Product Manual

OxiSelect™ Trolox Equivalent Antioxidant Capacity (TEAC) Assay Kit (ABTS)

Catalog Number

XAN-5040 **200 assays**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

CELL BIOLABS, INC.
Creating Solutions for Life Science Research

Introduction

Oxidative stress is a physiological condition where there is an imbalance between concentrations of reactive oxygen species (ROS) and antioxidants. However, excessive ROS accumulation will lead to cellular injury, such as damage to DNA, proteins, carbohydrates, and lipid membranes. The cellular damage caused by ROS has been implicated in the development of many disease states, such as cancer, diabetes, cardiovascular disease, atherosclerosis, and neurodegenerative diseases. Under normal physiological conditions, cellular ROS generation is counterbalanced by the action of cellular antioxidant enzymes, macro or micro molecules, as well as other redox molecules.

Antioxidants include both hydrophilic and lipophilic molecules for metabolizing ROS. These may be localized transiently within different tissues or cells. Due to their potential harmful effects, excessive ROS must be promptly eliminated from the cells by this variety of antioxidant defense mechanisms. Antioxidants commonly neutralize radicals via a hydrogen atom transfer (HAT) or single electron transfer (SET) mechanism. Although the products of ROS-induced oxidative stress are extensively used to monitor their biological effects, it is also important to evaluate the antioxidant capacity of biological fluids, cells, and extracts.

Cell Biolabs' OxiSelect™ Trolox Equivalent Antioxidant Capacity (TEAC) Assay Kit measures the total antioxidant capacity of biomolecules from a variety of hydrophilic or lipophilic samples. The TEAC Assay is based on the conversion of oxidized $ABTS^{\cdot+}$ radical to ABTS via SET or HAT antioxidant mechanisms. Antioxidants neutralize the radical ion in a concentration dependent manner, which correlates with a proportional decrease in color intensity. The antioxidant concentration, steric accessibility, and reaction kinetics on the radical adsorption will influence the antioxidant activity values. Antioxidant activity is compared to the water soluble vitamin E analog Trolox.

Cell Biolabs' OxiSelect™ TEAC Assay Kit is a versatile, fast and reliable kit for the direct measurement of total antioxidant capacity from plasma, serum, urine, cell lysates, tissue homogenates, and food extracts. Each kit provides sufficient reagents to perform up to 200 assays, including blanks, antioxidant standards and unknown samples. Both hydrophilic and lipophilic samples are compatible with the assay and it is stable over a broad pH range. The assay does not distinguish between hydrophilic and lipophilic antioxidants, thus the combined antioxidant capacity is measured. The assay is designed for use in single plate microplate readers as well as readers with high-throughput capabilities. The assay may be performed as an end point assay or run kinetically if needed. Please read the complete kit insert prior to performing the assay.

Assay Principle

Cell Biolabs' OxiSelect™ TEAC Assay Kit measures the total antioxidant capacity within a sample. Samples are compared to known concentrations of Trolox standards within a 96-well microtiter plate format. Samples and standards are added to the microplate well and, upon the addition of the primed ABTS probe, the reaction proceeds for a few minutes. The reaction is read with a standard 96-well spectrophotometric microplate reader at 405-415 nm. Antioxidant capacity is determined by comparison with the Trolox standards.

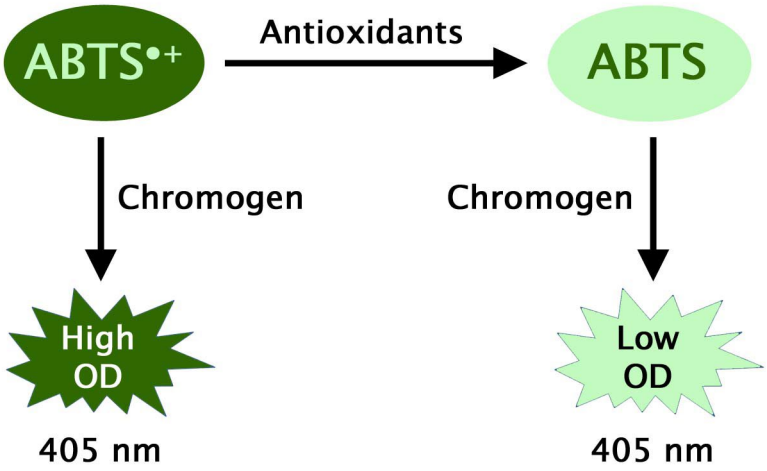

Figure 1. TEAC Assay Principle. The oxidized $ABTS^{\cdot+}$ radical is reduced to the ABTS form in the presence of antioxidants in a concentration-dependent manner, leading to a decrease in optical density at 405 nm.

Related Products

1. STA-312: OxiSelect™ Total Glutathione (GSSG/GSH) Assay Kit
2. STA-340: OxiSelect™ Superoxide Dismutase Activity Assay
3. STA-341: OxiSelect™ Catalase Activity Assay
4. STA-342: OxiSelect™ Intracellular ROS Assay Kit (Green Fluorescence)
5. STA-344: OxiSelect™ Hydrogen Peroxide/Peroxidase Assay (Fluorometric)
6. STA-345: OxiSelect™ ORAC Activity Assay
7. STA-346: OxiSelect™ HORAC Activity Assay
8. STA-347: OxiSelect™ In Vitro ROS/RNS Assay Kit (Green Fluorescence)
9. STA-349: OxiSelect™ Cellular Antioxidant Activity Assay Kit (Green Fluorescence)
10. STA-360: OxiSelect™ Total Antioxidant Capacity (TAC) Assay Kit
11. STA-844: OxiSelect™ Hydrogen Peroxide/Peroxidase Activity Assay (Colorimetric)
12. STA-859: OxiSelect™ Ferric Reducing Antioxidant Power (FRAP) Assay Kit
13. STA-860: OxiSelect™ Ascorbic Acid Assay Kit (FRASC)

Kit Components (shipped at room temperature)

1. ABTS Colorimetric Probe (Part No. 50401B): One 25 mg amber tube of powder
2. Trolox™ Standard (Part No. 50402C): One 200 µL amber tube of a 10 mM solution in ethanol
3. ABTS Primer (Part No. 50403B): One 200 mg amber tube of powder
4. Assay Buffer (10X) (Part No. 50404A): One 15 mL bottle

Materials Not Supplied

1. Standard 96-well microtiter plates for use in microplate reader
2. 10 kDa MWCO centrifugal filter (for high protein content samples)
3. 1X PBS and deionized water
4. Ethanol or other organic solvent for lipid-based samples
5. Sonicator or homogenizer for sample preparations
6. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips
7. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
8. Spectrophotometric microplate reader capable of reading 405-415 nm

Storage

Upon receipt store the Trolox Standard at -20ºC. Store all remaining kit components at room temperature.

Preparation of Reagents

Reagents may be prepared for either hydrophilic or lipophilic samples. Although many lipophilic samples are soluble upon dilution with 1X PBS, the kit reagents may be prepared in ethanol to ensure complete solubility.

- 10X ABTS Primer: Dissolve 10 mg of ABTS Primer in 1.5 mL deionized water. Vortex thoroughly until dissolved. Store the 10X ABTS Primer at 4ºC and protected from light for up to 24 hours.
- 50X ABTS Reagent: Prepare the ABTS Reagent by first priming the ABTS Colorimetric Probe. Dilute the 10X ABTS Primer solution 1:10 in 1.3 mL total volume with 5 mg of ABTS powder. (e.g. Dissolve 5 mg ABTS powder in 1.170 mL deionized water and then add 130 µL 10X ABTS Primer solution). Mix to homogeneity. Allow the 50X ABTS Reagent to incubate at room temperature protected from light for at least 4 hours. The solution will turn from light green to dark green. Once complete, store the 50X ABTS Reagent at 4ºC and protected from light for up to 48 hours.
- 1X Assay Buffer: Dilute the stock Assay Buffer (10X) 1:10 with deionized water. Mix to homogeneity. Store the 1X Assay Buffer at 4ºC up to six months.

Preparation of Samples

These preparation protocols are intended as a guide for preparing unknown samples. The user may need to adjust the sample treatment accordingly. All samples should be assayed immediately or stored for up to 2 months at -80°C. A trial assay with a representative test sample should be performed to determine the sample compatibility with the dynamic range of the standard curve. High levels of interfering substances may cause variations in results. Samples may be diluted in 1X Assay Buffer as necessary before testing. Run proper controls as necessary. Always run a standard curve with samples.

Note: Please see the Potential Interference and Compatibility section for chemicals that may interfere with assay results.

- Serum*: Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 30 minutes. Centrifuge at 2500 x g for 20 minutes. Remove the yellow serum supernatant without disturbing the white buffy layer. Samples should be tested immediately or frozen at -80°C.
- Plasma*: Collect blood sample and add to a blood collection tube containing heparin as the anticoagulant. Centrifuge at 3,000 rpm for 10-15 minutes at 4ºC. Remove the upper yellow plasma supernatant layer without disturbing the white buffy coat (leukocytes). Samples should be tested immediately or frozen at -80°C.

**Note: Hemolyzed plasma or serum should be avoided. Heparinized plasma is recommended over EDTA plasma.*

- Cell lysates: Lyse 1-2 x 10^6 cells/mL by sonication or multiple freeze-thaw cycles in 4 volumes of cold PBS or 1X Assay Buffer. Centrifuge at 12,000 rpm for 15 minutes at 4ºC and remove insoluble cell material. A high concentration of protein may interfere with the assay. In this case, filter the sample with a 10kDa MWCO centrifugal filter before assaying (to reduce protein interference and turbidity). Test samples immediately or store at -80°C.
- Tissue lysates/homogenates: Homogenize/sonicate approximately 10 mg of tissue in 1-2 mL of cold PBS or 1X Assay Buffer. Centrifuge the homogenate at 12,000 rpm for 15 minutes at 4ºC and collect the supernatant. A high concentration of protein may interfere with the assay. In this case, filter the sample with a 10kDa MWCO centrifugal filter before assaying (to reduce protein interference and turbidity). Test samples immediately or store at -80°C.
- Urine: Test neat or diluted with PBS or 1X Assay Buffer if appropriate.
- Lipophilic Fractions: Dissolve lipophilic samples in 100% ethanol or acetone and then dilute in 100% ethanol or 50% acetone. Incubate the mixture for 1 hour at room temperature with mixing. Further dilute samples as necessary prior to testing.
- Food Samples: Results may vary depending on sample source and purification. Dilution and preparation of these samples is at the discretion of the user, but use the following guidelines:
    - Solid or High Protein Samples: Weigh solid sample and then homogenize after adding deionized water (1:2, w/v). Centrifuge the homogenate at 10,000 x g for 10 minutes at 4ºC. Recover the supernatant which is the water-soluble fraction. The insoluble fraction (pulp) is further extracted by adding acetone (1:4, w(solid pulp)/v) and mixing at room temperature for 30-60 minutes. Centrifuge the extract/solid at 10,000 x g for 10 minutes at 4ºC. Recover the acetone extract and dilute with PBS, 1X Assay Buffer, ethanol or water as necessary prior to running the assay. The TEAC value is calculated by combining the results from the water-soluble fraction and the acetone extract from the pulp fraction.
    - Aqueous Samples: Centrifuge the sample at 10,000 x g for 10 minutes at 4ºC to remove any particulates. Dilute the supernatant in PBS or 1X Assay Buffer as necessary prior to running the assay.

Potential Interference and Compatibility

Table 1 contains some common substance compatibilities. The listed concentration values are prior to performing the assay. Dilution of the substance/buffer, and ultimately samples, may be required to

completely eliminate interference. Even with some interference, accurate quantitation can be achieved by running standards in the same buffer as samples, although kit sensitivity may be compromised.

Substance	Compatible Concentration
Acetone	50%
Deoxycholic acid	0.5%
Dithiothreitol	Not Compatible
DMSO	5%
EDTA	Not Compatible
Glycerol	50%
100 mM Hepes	Not Compatible
Methanol	50%
2-Mercaptoethanol	Not Compatible
NP-40	Not Compatible
PBS	undiluted
SDS	Not Compatible
Tris, pH 7.5	500 mM
Triton-X 100	0.1%
Tween-20	Not Compatible

Table 1. Substance Compatibilities

Preparation of Trolox Standard Curve

I. Hydrophilic (aqueous) Samples

1. Prepare fresh aqueous standards by diluting the 10 mM Trolox Standard stock solution to 0.3 mM in Assay Buffer (e.g. add 15 µL of Trolox Standard stock tube to 485 µL of Assay Diluent). Prepare a series of the remaining Trolox standards according to Table 1 below.

Tubes	10 mM Trolox™ Standard (µL)	Assay Diluent (µL)	Resulting Trolox™ Concentration (µM)
1	15	485	300
2	250 of tube #1	250	150
3	250 of tube #2	250	75
4	250 of tube #3	250	37.5
5	250 of tube #4	250	18.8
6	250 of tube #5	250	9.4
7	250 of tube #6	250	4.7
8	0	250	0

Table 2. Preparation of Standards for use when testing Hydrophilic Samples.

Note: Do not store diluted Trolox Standard solutions.

II. Lipophilic Samples

1. Prepare fresh lipophilic standards by diluting the 10 mM Trolox Standard stock solution to 0.3 mM in ethanol (e.g. add 15 µL of Trolox Standard stock tube to 485 µL of ethanol).
2. Prepare a series of the remaining Trolox standards according to Table 2 below.

Tubes	10 mM Trolox™ Standard (µL)	100% Ethanol (µL)	Resulting Trolox™ Concentration (µM)
1	15	485	300
2	250 of tube #1	250	150
3	250 of tube #2	250	75
4	250 of tube #3	250	37.5
5	250 of tube #4	250	18.8
6	250 of tube #5	250	9.4
7	250 of tube #6	250	4.7
8	0	250	0

Table 3. Preparation of Standards for use when testing Lipophilic Samples.

Note: Do not store diluted Trolox Standard solutions.

Assay 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.

1. Add 25 µL of the diluted Trolox Standards or samples to a 96-well microtiter plate.
2. Dilute the 50X ABTS Reagent 1:50 in either 1X Assay Buffer (Hydrophilic samples) or 75% ethanol (Lipophilic samples) (e.g. for 100 assays, combine 300 µL ABTS Reagent with 14.7 mL of either Assay Buffer or 75% ethanol). Vortex thoroughly.
3. Add 150 µL of the diluted ABTS Reagent to each well using either a multichannel pipette or a plate reader liquid handling system. Mix thoroughly. Immediately begin timing the reaction.
4. Incubate 5 minutes on an orbital shaker.
5. Read the plate at 405-415 nm immediately. The green colored product will fade over time.

Note: Trolox standards will completely react within 5 minutes. Different antioxidants may need more or less time to completely react. In these situations, the assay may also be performed kinetically by reading the plate at multiple time points.

Example of Results

The following figures demonstrate typical OxiSelect™ TEAC 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.

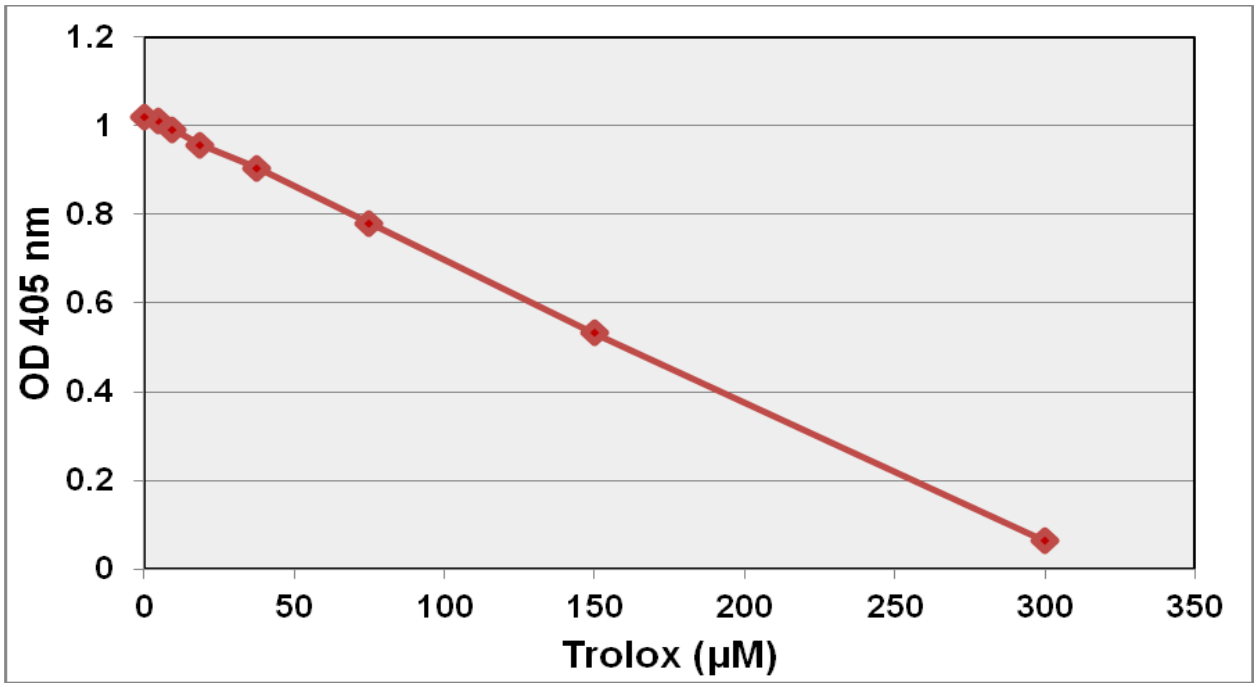

Figure 2: TEAC Assay Standard Curve (Hydrophilic).

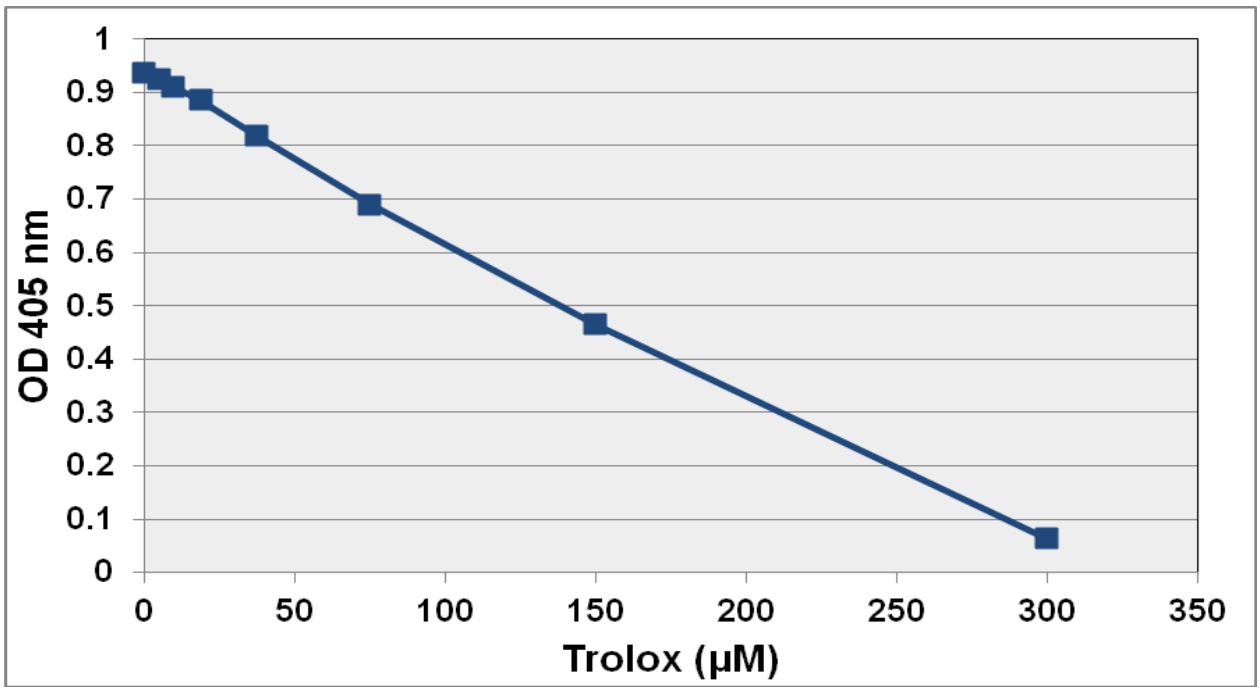

Figure 3: TEAC Assay Standard Curve (Lipophilic).

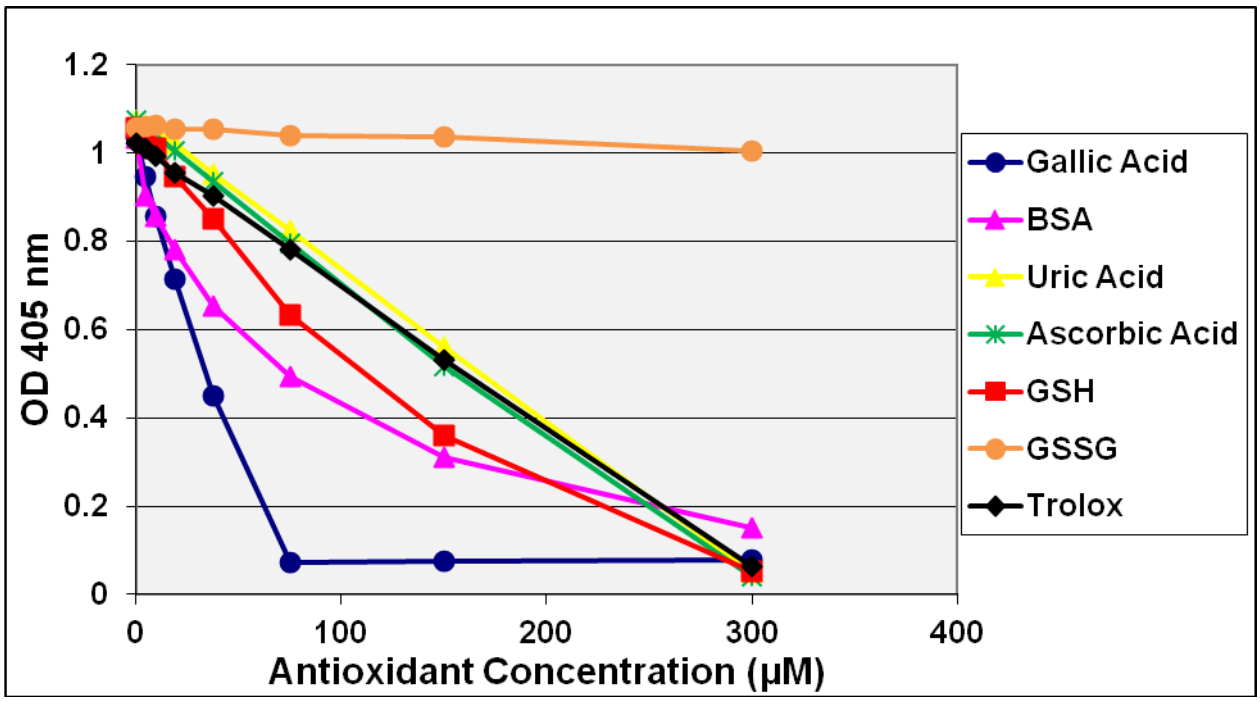

Figure 4: TEAC Assay of various hydrophilic antioxidants.

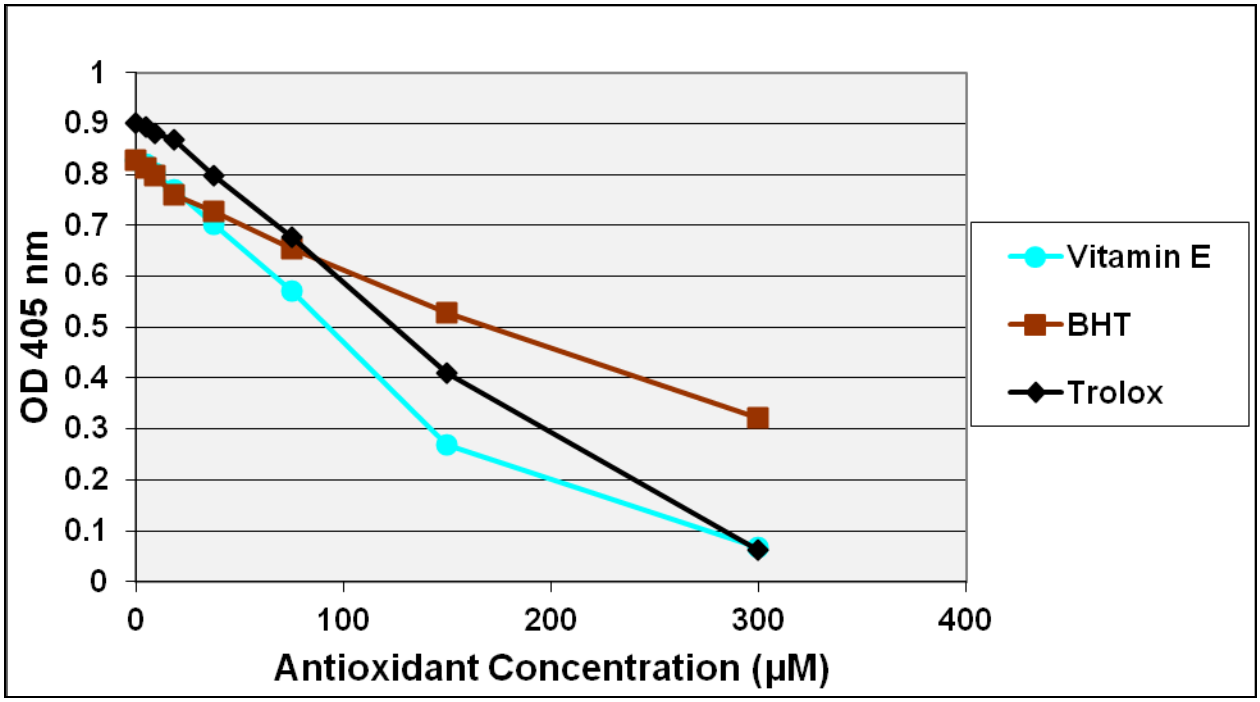

Figure 5: TEAC Assay results of various lipophilic antioxidants.

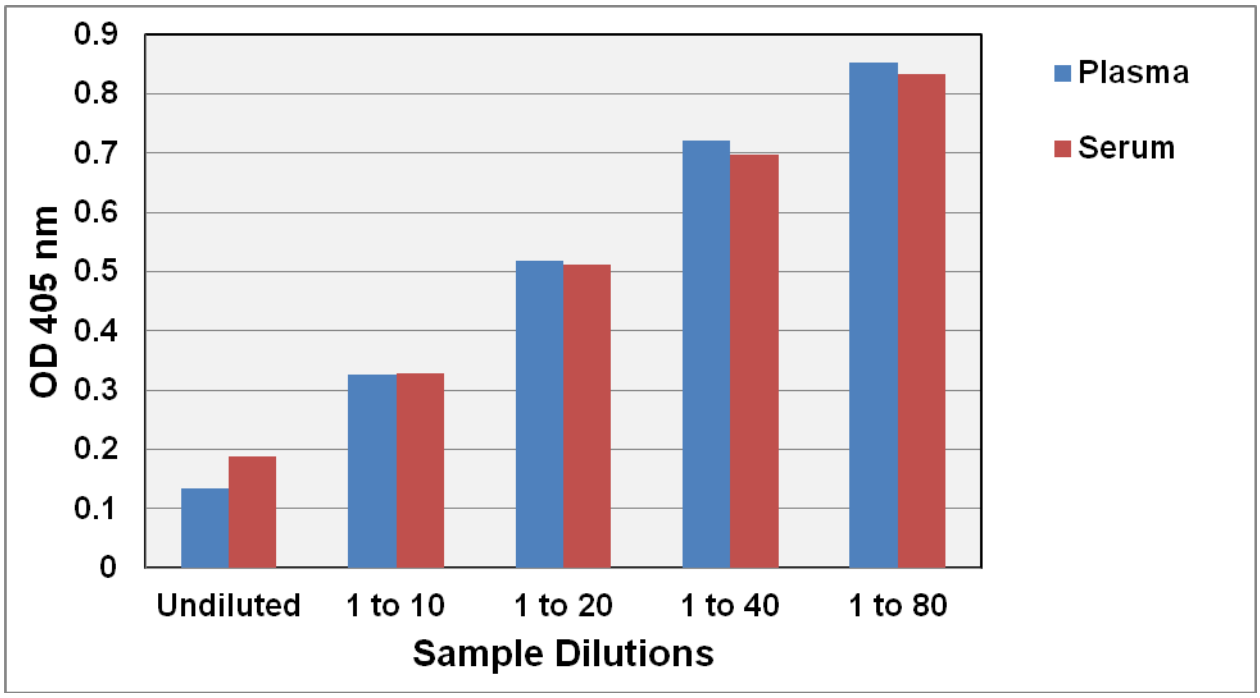

Figure 6: TEAC Assay results of human serum and plasma samples.

Calculation of Results

1. Calculate the average absorbance values for every standard, control, and sample.
2. Plot the average absorbance for the standards against the final concentration of the trolox standards from Table 2 (hydrophilic assays), or Table 3 (lipophilic assays) to determine the best curve. See Figures 2 and 3 for example standard curves.
3. Determine the antioxidant concentration, as µM Trolox equivalents (TEAC value), in of the samples with the equation obtained from the linear regression analysis of the standard curve. Substitute the average absorbance values for each sample. Remember to account for dilution factors. Only use values within the range of the standard curve.
4.

$$\textbf{Antioxidant (µM)} = \left[\frac{\textbf{(Sample average absorbance – (y-intercept))}}{\textbf{Slope}}\right] \textbf{ x Sample dilution}$$

Results may also be expressed as micromoles of Trolox equivalents per gram of sample (TE µmol / g). The formula weight of Trolox is 250.29 g / mol.

References

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2. Huang, H., et al. (2005) *J. Agric. Food Chem.* **53**: 1841-1856.
3. Kambayashi, Y., et al. (2009) *J. Clin. Biochem. Nutr.* **44**: 46-51.
4. Koracevic, D., et al. (2001) *J. Clin. Pathol.* **54**: 356-361.

5. Miller, N.J., et al. (1997) *Free Radic. Res.* **26**: 195-199.
6. Rice-Evans, C.A. (2000) *Free Radic. Res.* **33**: S59-66.

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Recent Product Citations

1. Elhassaneen, Y. A. et al. (2023). Influence of Novel Freezing and Storage Technology on Nutrient Contents, Bioactive Compounds and Antioxidant Capacity of Black Eggplant. *Journal of Agriculture and Crops*. **93**:338–352. doi: 10.32861/jac.93.338.352.
2. Pérez-Peiró, M. et al. (2023). Nitrosative and Oxidative Stress, Reduced Antioxidant Capacity, and Fiber Type Switch in Iron-Deficient COPD Patients: Analysis of Muscle and Systemic Compartments. *Nutrients*. **15**(6):1454. doi: 10.3390/nu15061454.
3. Qin, L. et al. (2021). Systemic Profiles of microRNAs, Redox Balance, and Inflammation in Lung Cancer Patients: Influence of COPD. *Biomedicines*. **9**(10):1347. doi: 10.3390/biomedicines9101347.
4. Nallan Chakravartula, S.S. et al. (2021). Stinging Nettles as Potential Food Additive: Effect of Drying Processes on Quality Characteristics of Leaf Powders. *Foods*. **10**(6):1152. doi: 10.3390/foods10061152.

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