Antioxidant Assay Kit (Colorimetric)

Antioxidant Assay Kit (Colorimetric) is a detection kit for the quantification of Antioxidant in serum, plasma, urine, saliva, food and beverage.

Catalog number: ARG82137

Package: 100 tests

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INTRODUCTION

Antioxidants are compounds that inhibit oxidation, a chemical reaction that can produce free radicals and chain reactions that may damage the cells of organisms. Antioxidants such as thiols or ascorbic acid (vitamin C) may act to inhibit these reactions. To balance oxidative stress, plants and animals maintain complex systems of overlapping antioxidants, such as glutathione.

The only dietary antioxidants are vitamins A, C, and E. The term antioxidant is also used for industrial chemicals added during manufacturing to prevent oxidation in synthetic rubber, plastics, and fuels, or as preservatives in food and cosmetics. [Provide by Wikipedia: Antioxidant]

PRINCIPLE OF THE ASSAY

This Antioxidant Assay Kit (Colorimetric) is a simple colorimetric assay that measures the amount of Antioxidant present in serum, plasma, urine, saliva, food and beverage. This assay measures total antioxidant capacity in which Cu^{2+} is reduced by antioxidant to Cu^{+}. The resulting Cu^{+} specifically forms a colored complex with a dye reagent. The color intensity at O.D. 570 nm is proportional to total antioxidant capacity (TAC) in the sample.

MATERIALS PROVIDED & STORAGE INFORMATION

The kit is shipped at room temperature. Store all components at-20°C upon receiving. Shelf life: 6 months after receipt.

Component	Quantity	Storage information
Reagent A	12 mL	-20°C
Reagent B	1 mL	-20°C
Standard (50 mM Trolox)	100 µL	-20°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at O.D. 570 nm
- Centrifuge
- Homogenizer or sonicator
- Clear flat-bottom 96 well microplate
- Deionized or Distilled water
- Pipettes, pipette tips and Multichannel micropipette reservoir

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.
- Samples should not contain any metal chelators (E.g., EDTA) and should be clear and free of any turbidity or particles.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- It is highly recommended assaying the Standards and samples in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum: Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 30 minutes. Centrifuge at 2500 x g for 20 minutes at 4°C. Collect the serum and assay directly.

Plasma: Collect blood with EDTA, heparin or citrate and centrifuge at 2000 x g for 10 minutes at 4°C. Collect the plasma layer and assay directly.

Cell lysate: Collect cells by centrifugation at 2,000 x g for 5 minutes at 4°C. For adherent cells, do not harvest cells using proteolytic enzymes; rather use a rubber policeman. Homogenize or sonicate cells in an appropriate volume of ice-cold 1X PBS (pH 7.4). Centrifuge at 10,000 x g for 10 min at 4°C. Collect the supernatant for assay.

Note:

1. Samples should not contain any metal chelators (E.g., EDTA) and should be clear and free of any turbidity or particles.
2. If not assayed immediately, freeze samples at-80°C (stable for 1 month).

REAGENT PREPARATION

- **Working Reagent:** for each 96 well assay, mix 100 µL of Reagent A and 8 µL of Reagent B. Fresh preparation before assay.
- **Standard:** Mix 5 µL of the standard with 245 µL of distilled water (final conc. 1 mM Trolox). Dilute standards as shown in the Table below.

Standard tube	Final Trolox conc. (µM)	Distilled water (µL)	Standard, 1 mM Trolox (µL)
S1	1000	0	100
S2	600	40	60
S3	300	70	30
S4	0	100	0

ASSAY PROCEDURE

Equilibrate reagents to desired reaction temperature (E.g., 25°C or 37°C). Briefly centrifuge tubes before use.

	Sample	Standard
Diluted Standard		20 µL
Samples	20 µL	
Working Reagent	100 µL	100 µL
Tap microplate to mix well. Incubate for 10 minutes at room temperature.		
Read the absorbance at O.D. 570 nm.		

Note:

1. For unknown samples, perform several dilutions to ensure that TAC is within the linear range of 1.5 to 1000 µM Trolox equivalents.
2. If calculated TAC is higher than 1000 µM Trolox equivalents, dilute sample in distilled water and repeat assay. Multiply the results by the dilution factor.

CALCULATION OF RESULTS

1. Subtract the Blank OD value (S4) from all standard and sample OD values. Plot the ΔOD_{570nm} against standard concentrations and determine the slope of the standard curve. Calculate the total antioxidant capacity (TAC) of samples,

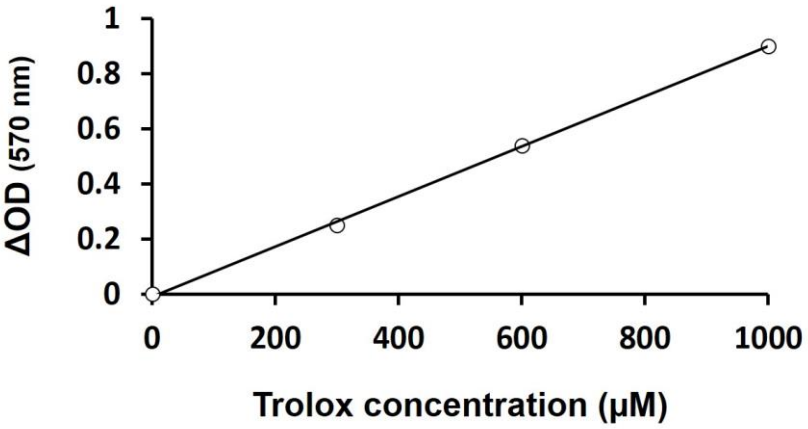
   TAC (μM Trolox equivalents) = $[(OD_{SAMPLE} - OD_{BLANK}) / \text{Slope } (\mu M^{-1})] \times n$

   Note:

   - OD_{SAMPLE} and OD_{BLANK}: the O.D. 570 nm values of the sample and standard 4 (S4).
   - n: the sample dilute factor.

EXAMPLE OF TYPICAL STANDARD CURVE

The following figures demonstrate typical results with the Antioxidant Assay Kit (Colorimetric). One should use the data below for reference only. This data should not be used to interpret actual results.



QUALITY ASSURANCE

Sensitivity

1.5 µM