Total Antioxidant Capacity (T-AOC) Assay Kit

Cat. No. KITG-081 **Lot. No.** (See product label)

SPECIFICATION

Product Overview	Total antioxidant capacity refers to the overall antioxidant level provided by various antioxidant substances and antioxidant enzymes, including antioxidant enzymes, vitamin C, vitamin E, and carotene. These components protect cells and organisms from oxidative stress damage caused by reactive oxygen species and free radicals. Measuring the total antioxidant capacity of plasma, saliva, urine and other body fluids, cell or tissue lysates, plant or traditional herbal extracts, and solutions of various antioxidants is important for a comprehensive and scientific evaluation of antioxidant capacity. This kit uses the FRAP (Ferric Reducing Ability of Plasma) method to determine total antioxidant capacity. Under acidic conditions, antioxidants reduce Fe^{3+}-TPTZ to the blue Fe^{2+}-TPTZ complex. The product has a characteristic absorption peak at 593 nm. The change in absorbance reflects the reducing capacity of the sample, namely its total antioxidant capacity.
Description	Method: FRAP (Ferric Reducing Ability of Plasma), visible spectrophotometry. Pack Size: 70 tests / 50 samples. Storage Temperature: 2-8°C. For Research Use Only. Not for Use in Diagnostic Procedures.
Form	Kit
Applications	Quantitative determination of total antioxidant capacity in plasma, serum, saliva, urine and other body fluids; cell or tissue lysates; plant or traditional herbal extracts; and solutions of antioxidant substances.
Notes	Instructions for Use Required Instruments and Reagents: Visible spectrophotometer,

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1 mL glass cuvette with a 10 mm optical path, mortar/homogenizer, adjustable pipette, benchtop centrifuge, thermostatic water bath/incubator, concentrated sulfuric acid, and distilled water. 1. Sample Treatment (adjust the sample amount and ratio appropriately according to preliminary test results) 1) Tissue: Process the sample at a tissue mass (g) to Extraction Solution volume (mL) ratio of 1:(5-10). It is recommended to weigh 0.1 g tissue and add 1 mL pre-cooled Extraction Solution. Homogenize in an ice bath, centrifuge at 10,000 × g for 5 min at 4°C, collect the supernatant, and keep it on ice until measurement. 2) Bacteria or cells: Collect bacteria or cells by centrifugation into a centrifuge tube. Process the sample at a bacterial or cell number (10^4) to Extraction Solution volume (mL) ratio of (500-1000):1. It is recommended to add 1 mL pre-cooled Extraction Solution to 5 million bacteria or cells. Disrupt the bacteria or cells by ultrasonication in an ice bath at 200 W, with 3 s sonication and 10 s intervals, repeated 30 times. Centrifuge at 10,000 × g for 5 min at 4°C, collect the supernatant, and keep it on ice until measurement. 3) Serum, plasma, saliva, urine, and other liquid samples: Heparin or sodium citrate may be used as the anticoagulant when preparing plasma; EDTA anticoagulation is not suitable. Centrifuge at 5,000 × g for 10 min at 4°C, collect the supernatant, and keep it on ice until measurement. Serum, saliva, or urine samples may be measured directly or stored at -80°C for no more than 30 days before measurement. 2. Assay Procedure 1) Preheat the spectrophotometer for at least 30 min, set the wavelength to 593 nm, and zero the instrument with distilled water. 2) Preheat the FRAP Working Solution at 37°C for 20 min before the assay. 3) Standard Curve Preparation: Dilute the 40 μmol/mL $FeSO_4$ standard solution with distilled water to prepare 0.1, 0.05, 0.025, 0.0125, 0.00625, and 0.003125 μmol/mL standard solutions. Standard 1: 500 μL of 0.1 μmol/mL standard solution + 500 μL Reagent 2; final Fe^{2+} concentration 0.05 μmol/mL. Standard 2: 500 μL of 0.05 μmol/mL standard solution + 500 μL Reagent 2; final Fe^{2+} concentration 0.025 μmol/mL. Standard 3: 500 μL of 0.025

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μmol/mL standard solution + 500 μL Reagent 2; final Fe^{2+} concentration 0.0125 μmol/mL. Standard 4: 500 μL of 0.0125 μmol/mL standard solution + 500 μL Reagent 2; final Fe^{2+} concentration 0.00625 μmol/mL. Standard 5: 500 μL of 0.00625 μmol/mL standard solution + 500 μL Reagent 2; final Fe^{2+} concentration 0.003125 μmol/mL. Standard 6: 500 μL of 0.003125 μmol/mL standard solution + 500 μL Reagent 2; final Fe^{2+} concentration 0.00156 μmol/mL. Blank Tube 1: 500 μL distilled water + 500 μL Reagent 2. Blank Tube 1 only needs to be measured 1-2 times. Mix thoroughly, allow color development at room temperature for 10 min, and measure absorbance at 593 nm. Record the values as A_standard and A_blank1, and calculate ΔA_standard = A_standard - A_blank1. Use the final Fe^{2+} concentrations of 0.05, 0.025, 0.0125, 0.00625, 0.003125, and 0.00156 μmol/mL as the x-axis values and the corresponding ΔA_standard values as the y-axis values to plot the standard curve and obtain the linear regression equation y = kx + b. 4) Sample Measurement: Test Tube: 900 μ FRAP Working Solution + 30 μ test sample + 90 μ distilled water. Blank Tube 2: 900 μ FRAP Working Solution + 120 μ distilled water. Mix thoroughly and allow color development at room temperature for 10 min. Absorbance Measurement: Measure absorbance at 593 nm and record the values as A_test and A_blank2. Calculate ΔA_test = A_test - A_blank2. Substitute ΔA_test into the standard curve equation y = kx + b to calculate the sample concentration x in μmol/mL. Blank Tube 2 only needs to be measured 1-2 times. 3. Total Antioxidant Capacity Calculations Unit Definition: The total antioxidant capacity of a sample is expressed as the concentration of standard ions required to produce the same absorbance change (ΔA). 1) Based on tissue protein concentration: Total antioxidant capacity (μmol/mg prot) = (x × V_reaction)/(V_sample × Cpr) = 34 × x/Cpr. 2) Based on tissue sample mass: Total antioxidant capacity (μmol/g) = (x × V_reaction × V_sample_total)/(V_sample × W) = 34 × x/W. 3) Based on bacterial or cell number: Total antioxidant capacity (μmol/10^4 cells) = (x × V_reaction × V_sample_total)/(V_sample × bacterial or cell count) = 34 × x/(bacterial or cell count). 4) Based on liquid sample volume: Total antioxidant capacity (μmol/mL) =

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	(x × V_reaction)/V_sample = 34 × x. Where V_sample_total is the total volume of the test sample, 1 mL; V_reaction is the total reaction volume, 1.02 mL; V_sample is the volume of test sample added to the reaction system, 0.03 mL; W is the sample mass in g; Cpr is the sample protein concentration in mg/mL; and the bacterial or cell count is expressed in units of 10^4. 4. Precautions 1) Reagent 2 is irritating. Avoid contact and take appropriate protective measures. Wear a laboratory coat and latex gloves during operation. 2) Avoid using samples that appear blue or nearly blue under acidic conditions, as they may interfere with the assay results. 3) Do not add detergents such as Tween or NP-40, or reducing agents that affect redox reactions, such as DTT or β-mercaptoethanol, to the samples. 4) If the measured absorbance is outside the linear absorbance range of the standards, dilute the test sample appropriately and repeat the measurement when the value is above the highest standard. When the value is below the lowest standard, appropriately increase the sample amount and repeat the measurement. Modify the calculation accordingly. 5) To ensure accurate results and avoid reagent loss, carefully read the instructions before measurement, confirm that reagent storage and preparation are adequate, verify that the operating steps are clear, and perform a preliminary test using 2-3 samples expected to show substantial differences. If any problem occurs during the procedure, seek technical assistance promptly.
Storage	Kit Components and Storage: Extraction Solution: liquid, 60 mL × 1 bottle; store at 4°C. Pre-cool to 4°C before use. Reagent 1: liquid, 60 mL × 1 bottle; store at 4°C. Reagent 2: liquid, 20 mL × 1 bottle; store at 4°C protected from light. Reagent 3: liquid, 8 mL × 1 bottle; store at 4°C protected from light. Standard: powder, 1 vial containing 10 mg $FeSO_4 \cdot 7H_2O$; store at 4°C. Before use, add 900 µL distilled water and 20 µL concentrated sulfuric acid to obtain a 40 µmol/mL $FeSO_4$ standard solution.
Reconstitution	Reagent and Standard Preparation: 1. Standard Solution: Add 900 µ distilled water

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	and 20 µ concentrated sulfuric acid to the vial containing 10 mg $FeSO_4 \cdot 7H_2O$ to obtain a 40 µmol/mL $FeSO_4$ standard solution. 2. FRAP Working Solution: Prepare immediately before use by mixing Reagent 1, Reagent 2, and Reagent 3 at a volume ratio of 10:1:1 according to the required amount. 3. Standard Dilutions: Dilute the 40 µmol/mL $FeSO_4$ standard solution with distilled water to prepare 0.1, 0.05, 0.025, 0.0125, 0.00625, and 0.003125 µmol/mL standard solutions. Required User-Supplied Reagent: Concentrated sulfuric acid (H_2SO_4, MW 98.078, CAS 7664-93-9).
Reaction conditions	Preheat the FRAP Working Solution at 37°C for 20 min. For sample measurement, add 900 µ FRAP Working Solution, 30 µ test sample, and 90 µ distilled water to the test tube; add 900 µ FRAP Working Solution and 120 µ distilled water to Blank Tube 2. Mix thoroughly, allow color development at room temperature for 10 min, and measure absorbance at 593 nm.
Unit definition	The total antioxidant capacity of a sample is expressed as the concentration of standard ions required to produce the same absorbance change (ΔA).

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