Total Antioxidant Capacity Assay Kit (Spectrophotometry)

Description

Antioxidants, also known as anti-free radicals, are substances that inhibit oxidative damage caused by free radicals. The total antioxidant capacity (T-AOC) consists of two systems: enzymatic and non-enzymatic. The antioxidant system of the human body is a system with perfect and complex functions that can be compared to the immune system. The stronger the body's antioxidant capacity, the healthier the body is, and the longer the lifespan. An increasing number of studies show that anti-oxidation is an important step in preventing aging. This assay method is suitable for detecting the total antioxidant capacity of various body fluids such as plasma, serum, saliva, and urine, lysates of cells or tissues, extracts of plants or herbal medicines, and various antioxidant solutions.

Detection Principle

Under acidic conditions, antioxidants reduce ferric tripyridyltriazine (Fe^{3+}-TPTZ) to the blue-colored ferrous tripyridyltriazine (Fe^{2+}-TPTZ) complex. The reducing capacity of the sample reflects its total antioxidant capacity.

Specifications

Composition and Storage Conditions (50T/48S)

Component	Specification	Storage Condition
CB0206S-ES	60 mL × 1 bottle	Store at 4°C. Pre-cool before use.
CB0206S-A	50 mL × 1 bottle	Store at 4°C protected from light (has an irritating odor).
CB0206S-B	20 mL × 1 bottle	Store at 4°C protected from light.
CB0206S-C	5 mL × 1 bottle	Store at 4°C protected from light.
Working Solution (Prepare Fresh Before Use): Mix CB0206S-A, CB0206S-B, and CB0206S-C at a ratio of 10:1:1. Pre-warm the mixture to 37°C before use.		
CB0206S-Standard	Powder × 1 vial	Store at 4°C

Note: Before formal measurement, it is recommended to select 2–3 samples with expected large differences for a preliminary experiment.

Instructions

I. Required Equipment and Materials:

Visible spectrophotometer, 1 mL glass cuvette, refrigerated centrifuge, water bath, adjustable pipette, mortar, ice, concentrated sulfuric acid, and double-distilled water.

II. Sample Preparation

1. Liquid Samples (Serum, Plasma, Saliva, or Urine)

For plasma samples, heparin or sodium citrate may be used as the anticoagulant during sample preparation. EDTA is not recommended as an anticoagulant.

Centrifuge plasma samples at 5000 rpm and 4°C for 10 min, and collect the supernatant for analysis.

Serum, saliva, and urine samples may be analyzed directly or stored at −80°C for subsequent analysis. Storage should not exceed 30 days.

2. Tissue Samples

Add CB0206S-ES according to the ratio of tissue weight (g): CB0206S-ES volume (mL) = 1:5–10 (it is recommended to weigh approximately 0.1 g of tissue and add 1 mL of CB0206S-ES).

Homogenize the sample in an ice bath, then centrifuge at 10,000 × g and 4°C for 10 min. Collect the supernatant and keep it on ice for analysis.

3. Cell Samples

Add CB0206S-ES according to the ratio of cell number (10^4 cells): CB0206S-ES volume (mL) = 500–1000:1 (it is recommended to add 1 mL CB0206S-ES to 5 × 10^6 cells).

Disrupt the cells by ultrasonication in an ice bath (power 200 W, sonicate for 3 s, interval 10 s, repeat 30 times).

Centrifuge at 10,000 × g and 4°C for 10 min. Collect the supernatant and keep it on ice for analysis.

III. Assay Procedure:

1. Instrument Preparation

Preheat the spectrophotometer for 30 min, set the wavelength to 593 nm, and zero the instrument with distilled water.

2. Standard Curve Preparation

Prior to use, add 900 μL distilled water followed by 20 μL concentrated sulfuric acid to prepare a 40 μmol/mL $FeSO_4$ standard solution.

Dilute the 40 μmol/mL $FeSO_4$ standard solution with distilled water to 0.1, 0.05, 0.025, 0.0125, 0.00625, and 0.003125 μmol/mL.

Aspirate 500 μL of the standard solution (use distilled water as blank) and add it to 500 μL of CB0206S-B. Mix thoroughly, incubate for 10 min, and measure the absorbance at 593 nm.

Calculate ΔA = A_standard – A_blank. At this point, the final concentrations of Fe^{2+} are 0.05, 0.025, 0.0125, 0.00625, 0.003125, and 0.00156 μmol/mL.

3. Sample Measurement

Add the following reagents sequentially:

Reagent	Blank Tube (μL)	Sample Tube (μL)
Working Solution	900	900
Sample		30
Distilled Water	120	90
Mix thoroughly and incubate for 10 min. Measure the absorbance at 593 nm. Calculate: ΔA = A_sample – A_blank (the blank tube only needs to be measured 1–2 times)		

Note: The Blank Tube only needs to be measured once.

IV. Calculation of Total Antioxidant Capacity:

1. Standard Curve

Plot the standard curve using the final Fe^{2+} concentration as the x-axis (x) and ΔA as the y-axis (y). Obtain the linear regression equation: y = kx + b. Substitute ΔA into the equation to calculate x (μmol/mL).

2. Calculation Formula

Definition of Unit: Total antioxidant capacity is expressed as the concentration of the standard solution (μmol/mL) required to produce the same absorbance change (ΔA) as the sample.

(1) Based on Protein Concentration

Total Antioxidant Capacity (μmol/mg prot) = x × V_total ÷ (V_sample × C_{pr}) = 34 × x ÷ C_{pr}

(2) Based on Fresh Sample Weight

Total Antioxidant Capacity (μmol/g tissue) = x × V_total ÷ (V_sample ÷ V_sample total × W) = 34 × x ÷ W

(3) Based on Cell Number

Total Antioxidant Capacity (μmol/10^4 cells) = x × V_total ÷ (V_sample × V_sample_total ÷ Cell number) = 34 × x ÷ Cell number

(4) Calculation Based on Liquid Sample Volume

Total Antioxidant Capacity (μmol/mL) = x × V_total ÷ V_sample = 34 × x

Note:

V_sample_total: Volume of extraction solution added, 1 mL

V_total: Total reaction volume, 1.02 mL

V_sample: Sample volume added to the reaction system, 0.03 mL

W: Sample weight (g)

C_{pr}: Protein concentration of the sample (mg/mL)

Cell number: Expressed in units of 10^4 cells

Precautions

1. Try to avoid using reagents that turn blue or near blue under acidic conditions, otherwise they will interfere with the test results of this kit.
2. Detergents such as Tween, Triton, and NP-40, and reducing agents that affect redox reactions such as DTT and β-mercaptoethanol, should not be added to the samples.
3. If the measured absorbance value of the sample falls outside the range of the standard curve, the sample needs to be appropriately diluted or concentrated before determination.
4. This product is restricted for scientific research use by professionals only. It must not be used for clinical diagnosis or treatment, must not be used in food or drugs, and must not be stored in ordinary residences.
5. For your safety and health, please wear a lab coat and disposable gloves during operation.