TECHNICAL MANUAL

Thiobarbituric Acid Reactants (TBARS) Colorimetric Assay Kit

- **SKU CODE:** MAES0174
- **SIZE:** 48 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Reagent preparation
- Standard curve preparation
- Sample preparation
- Operating steps
- Calculation

2. Intended use

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

3. Detection principle

TBARS and TBA can react under high temperature and acidic conditions to 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	Component	Size 1(48 T)	Size 2(96 T)	Size 3 (500 Assays)	Storage
Reagent 1	Clarificant	12 mL × 1 vial	12 mL×1 vial	60 mL × 1 vial	2-8°C, 12 months
Reagent 2	Acid Reagent	6 mL × 1 vial	12 mL×1 vial	60 mL × 1 vial	2-8°C, 12 months
Reagent 3	TBA Reagent	Powder×1 vial	Powder×1 vial	Powder×5 vials	2-8°C, 12 months shading light
Reagent 4	200 μmol/L Standard	5 mL × 1 vial	5 mL × 1 vial	15 mL × 2 vials	2-8°C, 12 months
	Microplate		96 wells		No requirement

Item	Component	Size 1(48 T)	Size 2(96 T)	Size 3 (500 Assays)	Storage
	Plate Sealer		2 pieces		

5. Materials prepared by users

Instruments:

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

Reagents:

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

6. Reagent preparation

1. Frozen storage (2-8°C) may cause the clarificant to freeze. To re-dissolve, place in a water bath (37°C) until the clarificant looks clear. Equilibrate other reagents to room temperature before use.
2. The preparation of acid application solution: Dilute 1.2 mL acid reagent with 34 mL double-distilled water. The acid application solution should be prepared fresh. Store at 2-8°C for up to 1 day.
3. The preparation of TBA application solution: Dissolve a vial of TBA reagent 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. Store at 2-8°C for up to 1 month protected from light.
4. The preparation of chromogenic agent: For each tube, prepare 4 mL of chromogenic agent (mix well 3 mL of acid application solution, 1 mL of TBA application solution). The chromogenic agent should be prepared fresh and must be used within 24 hours.
5. **The preparation of standard curve:** Always prepare a fresh set of standards. Discard working standard dilutions after use. 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.

7. Sample preparation

Sample preparation

Serum and plasma: detect directly. If not detected on the same day, the serum or plasma can be stored at -80°C for a month.

Tissue sample:

1. Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).

2. Wash tissue in cold PBS (0.01 M, pH 7.4).

3. Homogenize 20 mg tissue in 180 µL PBS (0.01 M, pH 7.4) with a dounce homogenizer at 4°C.

4. Centrifuge at 10000×g for 10 minutes to remove insoluble material. Collect supernatant and keep it on ice for detection.

5. Meanwhile, determine the protein concentration of supernatant (MAES0177).

Dilution of sample

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

- Human serum: 1

- Rat serum: 1

- 10% Mouse brain tissue homogenization: 1

- 10% Rat liver tissue homogenization: 1

Note: The diluent is normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4). For the dilution of other sample types, please perform a pretest to confirm the dilution factor.

8. 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 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.

9. Operating steps

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.

3. Add 4 mL of chromogenic agent into each tube.

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 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.

10. Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean OD value of the blank (Standard #①) from all standard readings. This is the absolute OD value.

3. Plot the standard curve by using absolute OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (y = ax + b) with graph software (or EXCEL).

The sample:

1. Serum (plasma) sample:

TBARS μmol/L = (ΔA - b) ÷ a × f

2. Tissue sample:

TBARS μmol/gprot = (ΔA - b) ÷ a × f ÷ C_{pr}

[Note]

f: Dilution factor of sample before test.

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

ΔA: Absolute OD (OD_{Sample} – OD_{Blank}).

11. Appendix I Performance Characteristics

Parameter:

Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay. (CV = Coefficient of Variation)

Inter-assay Precision

Three human serum samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Recovery

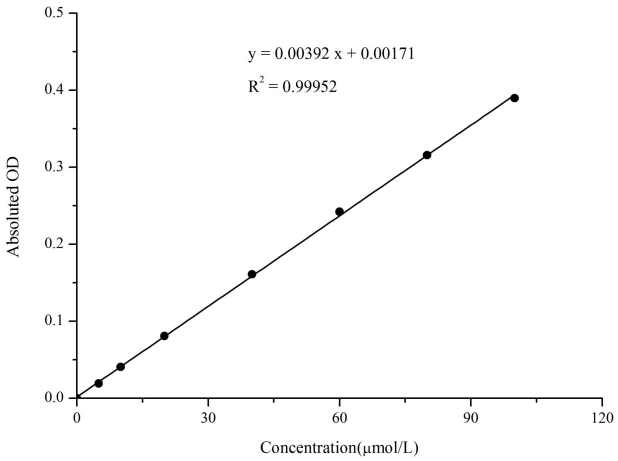
Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 101%.

Sensitivity

The analytical sensitivity of the assay is 0.85 µmol/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Standard curve

As the OD value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), the standard curve and data are provided as below for reference only:



12. Appendix II Example Analysis

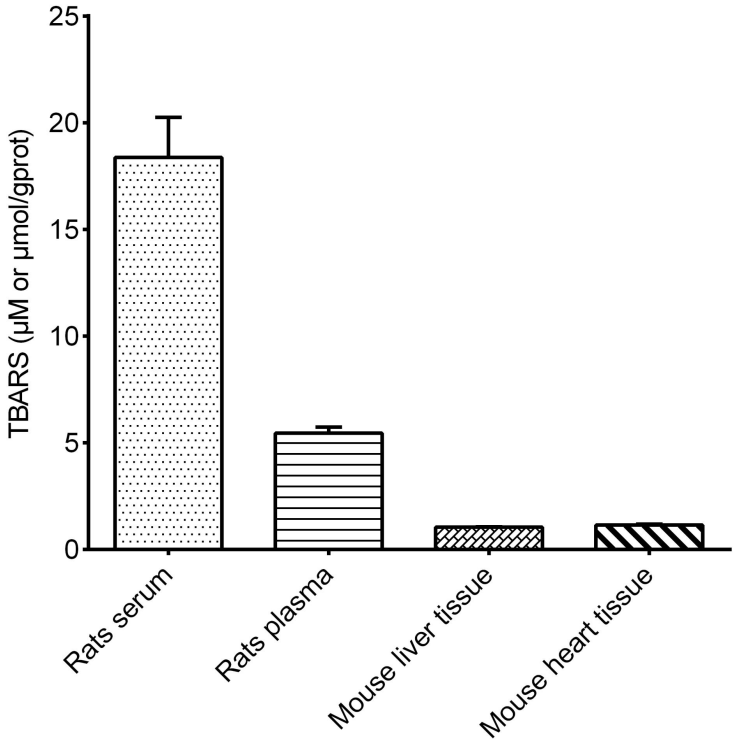
Example analysis:

For mouse liver tissue, take 0.1 mL of 10% mouse tissue homogenate, carry the assay according to the operation steps. The results are as follows:

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

TBARS content (μmol/gprot) = (0.106-0.047+0.0013) ÷ 0.0038 ÷ 15.15 = 1.05 μmol/gprot

Detect rat serum, rat plasma, mouse liver tissue (the concentration of protein in 10% tissue homogenate is 15.18 gprot/L), mouse heart tissue (the concentration of protein in 10% tissue homogenate is 5.55 gprot/L) according to the protocol, the result is as follows:



13. Statement

1. This assay kit is for Research Use Only. Assay Genie assumes no responsibility for any problems or legal liabilities arising from the use of this kit for clinical diagnosis or any other purpose.
2. Please read the instructions carefully and calibrate the instruments before performing the experiments. Follow the instructions strictly throughout the procedure.
3. Appropriate protective measures must be taken, including wearing a lab coat and latex gloves.
4. If the concentration of the substance falls outside the detection range, perform an additional dilution or concentration step on the sample.

5. It is recommended to perform a pre-test if your sample type is not listed in the instruction manual.
6. Experimental results are closely related to reagent quality, operator technique, environmental conditions, and other factors. Assay Genie guarantees the quality of the kits only and is NOT responsible for sample consumption resulting from use of the assay kits. It is advisable to estimate the expected sample usage and reserve sufficient samples before starting the experiment.

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.