EAGLE BIOSCIENCES

CUPRAC Food and Beverage Antioxidant Assay

Catalog Number:
CPC96-K01 (1 x 96 wells)
For Research Use Only. Not for use in diagnostic procedures.
v. 1.0

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INTENDED USE

The Eagle Biosciences CUPRAC Food and Beverage Antioxidant Assay has been designed to quantify the antioxidant levels in water-based foods and food extracts. The Eagle Biosciences CUPRAC Food and Beverage Antioxidant Assay is for research use only and not to be used in diagnostic procedures.

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 Food Science Division Aqueous CUPRAC Antioxidant Assay has been designed to quantify the antioxidant levels in water---based foods and food extracts. This assay is based on the changes in absorption characteristics of the neocuproine (Nc) copper (II) complex when it is reduced by an antioxidant. 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.

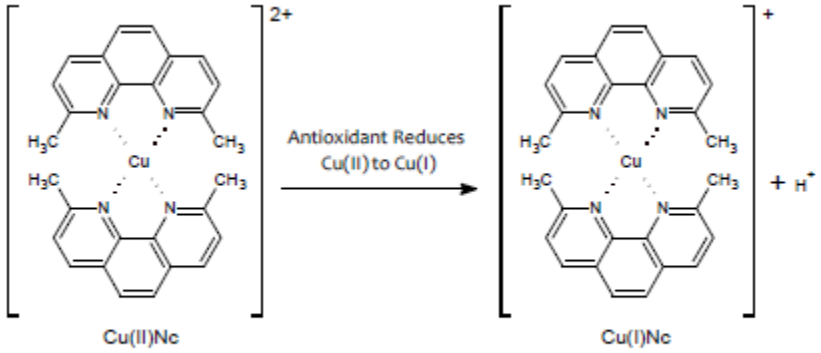

Figure 1. Reduction of the neocuproine/copper (II) complex.

KIT COMPONENTS

Dilution Buffer: A solution of chromogen used to dilute the standards and samples, 85 mL
Copper Solution: A solution used to produce the color reaction, 25 mL
Stop Solution: A solution used to stop the color reaction, 25 mL
Trolox Standard: A lyophilized vial of 2mM Trolox standard, 1 vial

SAMPLE COLLECTION

Solid samples should be homogenized in DI water and then centrifuged or filtered to clarify. Weights of solid samples and volume of water used to homogenize should be recorded and applied in the final calculations. Liquid samples should also be centrifuged or filtered to clarify.

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 insure a homogenous sample. Also, oxygen in air can reduce the antioxidant

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.

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 20 µL of sample into a glass test tube.
2. Add 780 µL of Dilution Buffer to each 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 Copper Solution until all samples and standards are diluted.
6. Proceed to Running Samples and Standards instructions below.

SAMPLE BLANK PREPARATION

1. Add 20 µL of sample into a glass test tube.
2. Add 980 µL of Dilution Buffer to each sample.
3. Mix well by using a vortex mixer.
4. Proceed to Running Samples and Standards instructions below.

STANDARD CURVE

STANDARD DILUTIONS

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 eb 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.

Standard	Trolox Conc. (mM)	Vol. of Deionized Water (µL)	Transfer Volume (µL)	Transfer Source	Final Volume (µL)
S_5	2.0	-	2000	2 mM	1500
S_4	1.0	500	500	S_5	500
S_3	0.5	500	500	S_4	500
S_2	0.25	500	500	S_3	500
S_1	0.125	500	500	S_2	1000
S_0	0	500	-	-	500

Table 1. Standard concentrations.

STANDARD PREPARATION

1. Add 20 µL of standard into a glass test tube.
2. Add 780 µL of Dilution Buffer to each 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 Copper Solution until all samples and standards are diluted.
6. Proceed to Running Samples and Standards instructions below.

RUNNING SAMPLES AND STANDARDS
Mixing samples and standards: It is very important that the standards and samples are mixed with the copper solution for the same amount of time. Do not run the Standard Curve and Samples separately.

STANDARDS AND SAMPLES

1. Add 200 µL of Copper Solution so each test tube containing Standards or Samples.
2. Allow to react for 10 minutes.
3. Add 200 µL of Stop Solution to each test tube.
4. Place each sample and standard into a 1.0 cm path length cuvette.
5. At 450 nm, blank the S_0 standard to set it to zero.
6. Read all samples and standards at 450 nm.

SAMPLE BLANKS

1. Allow to sit at room temperature for 10 minutes.
2. Add 200 µL of Stop Solution to each test tube.
3. Place each sample blank into a 1.0 cm path length cuvette.
4. Read all sample blanks at 450 nm.

NOTE: Do not add Copper Solution to the sample blanks.

CALCULATING SAMPLE TROLOX EQUIVALENT VALUES

1. If a Sample Blank was performed, subtract this value from the value of the Sample 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:

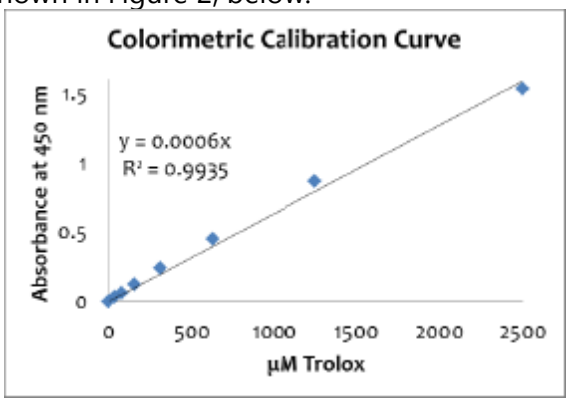

Figure 2: Trolox Calibration Curve

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 tht gave a corrected absorbance of 0.92 at 450 nm has a value of 1,533 µM Trolox equivalents.

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.

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