Total Antioxidant Capacity (TAC) Assay Kit

Quantitative Colorimetric Determination of Total Antioxidant Capacity

DESCRIPTION

An antioxidant is a molecule that can slow down or prevent the oxidation of other molecules. Antioxidants protect cells from damage caused by reactive oxygen species, which are produced during oxidation reactions within the cell. They can be small molecules, such as glutathione and vitamins, or macromolecules like catalase and glutathione peroxidase. Since oxidative stress contributes to the development of many diseases, including Alzheimer's disease, Parkinson's disease, diabetes, rheumatoid arthritis, and neurodegeneration, the use of antioxidants in pharmacology is extensively studied. Additionally, antioxidants are widely used as dietary supplements and as preservatives in food, cosmetics, rubber, and gasoline. Simple, direct, and high-throughput assays for TOS find wide applications in research, physiology, nutrition, botany, the food industry, environmental sciences, reproduction, and drug discovery.
Improved 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 630nm is proportional to TAC in the sample.

KEY FEATURES

Sensitive and accurate. Linear detection ranges from 1.5 to 1000 µM Trolox equivalents.
Simple and high-throughput. The procedure involves the addition of a single working reagent that can be readily automated as a high-throughput assay for thousands of samples per day.

APPLICATIONS

Direct Assays: serum, plasma, urine, saliva and other biological samples, food, milk, beverages and Drug Discovery/Pharmacology.

KIT CONTENTS 100 Reaction Kit

R1-60 ml **R2-** 7 ml
Standard: Prepare 30 mM Trolox ,
Take 1.87 mg of the give standard and dissolve in to 25ml of PBS buffer and then make serial dilution.

No	30 mM Trolox + PBS Buffer	Vol (µL)
1	500 µL + 500 µL	1000
2	250 µL + 500 µL	750
3	125 µL + 500µL	625
4	75 µL + 500 µL	575
5	37.5 +500 µL	537.5
6	0+ 500 µL	500

Storage conditions. The kit is shipped at room temperature. Store all components at room temperature for shelf life of 6months and store at -40°C for one year.

Precautions: reagents are for research use only. Normal precautions forlaboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

SAMPLE PREPARATION

Serum sample: Blood should be collected in a gel tube, then centrifuged at 4000 rpm for 5 min, and obtained clear solution on top of the cellular material. Separate it into a new Eppendorf tube and use it for further processing.

Tissue homogenate: Cell lysate is prepared by homogenizing tissue (25mg) or sonicating cells in ice-cold 1 x PBS (1mL) and centrifugation for 10 min at 14,000 rpm to pellet any debris. Use the clear supernatant for the assay. If not assayed immediately, freeze supernatant at -80°C (stable for 1 month).

ASSAY PROCEDURE

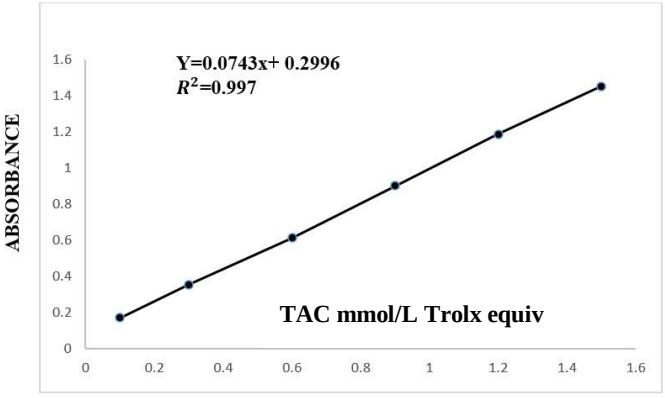
1. Label Eppendorf tubes on the based total number of samples.
2. Subsequently, in each Eppendorf tube, systematically introduce the following components one after the other.

Sr.	Reagents	Volume
1	Sample	15 µL
2	R1	600 µL
3	R2	60 µL

3. Following the addition of the specified components, all Eppendorf tubes were incubated for 4 minutes in the dark.
4. Read OD 630 nm on a plate reader.
5. Note: If the calculated TAC is higher than 1000 µM Trolox equivalents. Equivalents, dilute the sample in dH_2O and repeat the assay. Multiply the results by the dilution factor.

CALCULATION

Subtract blank OD value from all standard and sample OD values. Plot the OD 630 nm against standard concentrations and determine the slope of the standard curve. Calculate the Total antioxidant capacity (TAC) of the Sample,



TAC mmol/L Trolx equiv

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipetting devices, centrifuge tubes, clear flat-bottom uncoated 96-well plates, plate reader capable of reading optical density at 532 nm, homogenizer or sonicator etc.

PUBLICATIONS

1. Liza, Hussain, G., Malik, A., Akhtar, S., & Anwar, H. (2024). Artemisia vulgaris Extract as a Novel Therapeutic Approach for Reversing Diabetic Cardiomyopathy in a Rat Model. *Pharmaceuticals*, *17*(8), 1046.
2. Saleem, S., Mukhtar, I., Aati, H. Y., Muzaffar, H., Anwar, H., Hussain, M., Ahmad, M., & Umair, M. (2024). Effects of Withania somnifera (L.) Dunal in acute pulmonary pathophysiology in a rat model of smoke-induced lung injury and role of IRS-1 and SOX-2. *South African Journal of Botany*, *171*, 757.