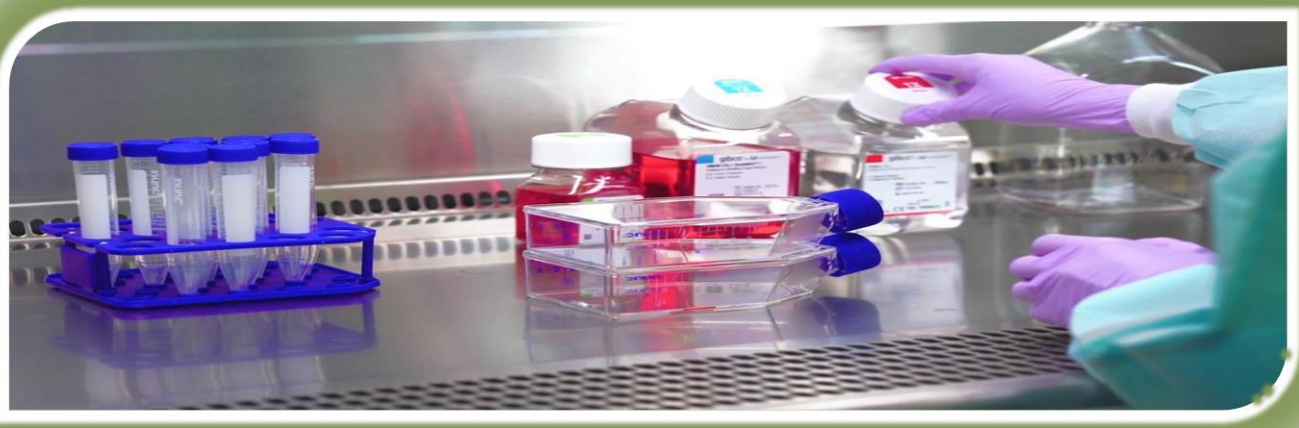
Knowledge based, technology- and research



Technical Manual

Thiobarbituric Acid Reactants (TBARS) Colorimetric Assay Kit

- ***Catalogue Code: kp-161md***
- ***Size: 96T- 48T***
- ***Research Use Only***

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1. Key Features and Sample Types

Detection method:

Colorimetric method

Specification:

96T

Range:

2.6-100 μmol/L

Sensitivity:

0.85 μmol/L

Storage:

2-8°C for 6 months

Expiry:

See Kit Label

Experiment Notes:

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.
- 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 OD values at 530-540 nm.

4. 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 (200 μmol/L)	• 5 mL × 1 vial	• 2-8°C, 6 months
• Microplate	• 96 wells	• No requirement
• Plate Sealer	• 2 pieces	

Materials required but not supplied

- Micropipettor
- Vortex mixer
- Water bath
- Microplate Reader (530-540 nm)
- Tips (10 μL, 200 μL, 1000 μL)
- EP tubes (1.5 mL, 2 mL)
- Double distilled water
- Acetic acid
- Normal saline (0.9% NaCl)
- PBS (0.01 M, pH 7.4)

Assay Notes:

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

5. Reagent Preparation:

- Bring all reagents to room temperature before use.
-
- Clarificant will solidify when stored at 2-8°C. Incubate the Clarificant at 37°C until transparent, liquid can be used.
- Preparation of Acid application solution: Mix 1.2 mL of acid reagent and 34 mL of double-distilled water fully. Prepare the fresh solution before use and it can be stored at 2-8°C for a day.

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

6. Sample Preparation

1. Serum sample:

- Fresh blood should be incubated at 25°C for 30 min to clot the blood. Centrifuge the sample 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:

- Place the fresh blood sample into a tube of anticoagulant and centrifuge at 700-1000g 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.

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 (4×106): 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: 400. 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.

3. 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.

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 (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 homogenization	1

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

Assay Protocol

Ambient Temperature: 25-30°C **Optimum detection wavelength:** 532 nm

7. Operation Steps

The preparation of standard curve

Dilute 200 μmol/L Standard with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 5, 10, 20, 40, 60, 80, 100 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. Cool the tubes to room temperature with running water, centrifuge the tubes at 1600 g for 10 min.

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

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

Operation Table

	Standard tube	Sample tube
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℃ for 60 min. Cool the tubes 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. Determine the OD value at 532 nm with microplate reader.		

8. Calculations

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) sample:

y: $OD_{Standard} - OD_{Blank}$ (OD_{Blank} is the OD value when the standard concentration is 0). x: The concentration of standard.

a: The slope of standard curve .

b: The intercept of standard curve.

ΔA: $OD_{Sample} - OD_{Blank}$ (OD_{Blank} is the OD value when the standard concentration is 0) f: Dilution factor of sample before test.

C_{pr}: Concentration of protein in sample, gprot/L.

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-Expert working group and different orientations.

-Up-to-date equipment for research and knowledge development.

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