TECHNICAL MANUAL

Thiobarbituric Acid Reactants (TBARS) Fluorometric Assay Kit

- **SKU CODE:** MAES0173
- **SIZE:** 48 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Reagent preparation
- Standard curve preparation
- Sample preparation
- Add standards and samples
- Incubate at 100°C for 60 minutes
- Cool and centrifuge
- Measure fluorescence values

2. Intended use

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

3. Detection principle

TBARS and TBA react under high temperature and acidic conditions to form a pink compound. The concentration of this compound is linearly related to the TBARS concentration in the sample. The TBARS concentration can be calculated by measuring fluorescence values at an excitation wavelength of 520 nm and an emission wavelength of 550 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 x 1 vial	12 mL x 1 vial	60 mL x 1 vial	2-8°C, 12 months
Reagent 2	Acid Reagent	6 mL x 1 vial	12 mL x 1 vial	60 mL x 1 vial	2-8°C, 12 months
Reagent 3	TBA Reagent	Powder x 1 vial	Powder x 1 vial	Powder x 5 vials	2-8°C, 12 months, protected from light

Item	Component	Size 1 (48 T)	Size 2 (96 T)	Size 3 (500 Assays)	Storage
Reagent 4	20 µmol/L Standard	5 mL x 1 vial	5 mL x 1 vial	12 mL x 2 vials	2-8°C, 12 months
	Black Microplate	96 wells	96 wells	96 wells	No requirement
	Plate Sealer	2 pieces	2 pieces	2 pieces	

5. Materials prepared by users

Instruments:

Fluorescent Microplate reader (Ex/Em = 520 nm/550 nm), Vortex mixer, Magnetic stirrers, Micropipette, Thermostat water bath

Reagents:

Double distilled water, Glacial 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 appears clear. Equilibrate other reagents to room temperature before use.
2. **Preparation of acid working solution::** Dilute 1.2 mL acid reagent with 34 mL double-distilled water. The acid working solution should be prepared fresh. Store at 2-8°C for up to 24 hours.
3. **Preparation of TBA working solution::** Dissolve one vial of TBA reagent with 60 mL double distilled water (90-100°C) and mix thoroughly. Then add 60 mL glacial acetic acid, mix thoroughly, and cool to room temperature. Store at 2-8°C for up to 1 month, protected from light.
4. **Preparation of chromogenic agent::** For each tube, prepare 4 mL of chromogenic agent by mixing 3 mL of acid working solution with 1 mL of TBA working solution. The chromogenic agent should be prepared fresh and must be used within 24 hours.
5. **Preparation of standard curve::** Always prepare a fresh set of standards. Discard working standard dilutions after use. Dilute 20 µmol/L standard with double

distilled water to create a serial dilution. The recommended concentrations are: 0, 0.5, 1, 2, 4, 6, 8, 10 μmol/L.

7. Sample preparation

Sample preparation:

Serum and plasma: Detect directly. If not analyzed on the same day, serum or plasma can be stored at -80°C for up to one month.

Tissue samples:

1. Harvest the required amount of tissue 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) using a Dounce homogenizer at 4°C.

4. Centrifuge at 10,000 x g for 10 minutes to remove insoluble material. Collect supernatant and keep on ice for detection.

5. Meanwhile, determine the protein concentration of the supernatant.

Cells:

1. Harvest the required number of cells for each assay (initial recommendation: 1×10^6 cells).

2. Wash cells with PBS (0.01 M, pH 7.4).

3. Homogenize 1×10^6 cells in 300 μL PBS (0.01 M, pH 7.4) using an ultrasonic cell disruptor at 4°C.

4. Centrifuge at 10,000 x g for 10 minutes to remove insoluble material. Collect supernatant and keep on ice for detection.

5. Meanwhile, determine the protein concentration of the supernatant.

Sample dilution:

The recommended dilution factors are provided for reference only. Use normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4) as diluent. For other sample types, perform a pretest to confirm the appropriate dilution factor.

8. The key points of the assay

1. The water bath temperature and incubation time must be precise and stable (95-100°C, 60 minutes).

2. During incubation in the 100°C water bath, do not close the tube caps directly. It is recommended to cover the tube openings with plastic film and make a small hole in the film.
3. The supernatant used for fluorometric measurement should not contain sediment, as this will affect the fluorescence values. Use a pipette to carefully collect the supernatant.

9. Operating steps

1. **Standard tubes: Add 0.1 mL of each standard concentration to numbered 10 mL glass tubes.:** Sample tubes: Add 0.1 mL of test sample to numbered 10 mL glass tubes.
2. Add 0.1 mL of clarificant to each tube.
3. Add 4 mL of chromogenic agent to each tube.
4. Cover tube openings with plastic film, mix thoroughly, and make a small hole in the film. Incubate tubes at 100°C for 60 minutes.
5. Remove tubes and place in an ice bath to stop the reaction. After cooling to room temperature with running water, centrifuge tubes at 1,600 x g for 10 minutes.
6. Transfer 0.25 mL of supernatant to the microplate wells using a micropipette (do not transfer any precipitate to the microplate).
7. Measure fluorescence values at excitation wavelength of 520 nm and emission wavelength of 550 nm.

10. Calculation

Standard curve:

1. Average the duplicate readings for each standard.

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

3. Plot the standard curve using the absolute F value of standards (y-axis) versus their corresponding concentrations (x-axis). Create the standard curve equation (y = ax + b) using graph software or Excel.

Sample calculations:

1. Serum (plasma) samples:

TBARS (μmol/L) = (ΔF - b) ÷ a × f

2. Tissue and cell samples:

TBARS ($\mu mol/g_{prot}$) = (ΔF - b) ÷ a × f ÷ C_{pr}

Where:

f: Dilution factor of sample before test

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

ΔF: Absolute fluorescence value of sample (F_{sample} - F_{blank})

11. Appendix I Performance Characteristics

Parameters:

Intra-assay Precision

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

Intra-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (µmol/L)	1.20	4.60	7.30
%CV	1.8	1.8	1.5

Inter-assay Precision

Three human serum samples were assayed 17 times in duplicate by three operators to determine precision between assays.

Inter-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (µmol/L)	1.20	4.60	7.30
%CV	2.5	2.6	3.3

Recovery

Three samples of high, medium, and low concentrations were tested with 6 parallel replicates each to obtain an average recovery rate of 96%.

Recovery

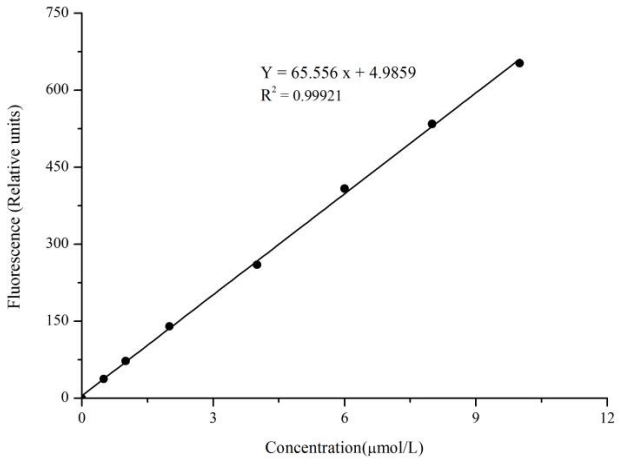
Parameters	Standard 1	Standard 2	Standard 3
Expected Conc. (µmol/L)	0.85	3.6	7.8
Observed Conc. (µmol/L)	0.8	3.5	7.6
Recovery rate (%)	94.2	96	96.9

Sensitivity

The analytical sensitivity of the assay is 0.09 µmol/L. This was determined by adding two standard deviations to the mean fluorescence value obtained when the zero standard was assayed 20 times and calculating the corresponding concentration.

Standard curve

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



Standard curve

Concentration (µmol/L)	Average fluorescence value	Absolute fluorescence value
0	50.2	0
0.5	87.5	37.3

Concentration (μmol/L)	Average fluorescence value	Absolute fluorescence value
1	122.7	72.5
2	190.1	140.0
4	310.0	260.0
6	458.5	408.3
8	584.4	534.3
10	702.8	652.6

12. Appendix II Example Analysis

Example analysis:

For mouse liver tissue, dilute 10% mouse liver homogenate 10-fold, take 0.1 mL of diluted sample, and perform the assay according to the operating steps. The results are as follows:

Standard curve: y = 63.104x + 13.471

Average fluorescence value of sample well: 70.527

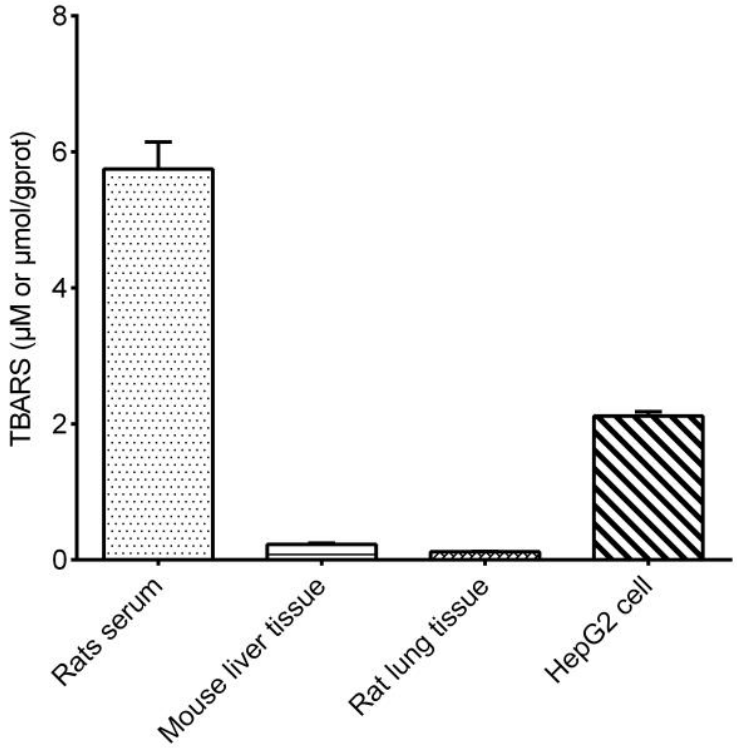
Average fluorescence value of blank well: 33.423

Protein concentration in sample: 16.56 g_{prot}/L

Calculation result:

TBARS content (μmol/g_{prot}) = (70.527 - 33.423 - 13.471) ÷ 63.104 × 10 ÷ 16.56 = 0.23 μmol/g_{prot}

Detect rat serum (diluted 10-fold), 10% rat liver tissue homogenate (protein concentration: 16.56 g_{prot}/L, diluted 10-fold), 10% rat lung tissue homogenate (protein concentration: 5.04 g_{prot}/L, diluted 2-fold), and HepG2 cells (protein concentration: 6.09 g_{prot}/L) according to the protocol. The results are shown below:



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 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 pretest 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.