# TECHNICAL MANUAL

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

- **SKU CODE:** MAES0168
- **SIZE:** 48 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

# 1. Assay summary

- Reagent preparation
- Sample preparation
- Add standard and sample
- Add ABTS working solution
- Measure absorbance at 734 nm

# 2. Intended use

This kit can be used to measure total antioxidant capacity (T-AOC) in serum, plasma, urine, saliva, tissue, cells and 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 appropriate oxidant, which can be inhibited if antioxidants are present. The T-AOC of the sample can be determined and calculated by measuring the absorbance of ABTS+ at 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 with 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 | ABTS Solution | 0.3 mL x 1 vial | 0.6 mL x 1 vial | 3.5 mL x 1 vial | -20 °C, 12 months, shading light |
| Reagent 2 | Oxidant Solution | 0.3 mL x 1 vial | 0.6 mL x 1 vial | 3.5 mL x 1 vial | -20 °C, 12 months |
| Reagent 3 | 5 mmol/L Trolox Standard | 0.5 mL x 1 vial | 0.5 mL x 1 vial | 1.5 mL x 2 vials | -20 °C, 12 months, shading light |

| Item | Component | Size 1 (48 T) | Size 2 (96 T) | Size 3 (500 Assays) | Storage |
|---|---|---|---|---|---|
| Reagent 4 | 10x PBS Solution | 1.5 mL x 1 vial | 1.5 mL x 2 vials | 20 mL x 1 vial | -20 °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 (730-740 nm), Micropipette, Centrifuge, Vortex mixer

**Reagents:**

Double distilled water, 1x PBS, 80% 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 volumes of reagents, please centrifuge before use to obtain sufficient amounts of reagents.

# 6. Reagent preparation

1. **Preparation of concentrated ABTS working solution:** Mix the ABTS solution and oxidant solution at a ratio of 1:1 thoroughly. The concentrated ABTS working solution can be used after storing at room temperature for 12-16 hours protected from light. Store at 2-8 °C for up to 2 days.
2. **Preparation of 1x PBS solution:** Dilute the 10x PBS solution with double distilled water 10-fold.
3. **Preparation of ABTS working solution:** Dilute the concentrated ABTS working solution with 1x PBS or 80% ethanol (self-prepared). The absorbance at 734 nm of blank tube (10 µL diluent + 200 µL ABTS working solution) should be 0.9-1.1. Note: If the sample to be tested is water-soluble, use PBS as diluent and dilute concentrated ABTS working solution with PBS 20-30 times. If the sample to be tested is water-insoluble, use 80% ethanol (self-prepared) as diluent and dilute concentrated ABTS working solution with 80% ethanol 22-32 times.
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 1x PBS

or 80% ethanol (self-prepared) to serial concentrations. The recommended dilution gradient is as follows: 0, 0.15, 0.3, 0.45, 0.6, 0.75, 0.9, 1 mmol/L. Dilution table: Concentration (mmol/L): 0, 0.15, 0.3, 0.45, 0.6, 0.75, 0.9, 1.0 5 mmol/L Trolox (µL): 0, 6, 12, 18, 24, 30, 36, 40 Diluent (µL): 200, 194, 188, 182, 176, 170, 164, 160 Note: If the sample to be tested is water-soluble, dilute the standard and samples with PBS. If the sample to be tested is water-insoluble, dilute the standard and samples with 80% ethanol.

# 7. Sample preparation

Sample requirements:

SDS, Tween, Triton, NP-40 and other detergents should not be added to the samples, and DTT, 2-mercaptoethanol and other reducing substances should not be added.

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

Urine: Collect fresh urine and centrifuge at 10,000 x g for 15 min at 4 °C. Take the supernatant and preserve it on ice for detection.

**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 10,000 x 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.

**Cells:**

1. Harvest the number of cells needed for each assay (initial recommendation 1 x $10^6$ cells).

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

3. Homogenize 1 x $10^6$ cells in 200 µL PBS (0.01 M, pH 7.4) with 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 it on ice for detection.

5. Meanwhile, determine the protein concentration of supernatant.

**Dilution of sample:**

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

20% Tomato tissue homogenate: 2-5 fold

10% Mouse heart tissue homogenate: 8-12 fold

10% Mouse liver tissue homogenate: 8-12 fold

10% Mouse lung tissue homogenate: 8-12 fold

Human saliva: 2-5 fold

Human urine: 15-30 fold

**Human serum:** 15-30 fold

**Human plasma:** 8-15 fold

**Note:** The diluent is 1x PBS or 80% ethanol. For the dilution of other sample types, please perform a pretest to confirm the dilution factor.

# 8. The key points of the assay

1. ABTS solution and ABTS working solution should be stored with light protection, otherwise the OD value will decrease.
2. If the sample to be tested is water-soluble, dilute the standard and samples with PBS. If the sample to be tested is water-insoluble, dilute the standard and samples with 80% ethanol.

# 9. Operating steps

1. Standard well: Add 10 µL of standard with different concentrations to the corresponding wells. Sample well: Add 10 µL of sample to the corresponding wells.
2. Add 200 µL of ABTS working solution to each well.
3. Mix thoroughly and incubate for 2-6 min at room temperature. Measure the OD values of each well at 734 nm with microplate reader.

# 10. Calculation

**The standard curve:**

1. Average the duplicate readings for each standard.

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

3. Plot the standard curve using absolute OD value of standard and corresponding 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) and other liquid samples:

T-AOC (mmol/L) = $(A_{734} - b) \div a \times f$

2. Tissue and cells samples:

T-AOC (mmol/$g_{prot}$) = $(A_{734} - b) \div a \div C_{pr} \times f$

**Note:**

$A_{734}$: $OD_{Blank} - OD_{Sample}$

f: Dilution factor of sample before test

$C_{pr}$: Concentration of protein in sample, $g_{prot}$/L

# 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)

## Intra-assay Precision

| Parameters | Sample 1 | Sample 2 | Sample 3 |
|---|---|---|---|
| Mean (mmol/L) | 0.25 | 0.45 | 0.76 |
| %CV | 4.5 | 4.1 | 3.7 |

## 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 (mmol/L) | 0.25 | 0.45 | 0.76 |
| %CV | 4.7 | 4.9 | 5.4 |

## Recovery

Three samples of high, middle, and low concentrations were tested with 6 replicates for each concentration to obtain an average recovery rate of 102%.

## Recovery

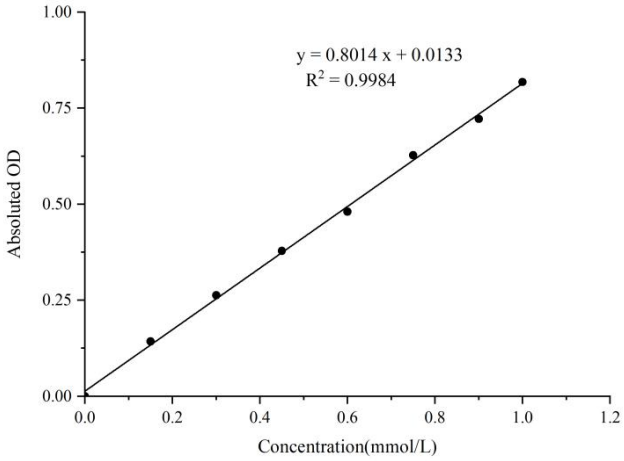
| | Standard 1 | Standard 2 | Standard 3 |
|---|---|---|---|
| Expected Conc. (mmol/L) | 0.2 | 0.55 | 0.85 |
| Observed Conc. (mmol/L) | 0.2 | 0.5 | 0.9 |
| Recovery rate (%) | 101 | 99 | 106 |

## Sensitivity

The analytical sensitivity of the assay is 0.05 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 data

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 | Absolute OD |
|---|---|---|
| 0 | 0.953 | 0 |
| 0.15 | 0.810 | 0.143 |
| 0.3 | 0.690 | 0.263 |
| 0.45 | 0.574 | 0.379 |
| 0.6 | 0.472 | 0.481 |
| 0.75 | 0.325 | 0.628 |
| 0.9 | 0.231 | 0.722 |
| 1 | 0.135 | 0.818 |

# 12. Appendix II Example Analysis

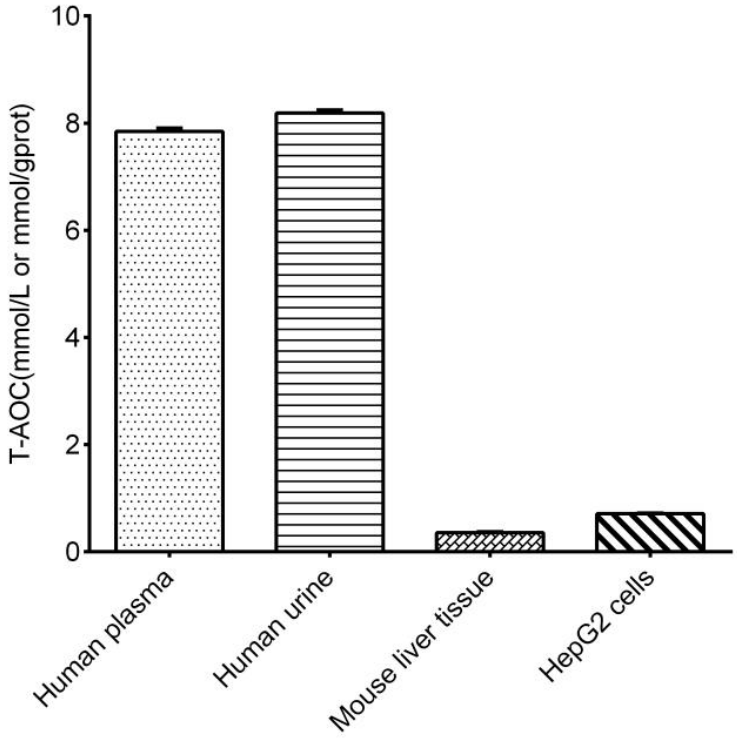
**Example analysis:**

Human plasma was diluted 12-fold with 1x PBS, then 10 µL of diluted sample was used and the assay was carried out according to the operating steps. The results are as follows:

**Standard curve:** y = 0.8014x + 0.0133, the average OD value of the sample well is 0.412, the average OD value of the blank well is 0.953, and the calculation result is:

T-AOC (mmol/L) = (0.953 - 0.412 - 0.0133) ÷ 0.8014 × 12 = 7.89 mmol/L

Human plasma (diluted 12-fold), human urine (diluted 20-fold), 10% mouse liver tissue homogenate (protein concentration 11.14 $g_{prot}$/L, diluted 9-fold) and HepG2 cells (protein concentration 3.18 $g_{prot}$/L, diluted 4-fold) were detected according to the protocol. The results are 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 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.**