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

Total Antioxidant Capacity (T-AOC) Assay Kit (FRAP Method)

Catalog No.: BC00019

Size: 100T

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

☎Tel: 0086-27-87002838

Website: www.enkilife.com

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

Product Name	Total Antioxidant Capacity (T-AOC) Assay Kit (FRAP Method)
Detection Method	Colorimetric
Sample Type	Tissue, cell, serum, plasma, saliva, urine, plant or herbal extracts
Assay Type	Quantitative
Detection Instrument	Microplate reader (585-605 nm, optimal detection wavelength 593 nm)
Range	0.0625-2mM
Sensitivity	0.05mM

Product Introduction

Reactive oxygen species (ROS) include hydroxyl radicals, superoxide radicals, and hydrogen peroxide. ROS are produced during normal physiological metabolism in cells or tissues, and environmental factors such as UV irradiation, γ-irradiation, smoking, and environmental pollution can also induce the production of ROS. ROS can cause oxidative damage to lipids, proteins, and DNA in cells, inducing oxidative stress, leading to various diseases such as tumors, atherosclerosis, rheumatoid arthritis, diabetes, liver damage, and central nervous system diseases.

The body contains various antioxidants, including antioxidant macromolecules, antioxidant small molecules, and enzymes, which can eliminate various ROS produced in the body to prevent the generation of oxidative stress induced by ROS. The total level of various antioxidant macromolecules, antioxidant small molecules, and enzymes in a system reflects the total antioxidant capacity of that system. Therefore, measuring the total antioxidant capacity in various body fluids such as plasma, serum, urine, saliva, cells, or tissue lysates is of great biological significance.

The detection of total antioxidant capacity in plant or 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 for drugs with strong antioxidant capacity.

Product Features

• This kit is convenient and fast. The absorbance can be measured 3-5 minutes after adding the sample, and usually 10-20 samples can be tested in more than ten minutes.

Principle

Under acidic conditions, antioxidants can reduce Ferric-tripyridyltriazine (Fe^{3+}-TPTZ) to produce blue Fe^{2+}-TPTZ, and then measure the blue Fe^{2+}-TPTZ at 593 nm to obtain the total antioxidant capacity of the sample. Since the reaction is carried out under acidic conditions, it can inhibit some endogenous interfering factors. Moreover, since the total concentration of iron ions or ferrous ions in samples such as plasma is usually below 10 μM, the iron ions or ferrous ions in samples such as plasma will not significantly interfere with the FRAP method detection reaction. Since the iron ions or ferrous ions in the reaction system are chelated with TPTZ, the small amount of metal ion chelating agents contained in the sample itself usually do not significantly affect the detection reaction.

$$Fe^{3+} - TPTZ \xrightarrow{\text{Antioxidant}} Fe^{2+} - TPTZ$$

Components

No.	Components	Size (100T)	Storage
Reagent 1	Assay Buffer	20 mL	-20℃, can be stored at 2-8℃ after opening.
Reagent 2	Matrix Solution	1 ml/vial, 2 vials	-20℃, protect from light, can be stored at 2-8℃ after opening.
Reagent 3	Substrate Solution	1 ml/vial, 2 vials	-20℃, protect from light, can be stored at 2-8℃ after opening.
Reagent 4	$FeSO_4 \cdot 7H_2O$ Standard	100 mg	-20℃, can be stored at 2-8℃ after opening.
Consumable 1	Microplate	1 plate	RT
Consumable 2	Plate Sealer	2 pieces	RT

Storage

The unopened kit can be stored at -20℃ for 12 months, and after opening, it can be stored

at 2-8℃ for 6 months.

Preparation

- **Sample handling**

1. Liquid samples such as serum and plasma: can be measured directly.
2. Tissue or cell samples: The homogenate medium is PBS (0.01 M, pH 7.4). After centrifugation of the homogenate, the supernatant is taken for measurement, and part of the supernatant is retained for protein measurement.
3. Sample dilution: Before the formal test, 2-3 samples with large expected differences can be selected and diluted into different concentrations for preliminary experiments. Based on the results of the preliminary experiment, select the dilution factor within the linear range of this kit: 0.0625-2 mmol/L.

- **Preparation of the kit**

1. Equilibrate the reagents in the kit to room temperature.
2. Prepare the FRAP working solution by mixing Reagent 1, Reagent 2, and Reagent 3 in a volume ratio of 10:1:1, prepare fresh and use within 2 hours.
3. Prepare the 100 mmol/L ferrous sulfate solution by accurately weighing 27.8 mg of Reagent 4 with an electronic balance, dissolving in 1 mL of double-distilled water, and mixing well. This reagent must be prepared fresh and used immediately, as ferrous sulfate solution is prone to oxidation, causing the solution color to change from light green to light yellow. If the solution color has turned obviously yellow, discard it and prepare it again.
4. Dilute different concentrations of standard samples by first diluting 100 mmol/L ferrous sulfate to 2 mmol/L with double-distilled water, then diluting to different concentrations such as 2, 1, 0.5, 0.25, 0.125, 0.0625, 0 (blank well) mmol/L with double-distilled water using the halving dilution method.

Operation process

1. Standard wells: Take 5μL of different concentrations of standards and add them to the corresponding wells of the plate; Measurement wells: Take 5μL of the sample to be tested and add them to the corresponding wells of the plate.
2. Add 180 μL of FRAP working solution to each well from step (1).
3. Incubate at 37℃ for 3-5 minutes, and measure the OD value at 593nm with a

microplate reader.

Note: When adding reagents to the plate wells, add them slowly to the bottom of the well to avoid bubbles (bubbles affect the measurement results).

The operation table is as follows:

	Standard Tube (Well)	Measurement Tube (Well)	Blank Tube (Well)
Different concentrations of ferrous sulfate (μL)	5	--	--
Double distilled water (μL)	--	--	5
Sample to be tested (μL)	--	5	--
FRAP working solution (μL)	180	180	180
Incubate at 37℃ for 3-5 minutes, and measure the OD value at 593 nm with a microplate reader.			

Calculation

Standard curve fitting: y = ax + b

Total antioxidant capacity calculation formula for liquid samples such as serum (plasma):

T-AOC (mmol/L) = (ΔA_{593} - b) ÷ a × f

Total antioxidant capacity calculation formula for tissue, cells:

T-AOC (mmol/gprot) = (ΔA_{593} - b) ÷ a × f ÷ C_{pr}

y: Standard OD value - blank OD value

x: Concentration of the standard

a: Slope of the standard curve

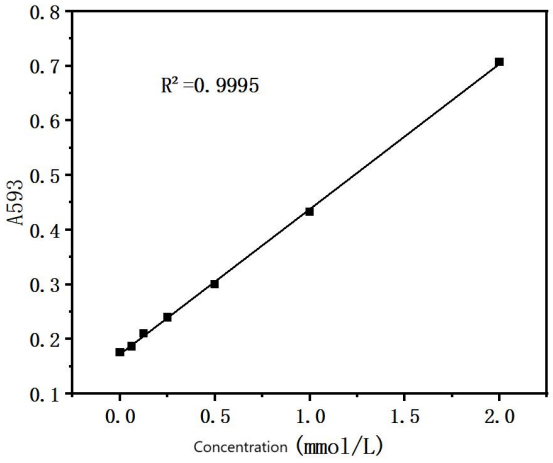
b: Intercept of the standard curve

ΔA_{593}: Sample OD value - blank OD value

f: Dilution factor before the sample is added to the detection system

C_{pr}: Protein concentration of the sample to be tested (gprot/L)

The following standard curve is for reference only:



Notes

1. Reagents that are blue or close to blue under acidic conditions will interfere with the detection of this kit and should be avoided.
2. If the sample contains added high concentrations of iron salts or ferrous salts, it will interfere with the measurement. However, the trace amounts of iron salts or ferrous salts contained in samples such as plasma, serum, cells, or tissue lysates will not interfere with the measurement.
3. Samples should not contain DTT, mercaptoethanol, or other substances that affect redox reactions, nor should they contain detergents such as Tween, Triton, and NP-40.
4. A microplate reader capable of measuring A593 (585-605nm is also acceptable) or a spectrophotometer capable of measuring trace samples is required for the measurement.
5. TPTZ is irritating to the human body, so be careful during operation and take appropriate precautions to avoid direct contact or inhalation.
6. This product is intended for scientific research use only by professionals and must not be used for clinical diagnosis or treatment, in food or drugs, or stored in ordinary residences.