TECHNICAL MANUAL

Total Antioxidant Capacity (T-AOC) Colorimetric Assay Kit (ABTS, Enzyme)

- **SKU CODE:** MAES0147
- **SIZE:** 48 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Sample preparation
- Reagent preparation
- Standard curve preparation
- Add standards and samples
- Add peroxidase application solution
- Add ABTS working solution
- Measure absorbance at 414 nm

2. Intended use

This kit can be used to measure total antioxidant capacity (T-AOC) in serum, plasma, tissue, cells, or other samples.

3. Detection principle

The principle of the ABTS method for determining T-AOC is as follows. ABTS is oxidized to green ABTS+ by an oxidant. This process can be inhibited by the presence of antioxidants. The T-AOC of the sample can be determined and calculated by measuring the absorbance of ABTS+ at 414 nm or 734 nm. Trolox is an analog of vitamin E and has similar antioxidant capacity to that of vitamin E. Trolox is used as a reference for other antioxidants. For example, if the T-AOC of Trolox is 1, then the antioxidant capacity of another substance at the same concentration is shown by the ratio of its antioxidant capacity to Trolox antioxidant capacity.

4. Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Size 3(500 Assays)	Storage
Reagent 1	Buffer Solution	12 mL × 1 vial	24 mL × 1 vial	24 mL × 5 vials	2-8°C, 12 months
Reagent 2	ABTS Solution	0.5 mL × 1 vial	1 mL × 1 vial	1 mL × 5 vials	2-8°C, 12 months, protect from light

Item	Component	Size 1(48 T)	Size 2(96 T)	Size 3(500 Assays)	Storage
Reagent 3	H2O2 Solution	0.25 mL × 1 vial	0.5 mL × 1 vial	0.5 mL × 5 vials	2-8°C, 12 months
Reagent 4	Peroxidase	0.1 mL × 1 vial	0.2 mL × 1 vial	0.2 mL × 5 vials	2-8°C, 12 months
Reagent 5	5 mmol/L Trolox Standard	0.3 mL × 1 vial	0.6 mL × 1 vial	0.6 mL × 5 vials	-20°C, 12 months, protect from light
	Microplate	48 wells	96 wells	/	No requirement
	Plate Sealer	2 pieces	2 pieces	2 pieces	

5. Materials prepared by users

Instruments:

Microplate reader (405-425 nm), Micropipettor

Reagents:

Double distilled water, Normal saline (0.9% NaCl), PBS (0.01 M, pH 7.4), 60% Ethanol

Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other. For small volume reagents, please centrifuge before use to ensure sufficient amount of reagent is obtained.

6. Reagent preparation

1. **Preparation of H2O2 application solution:** Before testing, prepare sufficient H2O2 application solution according to the number of test wells. For example, to prepare 200 µL of H2O2 application solution, mix 5 µL of H2O2 solution with 195 µL of double distilled water. The H2O2 application solution should be prepared fresh.
2. **Preparation of ABTS working solution:** For each well, prepare 170 µL of ABTS working solution by mixing 152 µL of buffer solution, 10 µL of ABTS solution, and 8 µL of H2O2 application solution. Store the prepared solution at room temperature, protected from light, and use within 30 minutes.

3. **Preparation of peroxidase application solution:** For each well, prepare 20 µL of peroxidase application solution by mixing 2 µL of peroxidase with 18 µL of buffer solution. Store the prepared solution at room temperature, protected from light, and use within 30 minutes.
4. **Preparation of standard curve:** Always prepare a fresh set of standards. Discard working standard dilutions after use. Dilute 5 mmol/L Trolox Standard with PBS or 60% ethanol to create a serial dilution. The recommended dilution gradient is: 0, 0.1, 0.2, 0.3, 0.4, 0.6, 0.8, 1.0 mmol/L. If the sample is water-soluble, dilute the standard with PBS. If the sample is water-insoluble, dilute the standard with 60% ethanol.

7. Standard curve preparation table

Item	1	2	3	4	5	6	7	8
Concentration (mmol/L)	0	0.1	0.2	0.3	0.4	0.6	0.8	1.0
5 mmol/L Trolox Standard (µL)	0	4	8	12	16	24	32	40
Diluent (µL)	200	196	192	188	184	176	168	160

8. 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 one month.

Urine: Collect fresh urine and centrifuge at 10,000×g for 10 minutes at 4°C. Take the supernatant and preserve on ice for detection. If not detected on the same day, the urine can be stored at -80°C for one 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 of 60% ethanol with a dounce homogenizer at 4°C.

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

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

Cells:

1. Harvest the number of cells needed 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 300-500 μL PBS (0.01 M, pH 7.4) with an ultrasonic cell disruptor at 4°C.

4. Centrifuge at 10,000×g for 10 minutes to remove insoluble material. Collect supernatant and keep 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):

10% Rat brain tissue homogenization: 1

10% Rat liver tissue homogenization: 1

10% Rat kidney tissue homogenization: 1

10% Epipremnum aureum tissue homogenization: 1

Human serum: 1

Human saliva: 1

Human urine: 1

Rat serum: 1

Note: When the sample is water-soluble, the diluent is PBS (0.01 M, pH 7.4); when the sample is water-insoluble, the diluent is 60% ethanol. For dilution of other sample types, please perform a pretest to confirm the dilution factor.

9. The key points of the assay

ABTS working solution should be stored at room temperature, protected from light, and used within 30 minutes.

10. Operating steps

1. Standard well: Add 10 µL of standard with different concentrations into the standard wells. Sample well: Add 10 µL of sample into the sample wells.
2. Add 20 µL of peroxidase application solution to each well.
3. Add 170 µL of ABTS working solution to each well.
4. Mix well and incubate for 6 minutes at room temperature. Measure the OD values of each well at 414 nm with a microplate reader.

11. Calculation

The standard curve:

1. Average the duplicate readings 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 using the absolute OD value of standards and corresponding concentrations as y-axis and x-axis respectively. Create the standard curve (y = ax + b) with graph software (or Excel).

The sample:

1. Serum (plasma) and other liquid samples:

T-AOC (mmol/L) = $(A_{414} - b) \div a \times f$

2. Tissue sample:

T-AOC (mmol/kg wet weight) = $(A_{414} - b) \div a \div (m/V) \times f$

3. Cell sample:

T-AOC (mmol/g prot) = $(A_{414} - b) \div a \div C_{pr} \times f$

[Note]

A_{414}: Average OD of sample.

f: Dilution factor of sample before test.

V: The volume of sample homogenate, mL.

m: The weight of tissue sample, g.

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

12. 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).

Intra-assay Precision Results

Parameters	Sample 1	Sample 2	Sample 3
Mean (mmol/L)	0.12	0.58	1.20
%CV	2.5	2.3	1.8

Inter-assay Precision

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

Inter-assay Precision Results

Parameters	Sample 1	Sample 2	Sample 3
Mean (mmol/L)	0.12	0.58	1.20
%CV	4.0	3.9	4.4

Recovery

Three samples of high, medium, and low concentrations were tested with 6 parallel measurements each to obtain an average recovery rate of 102%.

Recovery Results

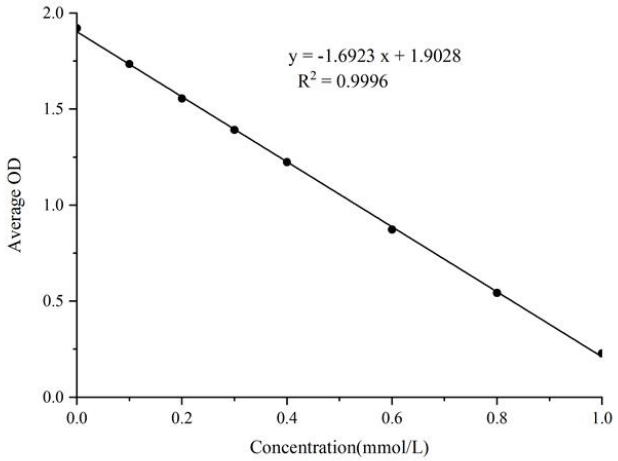
Parameters	Standard 1	Standard 2	Standard 3
Expected Conc. (mmol/L)	0.15	0.33	0.75
Observed Conc. (mmol/L)	0.2	0.3	0.8
Recovery rate (%)	101	105	100

Sensitivity

The analytical sensitivity of the assay is 0.047 mmol/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 below for reference only:



Standard curve data

Concentration (mmol/L)	Average OD
0	1.921
0.1	1.735
0.2	1.555
0.3	1.392
0.4	1.224
0.6	0.873
0.8	0.543
1.0	0.229

13. Appendix II Example Analysis

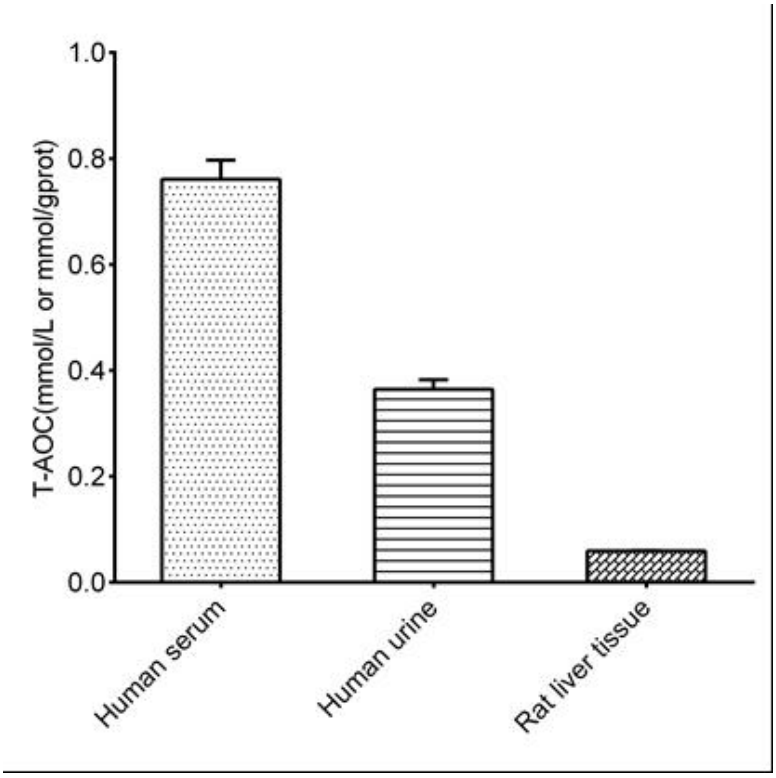
Example analysis:

Take 10 µL of human serum and perform the assay according to the operation steps. The results are as follows:

Standard curve: y = -1.122x + 1.7172, the average OD value of the sample is 0.863, and the calculation result is:

T-AOC (mmol/L) = (0.863 - 1.7172) ÷ (-1.122) = 0.76 mmol/L

Detect human serum, 10% rat liver tissue homogenate (protein concentration is 15.14 g prot/L), and human urine according to the protocol. The results are as follows:



14. 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.