（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

**Catalog No：E-BC-K271-M**

**Specification：48T(32 samples)/96T(80 samples)/ 500Assays(484 samples)**

**Measuring instrument：Microplate reader (730-740 nm)**

**Detection range：0.05-1.00 mmol/L**

# **Elabscience®** Total Antioxidant Capacity (T-AOC) Colorimetric Assay Kit (ABTS, Chemical Method)

This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
Tell: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

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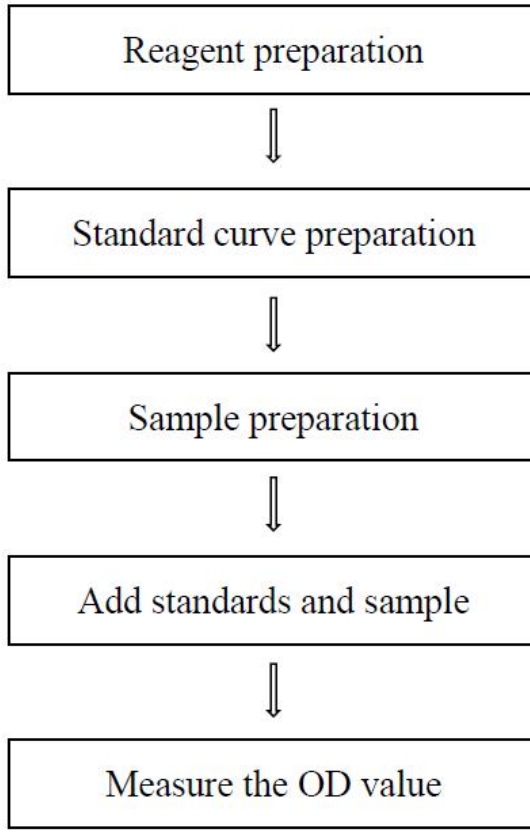
**Assay summary**



## Intended use

This kit can be used to measure total antioxidant capacity (T-AOC) in serum, plasma, urine, saliva, tissue, cells and other sample.

## Detection principle

The principle of the ABTS method for determining the T-AOC is as follows. ABTS is oxidized to green $ABTS^+$ by appropriate oxidant, which can be inhibited if there exist antioxidants. 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 VE and has a similar antioxidant capacity to that of VE. Trolox is used as a reference for other antioxidant antioxidants. For example, the T-AOC of Trolox is 1, then the antioxidant capacity of the other substance with the same concentration is showed by the ratio of its antioxidant capacity to Trolox antioxidant capacity.

## 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×1 vial | 0.6 mL×1 vial | 3.5 mL×1 vial | -20°C, 12 months shading light |
| Reagent 2 | Oxidant Solution | 0.3 mL×1 vial | 0.6 mL×1 vial | 3.5 mL×1 vial | -20°C, 12 months |
| Reagent 3 | 5 mmol/L Trolox Standard | 0.5 mL×1 vial | 0.5 mL×1 vial | 1.5 mL×2 vials | -20°C, 12 months shading light |
| Reagent 4 | 10×PBS Solution | 1.5 mL×1 vial | 1.5 mL×2 vials | 20 mL×1 vial | -20°C, 12 months |
| | Microplate | 48 wells | 96 wells | / | No requirement |
| | Plate Sealer | 2 pieces | | | |

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 a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

## Materials prepared by users

**Instruments:**

Microplate reader (730-740 nm), Micropipettor, Centrifuge, Vortex mixer

**Reagents:**

Double distilled water, 1 × PBS, 80% Ethanol

## Reagent preparation

① The preparation of concentrated ABTS working solution：
Mix the ABTS solution and oxidant solution at a ratio of 1: 1 fully, the concentrated ABTS working solution can be use after store at room temperature for 12-16 hours protected from light. Store at 2-8°C for 2 days.

② The preparation of 1×PBS solution：
Dilute the 10×PBS solution with double distilled water for 10 times.

③ The preparation of ABTS working solution：
Dilute the concentrated ABTS working solution with 1×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, the diluent is PBS, dilute concentrated ABTS working solution with PBS for 20-30 times. If the sample to be tested is water-insoluble, the diluent is 80% ethanol (self-prepared), dilute concentrated ABTS working solution with 80% ethanol for 22-32 times.

④ The preparation of standard curve：
Always prepare a fresh set of standards. Discard working standard dilutions after use.

Dilute 5 mmol/L trolox standard with 1×PBS or 80% ethanol (self-prepared) to a serial concentration. The recommended dilution gradient is as follows: 0, 0.15, 0.3, 0.45, 0.6, 0.75, 0.9, 1 mmol/L. Reference is as follows:

| Item | ① | ② | ③ | ④ | ⑤ | ⑥ | ⑦ | ⑧ |
|---|---|---|---|---|---|---|---|---|
| **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.

## Sample preparation

**① 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.

**② 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.

**Urine:** collect fresh urine and centrifuge at 10000×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 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 (E-BC-K318-M).

**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 200 μL PBS (0.01 M, pH 7.4) with a ultrasonic cell disruptor 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 (E-BC-K318-M).

### ③ Dilution of sample

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

| Sample type | Dilution factor |
|---|---|
| 20% Tomato tissue homogenization | 2-5 |
| 10% Mouse heart tissue homogenization | 8-12 |
| 10% Mouse liver tissue homogenization | 8-12 |
| 10% Mouse lung tissue homogenization | 8-12 |
| Human saliva | 2-5 |
| Human urine | 15-30 |
| Human serum | 15-30 |
| Human plasma | 8-15 |

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

## The key points of the assay

① ABTS solution and ABTS working solution should be stored with shading light, otherwise the OD value will be decreased.

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.

## Operating steps

① Standard well: add 10 μL of standard with different concentration to the corresponding well.

Sample well: add 10 μL of sample to the corresponding well.

② Add 200 μL of ABTS working solution to each well.

③ Mix fully and stand for 2-6 min at room temperature. Measure the OD values of each well at 734 nm with microplate reader.

## Calculation

**The standard curve**:

1. Average the duplicate reading for each standard.

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

3. Plot the standard curve by using absoluted 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) and other liquid sample:**

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

**2. Tissue and cells sample:**

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

[Note]

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

f: Dilution factor of sample before test.

$C_{pr}$: Concentration of protein in sample, gprot/L.

# Appendix I Performance Characteristics

## 1. Parameter:

### Intra-assay Precision

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

| 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.

| Parameters | Sample 1 | Sample 2 | Sample 3 |
|---|---|---|---|
| **Mean (mmol/L)** | 0.25 | 0.45 | 0.76 |
| **%CV** | 4.7 | 4.9 | 5.4 |

### 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 102%.

| | 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 |

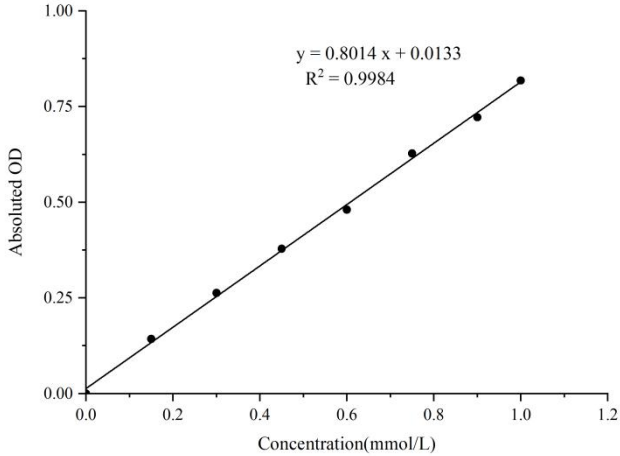
### Sensitivity

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.

**2. 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), so the standard curve and data are provided as below for reference only:

| Concentration (mmol/L) | 0 | 0.15 | 0.3 | 0.45 | 0.6 | 0.75 | 0.9 | 1 |
|---|---|---|---|---|---|---|---|---|
| Average OD | 0.953 | 0.810 | 0.690 | 0.574 | 0.472 | 0.325 | 0.231 | 0.135 |
| Absoluted OD | 0 | 0.143 | 0.263 | 0.379 | 0.481 | 0.628 | 0.722 | 0.818 |



# Appendix II Example Analysis

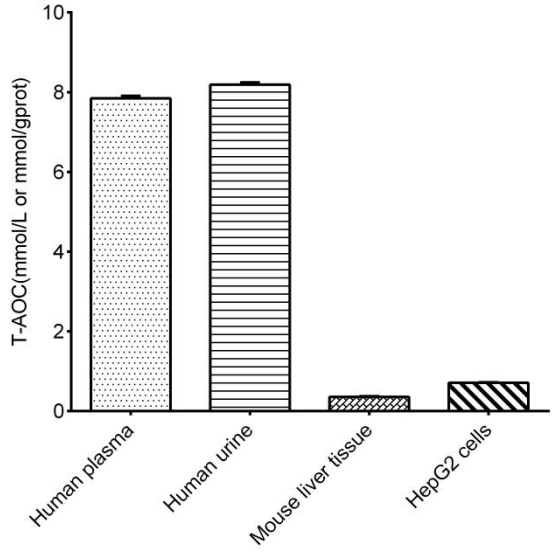
**Example analysis：**

Dilute the human plasma with 1×PBS for 12 times, then take 10 μL of diluted sample and carry the assay according to the operation steps. The results are as follows:

Standard curve: y = 0.8014 x + 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:

$$\text{T-AOC (mmol/L)} = (0.953 - 0.412 - 0.0133) \div 0.8014 \times 12 = 7.89 \text{ mmol/L}$$

Detect human plasma (dilute for 12 times), human urine (dilute for 20 times), 10% mouse liver tissue homogenate (the concentration of protein is 11.14 gprot/L, dilute for 9 times) and HepG2 cells (the concentration of protein is 3.18 gprot/L, dilute for 4 times) according to the protocol, the result is as follows：



# Appendix III Publications


1. Liu Q , Wang L , Wang Z ,et al.Preparation and characterization of carvacrol/soybean protein isolate composite film with efficient antimicrobial and antioxidant activities and its application in grape preservation[J].Food Chemistry, 2025, 464.DOI:10.1016/j.foodchem.2024.141572.
2. Bao L , Huang Y , Gu F ,et al.Zearalenone induces liver injury in mice through ferroptosis pathway[J].The Science of the total environment, 952:175875[2025-03-03].DOI:10.1016/j.scitotenv.2024.175875.
3. Marhamati S , Younesian O , Mir S M ,et al.The effects of high doses of selenium supplementation on mRNA and protein levels of cMLCK levels and total antioxidant capacity in rat heart tissue[J].Food and Chemical Toxicology, 2024, 191(000):4.DOI:10.1016/j.fct.2024.114886.
4. Wei S , Amevor F K , Du X ,et al.Quercetin mitigates iron-induced cell death in chicken granulosa cell[J].Journal of Animal Science and Biotechnology, 2024, 15(1).DOI:10.1186/s40104-024-01118-0.
5. Tefnu C L .The Interplay between Oxidative Stress and Fatty Acids Profile in Romanian Spotted Cows with Placental Retention[J].Veterinary Sciences, 2024, 11.DOI:10.3390/vetsci11100499.

## Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.