*Oxis*Research™

A Division of AOXRE LLC

BIOXYTECH® AOP-450™

Colorimetric, Quantitative Assay for Total Antioxidant Potential (Aqueous Samples)
For Research Use Only. Not For Use In Diagnostic Procedures.
Catalog Number #21053

INTRODUCTION

Oxidative stress has been implicated in a number of diseases such as atherosclerosis [1], chronic inflammatory disease[2], chronic renal failure[3], and cancer[4]. It is a condition where an imbalance exists between the production of reactive oxidizing species and the body's ability to neutralize these intermediates, resulting in cellular damage. The body has designed several physiological responses to oxidative stress including counterbalances[5] such as enzymes and variously functionalized molecules (see examples below) that effectively neutralize these damaging species. These antioxidants can be either water or lipid soluble, and are localized transiently throughout various tissues, cells and cell types.

Classification Examples

Enzymes:	Superoxide Dismutase
	Catalase
	Glutathione Peroxidase
Large Molecules:	Albumin
	Ferritin
	Ceruloplasmin
Small Molecule :	Ascorbic Acid
	α-Tocopherol
	β-Carotene
	Uric Acid

Given the multiplicity of antioxidant pathways, their centrality in the prevention of oxidant stress, and the influences of lifestyle and nutritional supplements on an individual's antioxidant capacity, it is important to be able to quantitatively measure the total antioxidant capacity or antioxidant power with biological specimens [6-11].

PRINCIPLES OF PROCEDURE

The reduction potential of the sample or standard effectively converts Cu+2 to Cu+1 , thus changing the ion's absorption characteristics. This reduced form of copper will selectively form a stable 2:1 complex with the chromogenic reagent with an absorption maximum at ca. 450 nm[12-16]. A known concentration of Trolox is used to create a calibration curve, with the data being expressed as mM Trolox equivalents or in µM copper reducing equivalents.
A study was performed to evaluate the correlation between the concentration of principal antioxidants in human serum and the value obtained for total antioxidant power using this method with the results presented in Figure 1. Multivariate analysis of the obtained results yields a very high significance. The results obtained with the Total Antioxidant Power Assay for a series of serum samples were also compared to the resistance to oxidation of the serum lipids in these samples. The results of this study also show that these two parameters are highly correlated. The higher the total antioxidant power, the more protected the serum lipids are from oxidation.

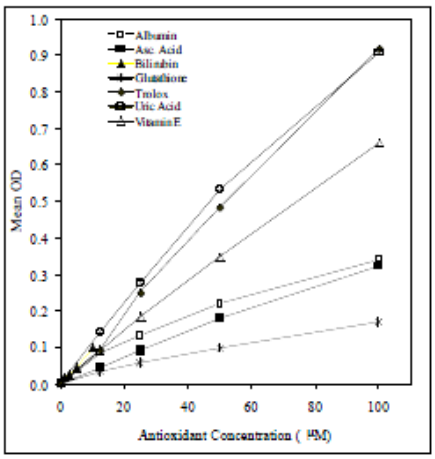


Figure 1: Relationship between antioxidant type and reduction from Cu^{+2} to Cu^{+1}.

MATERIALS PROVIDED

- R1 (Dilution Buffer) 60 ml
- R2 (Copper Solution) 5 ml
- Trolox Standard (2 mM) 1 vial
- Stop Solution 5 ml

MATERIALS NEEDED BUT NOT PROVIDED

- Microplate reader with a 450 nm filter
- Adjustable micropipettes (10 – 1000 µL) and tips
- Ethanol
- Deionized water
- Vortex

STORAGE

1. Store the components of this kit at the temperatures specified on the labels.
2. Unopened reagents are stable until the indicated kit expiration date.
3. Aliquot the Trolox Standard after reconstitution and freeze at -80°C.

WARNINGS AND PRECAUTIONS

1. Use aseptic technique when opening and dispensing reagents.
2. This kit is designed to work properly as provided and instructed. Additions, deletions or substitutions to the procedure or reagents are not recommended, as they may be detrimental to the assay

PROCEDURAL NOTES

1. Allow the Dilution Buffer, Copper Solution and Stop Solution to equilibrate to room temperature for 30 minutes prior to running the assay.
2. To minimize errors in absorbance measurements due to handling, wipe the exterior bottom of the microplate wells with a lint-free paper towel prior to inserting into the plate reader.

SAMPLE PREPARATION

All samples should be stored at -70°C at all times prior to assay. Apply the samples to the assay immediately upon thawing. Samples with a Trolox equivalent concentration greater than 2.0 mM should be diluted with pH 7.0 PBS prior to assay. Sample preparation is subject to the discretion and approval of the principal investigator, and changes may occur to optimize or better complement experimental design.
NOTE: Ethylene diamine tetraacetic acid (EDTA) is detrimental to the function of the assay and must not be present at any concentration in any sample type.
Urine: Collected samples may be assayed directly or diluted with pH 7.0 PBS where appropriate.
Tissue Lysate: Homogenize tissue sample on ice with ice-cold pH 7.0 PBS then centrifuge at 3000 × g for 12 minutes at 4°C. Aliquot the supernatant for storage at –70°C, protein determination and subsequent assays.
Cell Culture: Wash cells 2-3 times with ice-cold pH 7.0 PBS prior to lysis. Lyse cells by homogenization or sonication with ice-cold pH 7.0 PBS then centrifuge at 3000 × g for 12 minutes at 4°C. Aliquot the supernatant for storage at –70°C, protein determination and subsequent assays.
Plasma: Collect blood with sodium citrate and centrifuge at 3000 × g for 12 minutes at 4°C. Remove the plasma and aliquot for storage at –70°C, protein determination and subsequent assays. Sample results can alternatively be expressed in mL of plasma.
Food: This can vary considerably from one food to the next and requires foresight and discretion from the principal investigator. 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 pH 7.0 PBS then centrifuge at 3000 × g for 12 minutes at 4°C. Aliquot the supernatant for storage at –70°C, protein determination and subsequent assays.

Liquid Food: Centrifuge at 3000 × g for 12 minutes at 4°C. Aliquot the supernatant for storage at –70°C, protein determination and subsequent assays.

STANDARD CURVE PREPARATION

PLEASE NOTE:

1. The Trolox Standard vial is under vacuum.
2. Ethanol should be 200 proof. Do not use denatured ethanol as it contains antioxidants.
3. If 200 proof ethanol is not available, PBS can be used but care should be exercised to ensure the Trolox is completely dissolved. Loss of material will greatly affect the accuracy of your assay.

TO RECONSTITUTE THE TROLOX STANDARD:

Preferred Method – Add 2.0 mL of Ethanol directly to the Trolox Standard vial with a needle and syringe by puncturing the rubber stopper with the needle. Vortex the vial for 30-60 seconds, or until the standard is completely dissolved. The concentration is now 2.0 mM. Slowly remove the rubber stopper, allowing the vacuum seal to break and proceed to **Table 1** for the standard curve dilutions.
Alternative Method – Not Recommended - WARNING!!! The solid Trolox can become airborne due to the opening process or due to the turbulence created during pipetting and

reconstitution. Loss of material will greatly affect the accuracy of your assay – this method is performed at the user's risk and discretion. Slowly lift a corner of the rubber stopper until the vacuum seal is broken. Now remove the stopper and slowly add 2.0 mL of Ethanol to the vial. Recap the vial with the same rubber stopper and Parafilm or tape the stopper to the vial then vortex for 30-60 seconds, or until the standard is completely dissolved. The concentration of this standard is now 2.0 mM. Carefully remove the stopper and proceed to **Table 1** for the standard curve dilutions.
The reconstituted standard can be aliquoted and stored at –70°C for up to one year.
Use the following table to dilute the 2 mM Trolox Standard and construct a six-point standard curve.

Table 1: Standard Curve Preparation

Standard	Trolox Conc. (mM)	Vol. of Deionized Water (uL)	Transfer Volume (uL)	Transfer Source	Final Volume (uL)
S5	2.0	-	2000	2 mM Stock	1500
S4	1.0	500	500	S5	500
S3	0.5	500	500	S4	500
S2	0.25	500	500	S3	500
S1	0.125	500	500	S2	1000
S0	0	500	-	-	500

ASSAY PROCEDURE

1. Dilute both Samples and Standards 1:40 in the provided Dilution Buffer (e.g. 15 µL serum + 585 µL Dilution Buffer). **See Scheme I** for a suggested plate layout.
2. Place 200 µL of diluted Samples or Standards in each well. Reagent Blanks (BLK) should contain Dilution Buffer in place of Standard or Sample.
3. Read the plate at 450 nm for a reference measurement.
4. Add 50 µL of Copper Solution to each well and incubate for 3 minutes at room temperature.
5. Add 50 µL of Stop Solution.
6. Read the plate a second time at 450 nm.

Scheme I: Sample Plate Layout

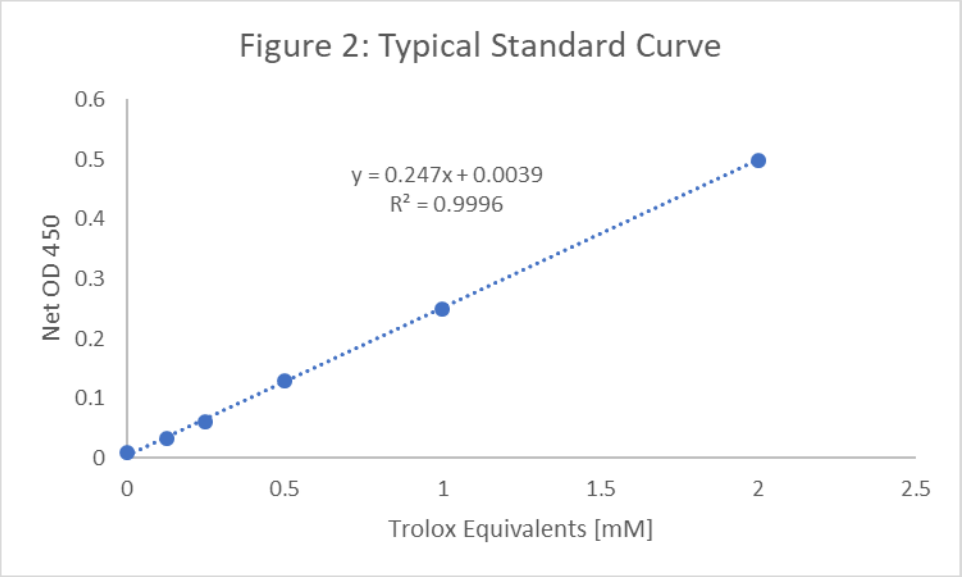
	1	2	3	4	5	6	7	8	9	10	11	12
A	S_0	S_0	U_3	U_3	U_{11}	U_{11}	U_{19}	U_{19}	U_{27}	U_{27}	U_{35}	U_{35}
B	S_1	S_1	U_4	U_4	U_{12}	U_{12}	U_{20}	U_{20}	U_{28}	U_{28}	U_{36}	U_{36}
C	S_2	S_2	U_5	U_5	U_{13}	U_{13}	U_{21}	U_{21}	U_{29}	U_{29}	U_{37}	U_{37}
D	S_3	S_3	U_6	U_6	U_{14}	U_{14}	U_{22}	U_{22}	U_{30}	U_{30}	U_{38}	U_{38}
E	S_4	S_4	U_7	U_7	U_{15}	U_{15}	U_{23}	U_{23}	U_{31}	U_{31}	U_{39}	U_{39}
F	S_5	S_5	U_8	U_8	U_{16}	U_{16}	U_{24}	U_{24}	U_{32}	U_{32}	U_{40}	U_{40}
G	U_1	U_1	U_9	U_9	U_{17}	U_{17}	U_{25}	U_{25}	U_{33}	U_{33}	U_{41}	U_{41}
H	U_2	U_2	U_{10}	U_{10}	U_{18}	U_{18}	U_{26}	U_{26}	U_{34}	U_{34}	BLK	BLK

CALCULATIONS

1. Calculate the net absorbance by subtracting the absorbance readings from step 3 from those in step 6.
2. Plot the net absorbance vs. Trolox concentration. A typical standard curve is shown in Figure 2.
3. Samples can be expressed in terms of µM Trolox equivalents by solving for "x" from your generated standard curve equation.
   y = mx + b
   where: y = y-axis value (OD readings) m = slope
   x = x-axis value (Trolox concentration) b = intercept

To express values in µM copper reducing equivalents (CRE's), multiply the "x" value coefficient by 2189 µM.

To express values in µM Uric Acid equivalents, divide the µM Trolox equivalents by 1.33.



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*Oxis*Research™

1499 Rollins Road
Burlingame, CA 94010 U.S.A.
E-mail: info@aoxre.com
Telephone: 650-289-8908
www.aoxre.com
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