Aqueous CUPRAC Antioxidant Assay

Catalogue Number: FS02

This test is sufficient for assaying 50 samples and two standard curves.

FOR RESEARCH USE ONLY

Not For Diagnostic Use

PRECAUTIONS AND STORAGE

Carefully read and understand these instructions before beginning any testing. This kit is for research use only, and is not for diagnostic use or for use in humans.

Wearing appropriate personal protective equipment such as gloves, lab coat and eye protection is highly recommended. In case of contact with skin or eyes, immediately rinse with plenty of water for 15 minutes and consult a physician.

This kit contains methanol which is a flammable solvent.

This kit will perform as specified until the date printed on the outside of the box.

IF YOU HAVE PROBLEMS

Technical Service Contact Information

Phone:	248.852.8815
E-Mail:	info@oxfordbiomed.com
Hours:	M-F 8:30 AM to 5:00 PM EST

For us to serve you best, please have the lot number from the kit ready for us to reference.

MATERIALS NEEDED BUT NOT SUPPLIED

1. A spectrophotometer capable of measuring absorbance at 450 nm
2. Adjustable micropipettes (10 – 1,000 µL) and tips
3. 10 mL glass test tubes
4. Vortex mixer
5. 1 cm path length cuvettes
6. Deionized water

INTRODUCTION

Antioxidants are plentiful in nature, designed to help preserve cells from oxidative damage due to injury or the environment. Many of these antioxidants are beneficial to humans, and are believed to be involved in preventing many diseases. Consumers have taken a great interest in their consumption of antioxidants for improved health, and the food industry has taken notice – oftentimes listing the amount on prepared, packaged foods. The OBR Food Science Division Aqueous CUPRAC Antioxidant Assay has been designed to quantify the antioxidant levels in water-based foods and food extracts[1].

This assay is based on the changes in absorption characteristics of the neocuproine (Nc) copper (II) complex when it is reduced by an antioxidant[2]. The reduction potential of the sample or standard effectively converts Cu^{+2} to Cu^{+1}, thus changing the absorbance maximum, as shown in **Figure 1**. This reduced copper complex gives an absorption maximum at 450 nm. The calibration curve is generated using a known concentration of Trolox, with the data being expressed as µM Trolox equivalents.

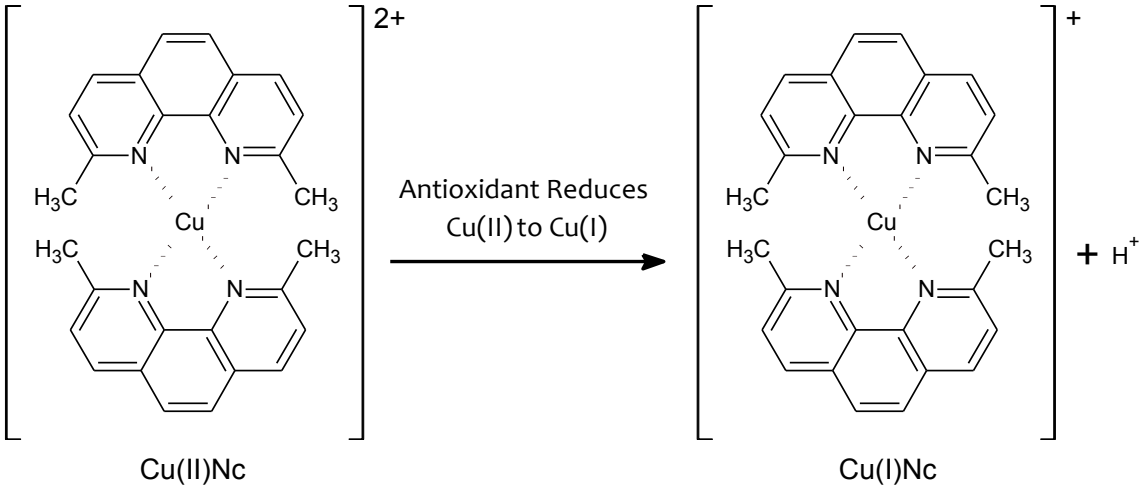

Figure 1. Reduction of the neocuproine/copper (II) complex.

KIT COMPONENTS

Developing Solution:	A solution of chromogen in methanol, 30 mL
Copper Solution:	A solution of copper (II) chloride in water, 10 mL
TRIS Solution:	A solution of 2-amino-2-(hydroxymethyl)-1,3-propanediol in water, 10 mL
Trolox Standard:	A 2,500 µM solution of (±)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid in methanol, 2.0 mL

REAGENT PREPARATION

Except for the Trolox standard which should be frozen upon receipt, the kit is shipped ready-to-use and can be stored at room temperature. The standard should be allowed to reach room temperature before use. Several minutes prior to running the assay, the **Indicator Solution** should be prepared as follows:

1. Add 10.0 mL of the **Developing Solution** into a plastic or glass vial.
2. Add 4.0 mL of the **Copper Solution** to the vial.
3. Add 6.0 mL of the **TRIS Solution** to the vial and mix well. The color of the solution should be a deep green/blue.

This is enough **Indicator Solution** to run 10 samples/standards, and is stable for several hours before a precipitate forms, making it unusable.

A WORD ON SAMPLE BLANKS…

This assay is colorimetric, with the reagent changing from blue-green to yellow in the presence of an antioxidant. This color change is most noticeable in the 440-460 nm region, which is also the color of many food products, such as tea and coffee. If the color of the food is not subtracted from the value obtained from the assay, then there will be a large error in the antioxidant value.

A simple way to correct for this is to run a sample blank, which simply measures the absorbance of the sample at 450nm. It is up to the operator to determine if the samples need to have a blank performed for each sample, as some food extracts and juices do not absorb at 450nm.

For example, freshly brewed coffee was prepared as a sample blank, and was found to have an absorbance of 0.260 at 450 nm. If the absorbance of the assayed sample was 0.56 after the reaction, this would account for an interference of 46%.

TECHNICAL NOTES

Some samples, such as fruit juices, contain pectin which is difficult to remove and will cause artificially high absorbance readings due to sample haziness. However, if diluted as instructed, the interference should be negligible as long as a sample blank is run.

Particulate matter in samples should be removed by centrifugation or by passing the sample through a 0.45 micron syringe filter.

SAMPLE AND STANDARD PREPARATION

SAMPLE PREPARATION

1. Add 1.0 mL of deionized water into a glass test tube.
2. Add 100 microliters of the aqueous sample.
3. Mix well by using a vortex mixer.
4. Prepare these dilutions no more than 30 minutes prior to running the assay as they can be affected by oxygen, heat and light.
5. DO NOT add the **Indicator Solution** until all samples and standards are diluted.

SAMPLE BLANK PREPARATION

1. Add 2.0 mL of deionized water into a glass test tube.
2. Add 100 microliters of the aqueous extract into the tube, mix well.
3. Record the absorbance of the sample at 450nm. DO NOT add **Indicator Solution** to the sample blank.
4. Discard the sample blank.

STANDARD CURVE

STANDARD PREPARATION

After dilution, the standard solutions should be used within one day and then discarded, as they are sensitive to oxygen. Trolox is provided as a solution in *n*-butanol and should be warmed to room temperature and mixed before using. **Table 1**, below, shows the standards and their respective concentrations.

Standard	Trolox Concentration, µM
S0	0
S1	39.06
S2	78.13
S3	156.25
S4	312.5
S5	625
S6	1,250
S7	2,500

Table 1. Standard concentrations.

Procedure:

Part I, Dilution of the **Trolox Standard**:

1. Label eight test tubes **D0** to **D7**.
2. Into tube **D7**, add 1.0 mL of the 2,500 µM **Trolox Standard** and set aside.
3. Into tubes **D6** thru **D0**, add 1.0 mL of deionized water and set tube **D0** aside.
4. Take 1.0 mL of the stock 2,500 µM **Trolox Standard**; add it to tube **D6** and mix.
5. Remove 1.0 mL of **D6**, add it to tube **D5** and mix.
6. Remove 1.0 mL of **D5**, add it to tube **D4** and mix.
7. Remove 1.0 mL of **D4**, add it to tube **D3** and mix.
8. Remove 1.0 mL of **D3**, add it to tube **D2** and mix.
9. Remove 1.0 mL of **D2**, add it to tube **D1** and mix.

Part II, Preparing the Standards for the assay:

1. Label six test tubes **S0** to **S7**.
2. Add 1.0 mL of deionized water into each tube.
3. Add 100 microliters of tube **D0** into tube **S0**, repeat for each standard.
4. DO NOT add the **Indicator Solution** until all samples and standards are diluted.

RUNNING SAMPLES AND STANDARDS

MIXING SAMPLES AND STANDARDS: *It is very important that the standards and samples are mixed with the Indicator Solution at the same time. Do not run the Standard Curve and Samples separately.*

STANDARDS AND SAMPLES

1. Add 1.0 mL of the **Indicator Solution** to all samples and standards and mix well. Tube **S7** should become noticeably more green-yellow than tube **S0**.
2. Allow to react for 10 minutes.
3. Place each sample and standard into a 1.0 cm path length cuvette.
4. At 450 nm, blank the **S0** standard to set it to zero.
5. Read all samples and standards at 450 nm.

CALCULATING SAMPLE TROLOX EQUIVALENT VALUES

1. If a Sample Blank was performed, subtract this value from the value of the assay value. This is called the "corrected absorbance".
2. Plot a standard curve using the corrected absorbance value for each Standard versus the Trolox concentration for each Standard. The equation of the line can be found using a linear fit method shown in **Figure 2**, below:

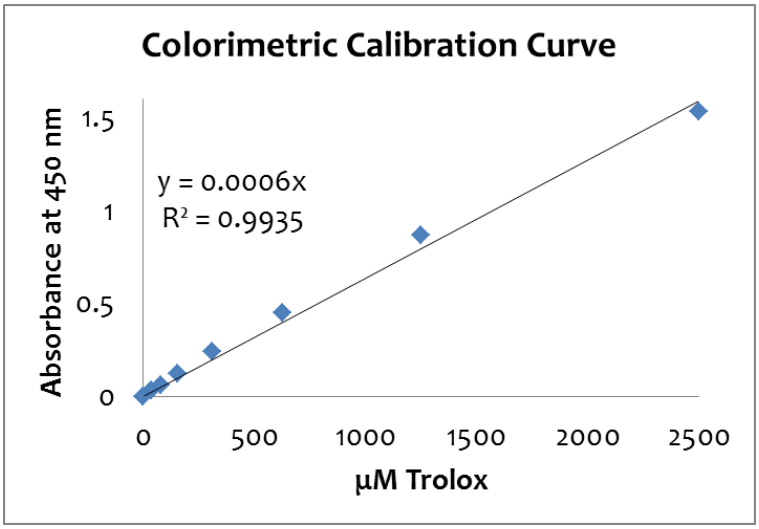

Figure 2: Trolox Calibration Curve

3. Calculate the Trolox concentration for each Sample using the absorbance (or corrected absorbance) value and the equation generated by the Standard Curve.

   EXAMPLE: Using the equation from the calibration curve shown in **Figure 2**.

   y = 0.0006x
   or **Absorbance = 0.0006x**
   which gives:
   x = Absorbance/0.0006
   Where (x) equals µM Trolox

So a tea extract sample that gave a corrected absorbance of 0.92 at 450 nm has a value of 1,533 µM Trolox equivalents.

SAMPLE COLLECTION

Antioxidants are unstable towards air, light and heat and 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 insure a homogenous sample. Also, oxygen in air can reduce the antoxidant concentration in the sample by mixing solutions and introducing bubbles, which is especially true for aqueous samples. Try to mix samples with a vortex mixer set to low speed to reduce the amount of oxygen exposure.

References:

1. Cekiç, S.D.; Cetinkaya, A.; Avan, A.N.; Apak, R. Correlation of Total Antioxidant Capacity with Reactive Oxygen Species (ROS) Consumption Measured by Oxidative Conversion. *J. Agric. Food Chem*. **2013**, *61*, 5260−5270.
2. a. Apak, R.; Guculu, K.; Ozyurek, M.; Karademir, Novel Total Antioxidant Capacity Index for Dietary Polyphenols and Vitamins C and E, Using Their Cupric Ion Reducing Capability in the Presence of Neocuproine: CUPRAC Method. *J. Agric. Food Chem.* **2004**, *52*, 7970-7981. b. Ozyurek, M.; Guculu, K.; Apak, R. The Main and Modified CUPRAC Methods of Antioxidant Measurement. *Trends in Analytical Chemistry*, **2011**, *30*, 652-644.

Warranty and Limitation of Remedy

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The Buyer's exclusive remedy and OBR's sole liability shall be limited to a refund of the purchase price of the kit, or at the discretion of OBR, the replacement of the kit at no cost to the buyer, of all material that fails to meet our specifications.

Said refund or replacement is conditional on the Buyer giving written notice to OBR within thirty (30) days of receipt of the material. Failure of Purchaser to give said notice within thirty (30) days shall constitute a waiver by Purchaser of al claims hereunder with respect to said material.