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

# Total Antioxidant Capacity (T-AOC) Assay Kit (ABTS Method)

Catalog No.: BC00013

Size: 300T

Please read the instructions carefully before use. If you have any questions or need further help during experiment, please don't hesitate to contact us through the following methods:


✉ Email (Sale) order@enkilife.com

✉ Email (Techsupport) techsupport@enkilife.com

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**Shelf life:** Please refer to the label on the outer package.

**Techsupport:** In order to provide you with better service, please inform us the lot number on the label of the outer package.

## Basic Information

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|---|---|
| **Product Name** | Total Antioxidant Capacity (T-AOC) Assay Kit (ABTS Method) |
| **Detection Method** | Colorimetric |
| **Sample Type** | Tissue, cell, serum, plasma, saliva, urine, plant or herbal extracts |
| **Assay Type** | Quantitative |
| **Detection Instrument** | Microplate reader (725-745 nm, optimal detection wavelength 734 nm; or 405 nm) |
| **Range** | 0.2–1.6mM |
| **Sensitivity** | 0.0513mM |

## Product Introduction

Reactive oxygen species (ROS) mainly include hydroxyl radicals, superoxide radicals and hydrogen peroxide. Reactive oxygen species can cause oxidative damage to intracellular lipids, proteins and DNA, induce oxidative stress, and then lead to various tumors, atherosclerosis, rheumatoid arthritis, diabetes, liver damage, and central nervous system diseases.

There are many kinds of antioxidants in the body, including antioxidant macromolecules, antioxidant small molecules and enzymes, which can remove various reactive oxygen species produced in the body to prevent the generation of oxidative stress induced by reactive oxygen species. The total level of various antioxidant macromolecules, antioxidant small molecules and enzymes in a system reflects the total antioxidant capacity in the system. Therefore, it is of great biological significance to measure the total antioxidant capacity in various body fluids such as plasma, serum, urine, saliva, and lysates of cells or tissues.

The detection of the total antioxidant capacity of plant or Chinese herbal extracts, or various antioxidant solutions can be used to detect the strength of the antioxidant capacity of various solutions, and can be used to screen drugs with strong antioxidant capacity.

## Product Features

●This kit is convenient and quick. The absorbance measurement can be performed 2-6 minutes after adding the sample to be tested. Usually 10-20 samples can be tested in more than ten minutes.

## Principle

This kit uses ABTS (2,2'-hydrazinobis[3-ethylbenzothiazole-6-sulfonic acid]) as a colorimetric agent, which can accurately determine the total antioxidant capacity in the sample. ABTS is oxidized to green ABTS+ in the presence of an appropriate oxidant. The production of ABTS+ will be inhibited in the presence of antioxidants. The total antioxidant capacity of the sample can be determined and calculated by measuring the absorbance of ABTS+ at 734nm or 405nm. Trolox is an analog of vitamin E with an antioxidant capacity similar to that of vitamin E. It is used as a reference for the total antioxidant capacity of other antioxidants. For example, the total antioxidant capacity of Trolox is 1. Under the same concentration, the antioxidant capacity of other substances is expressed as a multiple of their antioxidant capacity compared to Trolox.

## Components

| Serial number | Components | Size(300T) | Storage |
|---|---|---|---|
| Reagent 1 | ABTS Solution | 1ml | -20℃, keep away from light. |
| Reagent 2 | Oxidant Solution | 1ml | -20℃ |
| Reagent 3 | Trolox Solution (10 mM) | 0.5ml | -20℃, keep away from light. |
| Consumable 1 | Microplate(96 wells) | 3 plates | RT |
| Consumable 2 | Plate Sealer | 6 pieces | RT |

## Storage

The unopened kit can be stored at -20℃ for 12 months.

## Experimental Preparation

- Sample processing

1. Preparation of serum, plasma, saliva or urine samples

Serum, plasma, saliva or urine samples require 10μl each and can be used directly for measurement. Serum, plasma, saliva or urine samples can be measured using fresh samples or frozen at -80ºC before measurement. There is no significant change in the data measured for samples frozen at -80ºC for at least one month.

Note: Heparin or sodium citrate can be used for anticoagulation when preparing plasma, but EDTA should not be used for anticoagulation. According to literature reports, the total antioxidant capacity in human serum or plasma is 0.5-2mM, the total antioxidant capacity in human saliva is 0.3-1mM, and the total antioxidant capacity in human urine is 0.2-3mM.

2. Cell or tissue sample preparation

For cell samples, collect about 1 million cells (no need to count accurately, scrape directly, trypsin and EDTA digestion is not suitable), place in 200μl of ice-cold PBS or HBSS solution, homogenize or sonicate to fully disrupt the cells and To release the antioxidants, centrifuge at about 12,000 x g for 5 minutes at 4ºC, and take the supernatant for subsequent determination.
For tissue samples, add 100 μl of ice-cold PBS or HBSS solution per 20 mg of tissue, homogenize or ultrasonicate to fully break the tissue and release the antioxidants in it, centrifuge at about 12000 x g for 5 minutes at 4ºC, and take the supernatant for subsequent determination. All the above operations must be performed at 4ºC or on ice. The supernatant of the prepared cell or tissue sample can be frozen at -80ºC if not used immediately for assay. There is no significant change in the data measured for samples frozen at -80ºC for at least one month. When measuring the total antioxidant capacity of cell or tissue samples, the protein concentration needs to be measured at the same time. The total antioxidant capacity obtained by the final measurement is usually expressed as the total antioxidant capacity per milligram or per gram of protein weight, expressed in mmol/mg or mmol/g.

3. Preparation of other samples

Plant or Chinese herbal extracts can be used directly for testing. It should be noted that the color of the sample itself will not interfere with the test. The antioxidant capacity of plant or Chinese herbal extracts can be expressed as the total antioxidant capacity per milligram or gram of dry weight of the extract, expressed in mmol/mg or mmol/g. When various antioxidants are tested for their antioxidant capacity, they are usually prepared into 0.15-1.5mM and then tested. When the concentration of antioxidants can be expressed in molar concentration, the total antioxidant capacity obtained by the test can be expressed as the relative total antioxidant capacity. For example, if the OD value obtained by the test of 0.5mM of an antioxidant is the same as the OD value obtained by the test of 1mM Trolox, then its relative total antioxidant capacity is 2. For example, if the OD value obtained by the test of 0.2mM of an antioxidant is the same as the OD value obtained by the test of 1mM Trolox, then its relative total antioxidant capacity is 5. According to literature reports and actual measurement results, the antioxidant capacity of vitamin C is 1.0, the antioxidant capacity of vitamin E is 1.0, the antioxidant capacity of GSH is 1.3, the antioxidant capacity of Uric acid is 1.0, the antioxidant capacity of β-Carotene is 2.6, the antioxidant capacity of Lycopene is 3.1, the antioxidant capacity of Quercetin is 3.0, and the total antioxidant capacity of fresh orange juice is 2.2.

- Preparation of the assay kit

1. Preparation of ABTS working solution

Refer to the table below and prepare an appropriate amount of ABTS working solution according to the number of samples to be measured (including the standard curve). First, use equal volumes of ABTS solution and oxidant solution to prepare ABTS working stock solution as follows:

| Sample No. to be measured | About 12-20 | About 30-50 | About 60-100 | About 120-200 |
|---|---|---|---|---|
| ABTS solution | 40 μl | 100 μl | 200 μl | 400 μl |

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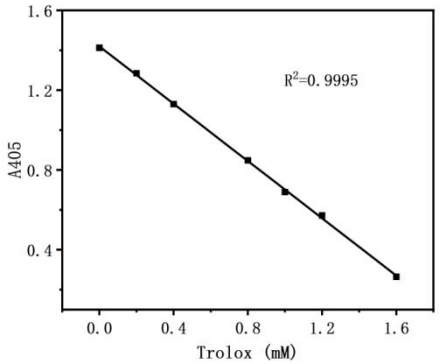
|---|---|---|---|---|
| Oxidant solution | 40 μl | 100 μl | 200 μl | 400 μl |
| ABTS working stock solution | 80 μl | 200 μl | 400 μl | 800 μl |

After the ABTS working stock solution is prepared, it can be used after being stored at room temperature away from light for 12-16 hours. The prepared ABTS working stock solution is stored at room temperature away from light and is stable for 2-3 days. Before use, dilute the ABTS working stock solution with PBS or 80% ethanol to make the ABTS working solution. The absorbance of the ABTS working solution minus the corresponding PBS or 80% ethanol blank control is required to be 0.7±0.05, and the corresponding A405 is about 1.4. When the sample to be tested is a water-soluble sample, dilute it with PBS. At this time, the dilution multiple of the ABTS working stock solution is about 30-50 times; when the sample to be tested is a non-water-soluble sample, dilute it with 80% ethanol. At this time, the dilution multiple of the ABTS working stock solution is about 35-55 times.

2. Preparation of standard curve assay

Dilute the standard with distilled water or sample preparation solution. For serum, plasma, saliva or urine samples, dilute the standard directly with distilled water or PBS. For cell or tissue samples, use the solution used for cell or tissue homogenization to dilute the standard. For other samples, dilute the standard with sample preparation solution, or choose PBS or 80% ethanol to dilute the standard. After diluting the 10mM Trolox standard solution to 1.6mM, use the half-dilution method to obtain 1.6, 0.8, 0.4, 0.2, 0mM standards for the standard curve. The figure below shows a typical graph of the standard curve determined by this kit.




## Operation process

1. Add 200 μl of ABTS working solution to each well of the 96-well plate.
2. Add 10 μl of distilled water or PBS or other appropriate solution to the blank control well; add 10 μl of Trolox standard solution of various concentrations to the standard curve detection well; add 10 μl of various samples to the sample detection well. Mix gently.
3. After incubation at room temperature for 2-6 minutes, measure A734. If it is difficult to measure A734, you can also measure it in the range of 725-745nm. If the sample is difficult to measure in the range of 725-745nm or is interfered by the sample, you can also try to measure A405. For cell or tissue samples, when measuring A405, it is likely that the

absorbance of the sample itself will cause some interference to the test results.
4. The total antioxidant capacity of the sample is calculated based on the standard curve. If the absorbance of the sample is outside the range of the standard curve, the sample needs to be appropriately diluted or concentrated before measurement.

## Result calculation

When Trolox is used as a standard for total antioxidant capacity detection, the antioxidant capacity of the sample can be expressed by Trolox-Equivalent Antioxidant Capacity (TEAC). For the antioxidant capacity of a mixture such as plasma, serum, saliva or urine, it can be directly expressed by the molar concentration of Trolox. For example, if the inhibition rate obtained by a certain sample is the same as that of 0.6mM Trolox, then the total antioxidant capacity of the sample is 0.6mM; for another example, if the inhibition rate obtained by a certain sample after dilution 5 times is the same as that of 0.5mM Trolox, then the total antioxidant capacity of the sample is 2.5mM. For cell or tissue lysate, for example, if the protein concentration of a lysate sample is 0.15mg/ml, and the inhibition rate obtained by the measurement is the same as that of 0.3mM Trolox, then the total antioxidant capacity of the lysate sample is 0.3mM/0.15mg/ml, that is, 2mmol/g. For plant or herbal extracts, for example, if the concentration of a sample is 0.1 mg/ml, and the inhibition rate obtained by the test is the same as that of 0.5 mM Trolox, then the total antioxidant capacity of the sample is 0.5 mM/0.1 mg/ml, that is, 5 mmol/g. For pure compounds such as vitamin C, GSH, etc., for example, when the inhibition rate of a sample at 1 mM is equivalent to that of 1.5 mM Trolox, the antioxidant capacity of the sample is 1.5 mM. For example, when the inhibition rate of a sample at 0.5 mM is equivalent to that of 0.8 mM Trolox, the antioxidant capacity of the sample is 1.6 mM.

## Notes

1. Substances that affect redox reactions, such as DTT and mercaptoethanol, should not be added to the sample, and detergents such as Tween, Triton and NP-40 should not be added.
2. You need to use a microplate reader reader that can measure A734 (725-745nm is also acceptable ) or a spectrophotometer that can measure trace samples.
3. ABTS is irritating to the human body. Please be careful when handling it and take appropriate protective measures to avoid direct contact with the human body or inhalation.
4. This product is for Research Use Only and shall not be used for clinical diagnosis or treatment, food or medicine, or stored in ordinary residences.