



## TECHNICAL MANUAL

### Total Antioxidant Capacity (T-AOC) Colorimetric Assay Kit

- **SKU CODE:** MAES0109
- **SIZE:** 48 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

## 1. Assay summary

- Reagent preparation
- Sample preparation
- Add reagents and samples
- Incubate at 37°C
- Add stop solution
- Measure absorbance at 520 nm

## 2. Intended use

This kit is used for the determination of total antioxidant capacity (T-AOC) in serum, plasma, whole blood, tissue, cell and culture supernatant samples.

## 3. Detection principle

A variety of antioxidant macromolecules, antioxidant molecules and enzymes in a system can eliminate all kinds of reactive oxygen species and prevent oxidative stress induced by reactive oxygen species. The total level reflects the total antioxidant capacity in the system.

Many antioxidants in the body can reduce  $\text{Fe}^{3+}$  to  $\text{Fe}^{2+}$  and  $\text{Fe}^{2+}$  can form stable complexes with phenanthroline substance. The antioxidant capacity (T-AOC) can be calculated by measuring the absorbance at 520 nm.

## 4. Kit components & storage

| Item      | Component         | Size 1(48 T)  | Size 2(96 T)   | Size 3<br>(500Assays) | Storage                         |
|-----------|-------------------|---------------|----------------|-----------------------|---------------------------------|
| Reagent 1 | Buffer Solution   | 6 mL×1 vial   | 12 mL × 1 vial | 60 mL×1 vial          | 2-8°C, 12 months                |
| Reagent 2 | Chromogenic Agent | Powder×1 vial | Powder×2 vials | Powder×10 vials       | 2-8°C, 12 months, shading light |

| Item      | Component                  | Size 1(48 T)   | Size 2(96 T)    | Size 3<br>(500Assays) | Storage                         |
|-----------|----------------------------|----------------|-----------------|-----------------------|---------------------------------|
| Reagent 3 | Ferric Salt Stock Solution | 0.2 mL×1 vial  | 0.4 mL×1 vial   | 2 mL×1 vial           | 2-8°C, 12 months, shading light |
| Reagent 4 | Ferric Salt Diluent        | 4 mL×1 vial    | 8 mL×1 vial     | 40 mL×1 vial          | 2-8°C, 12 months                |
| Reagent 5 | Stop Solution              | 1.25 mL×1 vial | 1.25 mL×2 vials | 12.5 mL×1 vial        | 2-8°C, 12 months                |
| Reagent 6 | Clarificant                | 1.25 mL×1 vial | 1.25 mL×2 vials | 12.5 mL×1 vial        | 2-8°C, 12 months                |
|           | Microplate                 | 48 wells       | 96 wells        | /                     | No requirement                  |
|           | Plate Sealer               | 2 pieces       | 2 pieces        | 2 pieces              |                                 |

## 5. Materials prepared by users

### Instruments:

Microplate reader (500-520 nm, optimum wavelength: 520 nm), Micropipettor, Centrifuge, Incubator, Vortex mixer

### Reagents:

Double distilled water, Normal saline (0.9% NaCl), PBS (0.01 M, pH 7.4)

## 6. Reagent preparation

1. Equilibrate all reagents to room temperature before use.
2. **Preparation of chromogenic working solution:** Dissolve one vial of chromogenic agent with 20 mL of double distilled water. It can be dissolved by incubating in 80-90°C water bath and used after cooling to room temperature. Store at 2-8°C for 7 days.
3. **Preparation of ferric salt working solution:** Before testing, prepare sufficient ferric salt working solution according to the test wells. For example, prepare 100 µL of ferric salt working solution (mix well 5 µL of ferric salt stock solution and 95 µL of ferric salt diluent). The ferric salt working solution should be prepared on spot and protected from light. Store at 2-8°C for 2 days.

4. Clarificant will freeze in cold weather. Dissolve by incubating in 37°C water bath until clarified before experiment.

## 7. Sample preparation

### Sample preparation:

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

**Cell culture supernatant:** Detect the cell culture supernatant directly. If there is turbidity, centrifuge at 3100 g for 10 min, take the supernatant and preserve it on ice for detection. If not detected on the same day, the cell culture supernatant 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 min to remove insoluble material. Collect supernatant and keep it on ice for detection.
5. Meanwhile, determine the protein concentration of supernatant (MAES0177).

Cell (adherent or suspension) samples:

1. Harvest the number of cells needed for each assay (initial recommendation  $1 \times 10^6$  cells).
2. Wash cells with PBS (0.01 M, pH 7.4).
3. Homogenize  $1 \times 10^6$  cells in 300-500 µL PBS (0.01 M, pH 7.4) with an ultrasonic cell disruptor at 4°C.
4. Centrifuge at 10000×g for 10 min 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
- Human urine: 1-2
- 10% Rat liver tissue homogenate: 1

- 10% Epipremnum aureum tissue homogenate: 1
- HepG2 cells: 1
- HepG2 cell culture supernatant: 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 do pretest to confirm the dilution factor.

## 8. The key points of the assay

1. The supernatant of sample preparation after centrifugation must be clarified, otherwise centrifuge again.
2. After the sample reaction, ensure to complete the detection within 25 min.

## 9. Operating steps

For serum (plasma) and other liquid samples

① Sample tube: Add 100  $\mu$ L of buffer solution to 1.5 mL EP tube.

Control tube: Add 100  $\mu$ L of buffer solution to 1.5 mL EP tube.

② Sample tube: Add 10  $\mu$ L of sample to the tube.

Control tube: Add nothing.

③ Add 200  $\mu$ L of chromogenic working solution and 50  $\mu$ L of ferric salt working solution to sample tube and control tube.

④ Mix well and incubate the tubes at 37°C for 30 min.

⑤ Add 10  $\mu$ L of stop solution to sample tube and control tube.

⑥ Sample tube: Add nothing.

Control tube: Add 10  $\mu$ L of sample to the tube.

⑦ Mix fully and stand for 5 min at room temperature. Take 300  $\mu$ L of reaction liquid to 96 well microplate and measure the OD value of each well at 520 nm with microplate reader.

2. For tissue and cells samples

① Sample tube: Add 100  $\mu$ L of buffer solution to 1.5 mL EP tube.

Control tube: Add 100  $\mu$ L of buffer solution to 1.5 mL EP tube.

② Sample tube: Add 10  $\mu$ L of sample to the tube.

Control tube: Add nothing.

③ Add 200  $\mu$ L of chromogenic working solution and 50  $\mu$ L of ferric salt working solution to sample tube and control tube.

④ Mix fully and incubate the tubes at 37°C for 30 min.

⑤ Add 20 µL of stop solution to sample tube and control tube.

⑥ Sample tube: Add nothing.

Control tube: Add 10 µL of sample to the tube.

⑦ Add 20 µL of clarificant to sample tube and control tube.

⑧ Mix fully and stand for 5 min at room temperature. Take 300 µL of reaction liquid to 96 well microplate and measure the OD value of each well at 520 nm with microplate reader.

### 3. For whole blood samples

① Sample tube: Add 100 µL of buffer solution to 1.5 mL EP tube.

Control tube: Add 100 µL of buffer solution to 1.5 mL EP tube.

② Sample tube: Add 10 µL of sample to the tube.

Control tube: Add nothing.

③ Add 200 µL of chromogenic working solution and 50 µL of ferric salt working solution to sample tube and control tube.

④ Mix fully and incubate the tubes at 37°C for 30 min.

⑤ Add 20 µL of stop solution to sample tube and control tube.

⑥ Sample tube: Add nothing.

Control tube: Add 10 µL of sample to the tube.

⑦ Mix fully and stand for 5 min at room temperature. Take 300 µL of reaction liquid to 96 well microplate and measure the OD value of each well at 520 nm with microplate reader.

## 10. Calculation

Serum (plasma), whole blood and other liquid samples

**Definition:** At 37°C, the OD value of the reaction system was increased 0.01 by 1 mL of sample per minute is defined as a unit of total antioxidant capacity.

$$\text{T-AOC activity (U/mL)} = \Delta A / (0.01 \div 30) \times (V_1/V_2) \times f$$

### 2. Tissue and cell samples

**Definition:** At 37°C, the OD value of the reaction system was increased 0.01 by 1 mg of protein per minute is defined as a unit of total antioxidant capacity.

$$\text{T-AOC activity (U/mgprot)} = \Delta A / (0.01 \div 30) \times (V_1/V_2) \times f \div C_{pr}$$

**Note:**

$$\Delta A: \text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}}$$

30: The reaction time, 30 min.

$V_1$ : The total volume of reaction, mL.

$V_2$ : The volume of sample added to the reaction, mL.

f: Dilution factor of sample before tested.

$C_{pr}$ : Concentration of protein in sample, mgprot/mL.

## 11. 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)

### Intra-assay Precision

| Parameters  | Sample 1 | Sample 2 | Sample 3 |
|-------------|----------|----------|----------|
| Mean (U/mL) | 3.50     | 64.50    | 138.50   |
| %CV         | 5.1      | 4.7      | 4.6      |

### Inter-assay Precision

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

### Inter-assay Precision

| Parameters  | Sample 1 | Sample 2 | Sample 3 |
|-------------|----------|----------|----------|
| Mean (U/mL) | 3.50     | 64.50    | 138.50   |
| %CV         | 5.3      | 5.7      | 5.8      |

## 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 96%.

## Recovery

| Standard 1 | Standard 2 | Standard 3 |
|------------|------------|------------|
| 22.5       | 84.6       | 164.5      |
| 22.3       | 80.4       | 154.6      |
| 99         | 95         | 94         |

## Sensitivity

The analytical sensitivity of the assay is 0.62 U/mL. 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.

## 12. Example Analysis

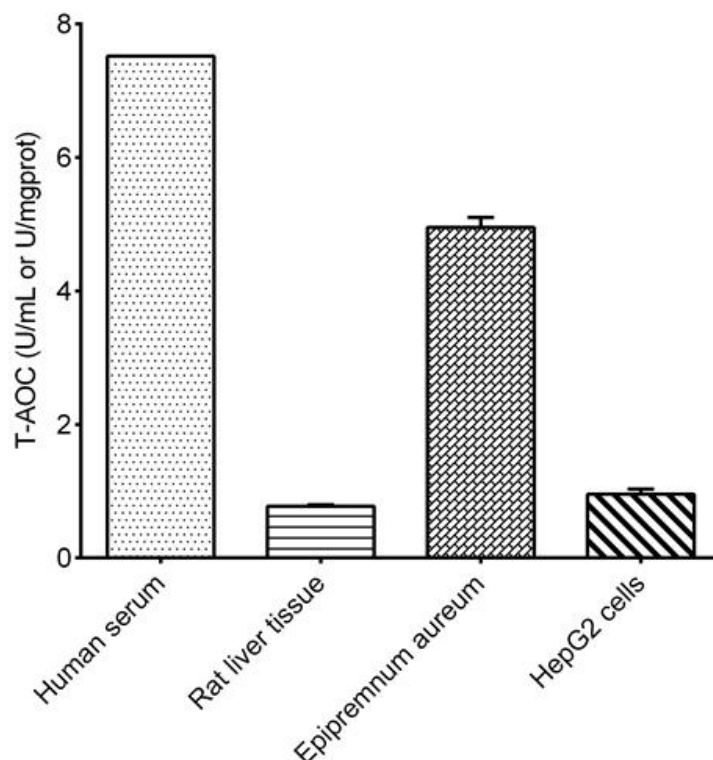
### Example analysis:

Take 10  $\mu$ L of human serum sample and carry the assay according to the operation steps. The results are as follows:

The average OD value of the sample is 0.081, the average OD value of the control is 0.020, and the calculation result is:

$$\text{T-AOC (U/mL)} = (0.081 - 0.020) / (0.01 \div 30) \times (0.37/0.01) = 7.52 \text{ U/mL}$$

Detect human serum, 10% rat liver tissue homogenate (the concentration of protein in sample is 13.99 mgprot/mL), 10% Epipremnum aureum tissue homogenate (the concentration of protein in sample is 1.59 mgprot/mL) and HepG2 cells (the concentration of protein in sample is 5.02 mgprot/mL) 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.**