EzWay™ Total Antioxidant Capacity (TAC) Assay Kit

1. Catalog No. K23000

2. Quantity 100 Tests

3. Storage & Stability The kit is shipped at room temperature. Store at 4°C after receipt until their expiration dates. Discard the remaining reconstituted Trolox standard solution after use.

4. Introduction Oxidative stress is a physiological condition where there is an imbalance between concentrations of reactive oxygen species (ROS) and antioxidant. However, excessive ROS accumulation will lead to cellular injury, such as damage to DNA, proteins, and lipid membranes. The cellular damage caused by ROS has been implicated in the cause of many diseases, such as cancer, diabetes, cardiovascular disease, atherosclerosis and neurodegenerative diseases.
The body's physiological response to oxidative stress activates several antioxidant pathways that include enzymes and varying sized molecules (see example below). These antioxidants can be found as water soluble or lipid soluble molecules and they are localized transiently throughout tissues and cells.

Classification	Examples
Enzymes	Superoxide dismutase, Catalase, Glutathione Peroxidase
Large Molecule	Albumin, Ferritin, Ceruloplasmin
Small Molecule	Ascorbic Acid, α-Tocopherol, β-Carotene, Uric Acid

5. Principle Total Antioxidant Capacity (TAC) Assay Kit measures the total antioxidant capacity of biomolecules from a variety of samples. The TAC Assay is based on the reduction of copper (II) to copper (I) by antioxidants such as Trolox. Upon reduction, the copper (I) ion further reacts with a coupling chromogenic reagent that produces a color with a maximum absorbance at 450nm. The net absorbance values of antioxidants are compared with a known Trolox standard curve. Absorbance values are proportional to the sample's total reductive capacity.

Samples are compared to a known concentration of Trolox standard within a 96-well microplate format. Samples and standards are diluted with a reaction reagent and, upon the addition of copper, the reaction proceeds for a few minutes and are read with a standard 96-well spectrophotometric microplate reader at 450 nm. Antioxidant capacity of the sample is determined by comparison with the Trolox standards. The assay is compatible with both hydrophilic and lipophilic samples.

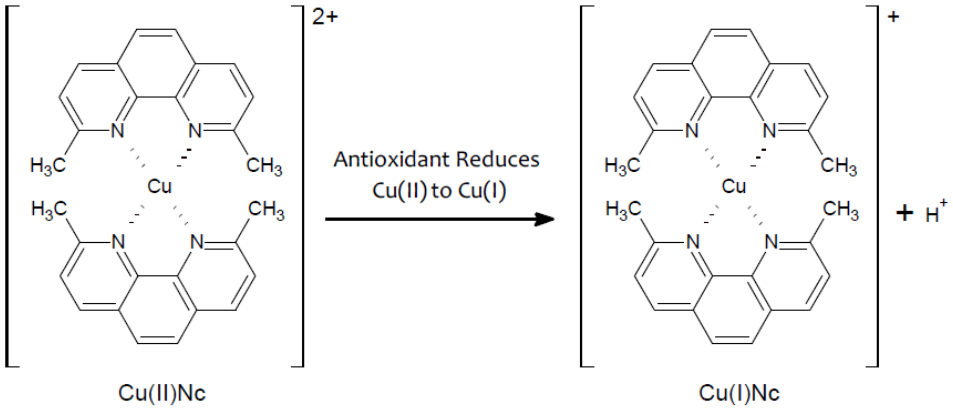


Figure 1. Reduction of the neocuproine/copper (II) complex.

6. Kit Contents

Component	Amount
Copper solution, 6ml	1 EA
Reaction buffer, 6ml	1 EA
Chromogenic solution, 6ml	1 EA
Trolox standard (dried powder)	5 vials

Materials needed but not provided

1. Adjustable pipettes (10 - 1,000 ul) and disposable tips.
2. Deionized water (D.W.)
3. Ethanol
4. Optically clear 96 well microplate
5. Microplate reader with 450 nm filter.
6. Vortex mixer.

Note: Reconstituted Trolox standard can’t store and do not reuse.

7. Preparation of Reagent

Reaction mixture preparation

1) Mix the 3 solutions with equal volumes.
   (Copper solution : Reaction buffer : Chromogenic solution = 1 : 1 : 1)
2) This reaction mixture is used 150 ul per test (150 ul/well).
   Prepare fresh prior to use and discard unused reaction mixture.
   (Do not use the remaining mixture the next day.)

Standard preparation

1) Add 100 ul Ethanol to 1 vial of standard Trolox and vortex the vial until the standard is completely dissolved. After reconstitution, add 900 ul D.W. to the vial and mix well.
   This will be used as the highest standard (1 mM Trolox).
2) Prepare a series of the remaining standards according to Table 1 below.

Table 1.

Tubes	Trolox Standard Dilution Method	Trolox Concentration
1	100 ul of Ethanol + 900 ul of D.W.	1 mM
2	500 ul of tube 1 + 500 ul of D.W.	0.5 mM
3	500 ul of tube 2 + 500 ul of D.W.	0.25 mM
4	500 ul of tube 3 + 500 ul of D.W.	0.125 mM
5	500 ul of tube 4 + 500 ul of D.W.	0.0625 mM
6	500 ul of tube 5 + 500 ul of D.W.	0.03125 mM
7	500 ul of tube 6 + 500 ul of D.W.	0.0156 mM
8	Add 500 ul of D.W.	0.0 mM

8. Preparation of Samples

All samples should be stored at -70°C at all times prior to assay. Apply samples to the assay immediately upon thawing. Samples with a Trolox equivalent concentration greater than 1.0 mM should be diluted with PBS (pH 7.2) or D.W. prior to assay.

NOTE: Chelators such as EDTA may be detrimental to the function of the assay and should be avoided in the use of this kit.

Tissue Lysate: Homogenize tissue sample on ice with ice cold pH 7.0 PBS then centrifuge at 3,000 x g for 10 minutes at 4°C. Aliquot the supernatant for -70°C storage, protein determination, and Total Antioxidant Assay.

Cell lysate: Wash cells 2-3 times with ice cold PBS (pH 7.2) prior to lysis. Lyse cells by homogenization or sonication with ice cold PBS then centrifuge at 3,000 x g for 10 minutes at 4°C. Aliquot the supernatant for -70°C storage, protein determination, and Total Antioxidant Assay.

Urine: Collected samples may be assayed directly or diluted with PBS (pH 7.2) if appropriate.

Plasma: Collect blood with sodium citrate and centrifuge for 10 minutes at 3,000 x g within 30 minutes of blood collection. Remove plasma and assay immediately or aliquot for -70°C storage. Avoid multiple (>2) freeze/thaw cycles. The sample should be diluted properly to fall within the concentration range of the standards.

Serum: Allow the blood to clot for at least 30 minutes before centrifugation for 10 minutes at 3,000 x g. Remove serum and assay immediately or aliquot for -70°C storage. Avoid multiple (>2) freeze/thaw cycles. The sample should be diluted properly to fall within the concentration range of the standards.

Food: This can vary considerably from one food to the next. In many cases liquid food samples such as juice and tea can be assayed directly without any processing. Liquid samples that contain high amounts of protein and/or fiber or solid foods should be processed as indicated below.

Solid Food: Homogenize food sample on ice in ice cold PBS (pH 7.2) then centrifuge at 3,000 x g for 10 minutes at 4°C. Aliquot the supernatant for -70°C storage, protein determination, and Total Antioxidant Assay.

Liquid Food: Centrifuge at 3,000 x g for 10 minutes at 4°C. Aliquot the supernatant for -70°C storage, protein determination, and Total Antioxidant Assay.

Lipophilic sample: Dissolve lipophilic samples in 100% methanol/ethanol and assay directly or dilute with methanol/ethanol if appropriate. When this diluted sample is tested, dilute the Trolox standard also with methanol/ethanol.

9. Protocol

Each Trolox Standard and sample should be assayed in duplicate or triplicate. A freshly prepared standard curve should be used each time the assay is performed.

Antioxidants are unstable towards air, light and heat. Samples should be stored frozen until testing. Prior to use, the sample should be allowed to come to room temperature and be well-mixed to ensure a homogeneous sample. Also, introducing bubbles (oxygen in the air) during mixing can reduce the antioxidant concentration in the sample. Try to mix samples with a vortex mixer, which is set to low speed, to reduce the amount of oxygen exposure.

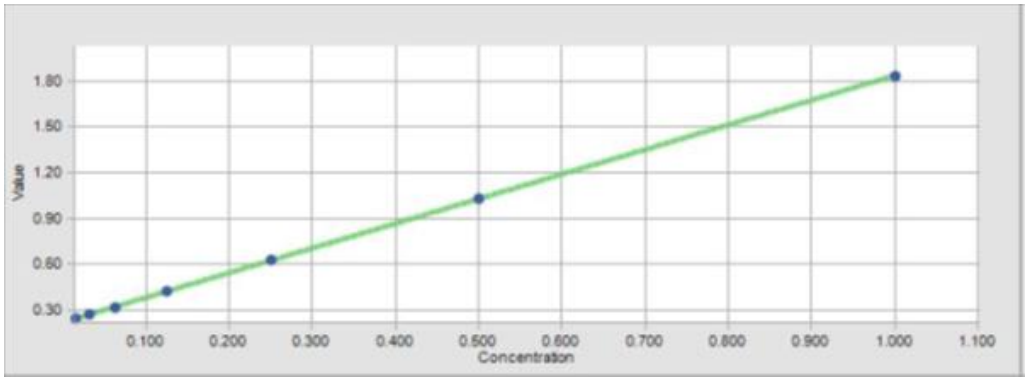
If the sample (even diluted) is colored or turbid which interrupt the absorbance at 450 nm, a simple way to correct this is to run each Sample Blank, which simply measures the absorbance of the sample reaction mixture with D.W. instead of reaction mixture. Then the substracted value the blank sample absorbance from the sample reaction absorbance should be used for result calculation.

1. Add 50 ul of each Standard and samples to the 96-well microplate.
2. Add 150 ul of the Reaction mixture to each well using either a multichannel pipette or a plate reader liquid handling system. Mix thoroughly.
3. Incubate 5 - 10 minutes at room temperature.
4. Read the plate at 450 nm.

10. Example Results

The following figures demonstrate typical TAC Assay results (hydrophilic). One should use the data below for reference only. This data should not be used to interpret or calculate actual sample results.

Figure 2. Actual sample result



11. Calculation of Results

1. If a Sample Blank was run, subtract this blank value from the sample's absorbance at 450 nm of reaction value.

2. Plot a standard curve using absorbance value for each Trolox standard concentration. (Figure 2).

3. Calculate the antioxidant capacity of unknown samples by comparing the absorbance of samples (if sample blank was run, the subtracted values is the absorbance of sample) to the Trolox standard curve.