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Product Manual

# OxiSelect™ Total Antioxidant Capacity (TAC) Assay Kit

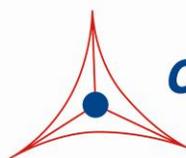
Catalog Number

STA-360

200 assays

**FOR RESEARCH USE ONLY**  
Not for use in diagnostic procedures

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**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, 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 also include both hydrophilic and lipophilic molecules for metabolizing ROS. These may be localized transiently within different tissues or cells. Because of their potential harmful effects, excessive ROS must be promptly eliminated from the cells by this variety of antioxidant defense mechanisms.

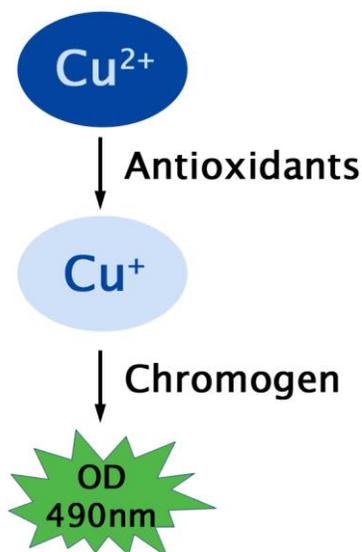
Although the products of ROS-induced oxidative stress are extensively used to monitor their biological effects, it is also important to evaluate the antioxidant capacity of biological fluids, cells, and extracts. Antioxidants commonly neutralize radicals via a hydrogen atom transfer (HAT) or single electron transfer (SET) mechanism. SET assays quantify the capability of an antioxidant to transfer one electron to reduce any compound, such as free radicals, carbonyls, and metals.

Cell Biolabs' OxiSelect™ Total Antioxidant Capacity (TAC) Assay Kit measures the total antioxidant capacity of biomolecules from a variety of samples via a SET mechanism. The TAC Assay is based on the reduction of copper (II) to copper (I) by antioxidants such as uric acid. Upon reduction, the copper (I) ion further reacts with a coupling chromogenic reagent that produces a color with a maximum absorbance at 490 nm. The net absorbance values of antioxidants are compared with a known uric acid standard curve. Absorbance values are proportional to the sample's total reductive capacity. Results are expressed as "µM Copper Reducing Equivalents" or "mM Uric Acid Equivalents". Copper is advantageous over iron-based antioxidant assays because all classes of antioxidants, including thiols, are detected with marginal radical interference. In addition, the kinetics of the copper-based reaction is also faster than the iron-based reaction, which makes the TAC Assay an ideal tool for estimating reductive or antioxidant capacity efficiently and accurately.

Cell Biolabs' OxiSelect™ TAC Assay Kit is a fast and reliable kit for the direct measurement of total antioxidant capacity from cell lysate, plasma, serum, urine, tissue homogenates, and food extracts. Each kit provides sufficient reagents to perform up to 200 assays, including blanks, antioxidant standards and unknown samples. Hydrophilic and lipophilic samples are compatible with the assay.

## **Assay Principle**

Cell Biolabs' OxiSelect™ TAC Assay Kit measures the total antioxidant capacity within a sample. Samples are compared to a known concentration of uric acid standard within a 96-well microtiter plate format. Samples and standards are diluted with a reaction reagent and, upon the addition of copper, the reaction proceeds for a few minutes. The reaction is stopped and read with a standard 96-well spectrophotometric microplate reader at 490 nm (Figure 1). Antioxidant capacity is determined by comparison with the uric acid standards.



**Figure 1. TAC Activity Assay Principle**

### **Related Products**

1. STA-310: OxiSelect™ Protein Carbonyl ELISA Kit
2. STA-312: OxiSelect™ Total Glutathione (GSSG/GSH) Assay Kit
3. STA-320: OxiSelect™ Oxidative DNA Damage ELISA Kit (8-OHdG Quantitation)
4. STA-330: OxiSelect™ TBARS Assay Kit (MDA Quantitation)
5. STA-340: OxiSelect™ Superoxide Dismutase Activity Assay
6. STA-341: OxiSelect™ Catalase Activity Assay
7. STA-342: OxiSelect™ Intracellular ROS Assay Kit (Green Fluorescence)
8. STA-344: OxiSelect™ Hydrogen Peroxide/Peroxidase Assay (Fluorometric)
9. STA-345: OxiSelect™ ORAC Activity Assay
10. STA-346: OxiSelect™ HORAC Activity Assay
11. STA-347: OxiSelect™ In Vitro ROS/RNS Assay Kit (Green Fluorescence)

### **Kit Components**

1. Uric Acid Standard (Part No. 236001): One 100 mg tube of powder.
2. Reaction Buffer (100X) (Part No. 236002): One 400  $\mu\text{L}$  amber tube.
3. Copper Ion Reagent (100X) (Part No. 236003): One 1.0 mL tube.
4. Stop Solution (10X) (Part No. 236004): One 1.5 mL tube.

## **Materials Not Supplied**

1. Standard 96-well microtiter plates for use in microplate reader
2. 1N NaOH, 1X PBS and deionized water
3. Methanol or other organic solvent for lipid-based samples
4. Sonicator or homogenizer for sample preparations
5. 10  $\mu$ L to 1000  $\mu$ L adjustable single channel micropipettes with disposable tips
6. 50  $\mu$ L to 300  $\mu$ L adjustable multichannel micropipette with disposable tips
7. Spectrophotometric microplate reader capable of reading 490 nm

## **Storage**

Upon receipt store the Reaction Buffer (100X) at 4°C. 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, kit reagents and samples may be prepared in methanol to ensure complete solubility.

- 1X Reaction Buffer: Dilute the Reaction Buffer 1:100 with 1X PBS (hydrophilic) or with methanol (lipophilic). Mix to homogeneity. Store the 1X Reaction Buffer at 4°C up to three months.
- 1X Copper Ion Reagent: Dilute the Copper Ion Reagent 1:100 with deionized water (hydrophilic) or with methanol (lipophilic). Mix to homogeneity. Store the 1X Copper Ion Reagent at 4°C up to three months.
- 1X Stop Solution: Dilute the Stop Solution 1:10 with deionized water (hydrophilic) or with methanol (lipophilic). Mix to homogeneity. Store the 1X Stop Solution at 4°C up to three months.

## **Preparation of Samples**

Samples should be stored at -80°C prior to performing the assay. Samples should be prepared at the discretion of the user. The following recommendations are only guidelines and may be altered to optimize or complement the user's experimental design.

*Note: EDTA can interfere with the TAC assay and should not be present in any sample.*

- Tissue Lysate: Sonicate or homogenize tissue sample on cold PBS and centrifuge at 10,000 x g for 10 minutes at 4°C. Aliquot the supernatant for storage at -80°C, protein determination and subsequent TAC assay.
- Cell Culture: Wash cells 3 times with cold PBS prior to lysis. Lyse cells with sonication or homogenation in cold PBS and centrifuge at 10,000 x g for 10 minutes at 4°C. Aliquot the supernatant for storage at -80°C, protein determination and subsequent TAC assay.
- Plasma: Collect blood with heparin and centrifuge at 4°C for 10 minutes. Remove the plasma and aliquot samples for testing.
- Urine: Test neat or diluted with PBS if appropriate.

- Lipophilic Fractions: Dissolve lipophilic samples in 100% methanol or acetone and then dilute in 50% methanol or 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 4°C. Recover the supernatant which is the water-soluble fraction. The insoluble fraction (pulp) is further extracted by adding pure 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 4°C. Recover the acetone extract and dilute with PBS or water as necessary prior to running the assay. The TAC 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 4°C to remove any particulates. Dilute the supernatant in PBS as necessary prior to running the assay. Certain liquids such as juice extracts may be tested without dilution.

### **Preparation of Uric Acid Standard Curve**

1. Prepare fresh Uric Acid standards by weighing out the Uric Acid powder for a 10 mg/mL solution in 1N NaOH. This 10 mg/mL is equivalent to a concentration of 60 mM. Use the 60 mM Uric Acid solution to prepare a 2 mM solution of Uric Acid (eg. add 100  $\mu$ L of the 60 mM Uric Acid standard to 2.900 mL of deionized water).
2. Prepare a series of the remaining Uric Acid standards according to Table 1 below. Prepare in deionized water for hydrophilic testing or methanol for lipophilic testing.

<b>Tubes</b>	<b>2 mM Uric Acid Antioxidant Standard (<math>\mu</math>L)</b>	<b>Deionized Water or Methanol (<math>\mu</math>L)</b>	<b>Resulting Uric Acid Concentration (mM)</b>
1	500	500	1
2	500 of tube #1	500	0.5
3	500 of tube #2	500	0.25
4	500 of tube #3	500	0.125
5	500 of tube #4	500	0.0625
6	500 of tube #5	500	0.03125
7	500 of tube #6	500	0.0156
8	500 of tube #7	500	0.0078
9	500 of tube #8	500	0.0039
10	0	500	0.0

**Table 1. Preparation of Uric Acid Standards.**

*Note: The 60 mM stock Uric Acid standard solution may be aliquotted and stored at -70°C for up to one week. Do not store diluted Uric Acid Standard solutions.*

## **Assay Protocol**

Each Uric Acid 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 20  $\mu\text{L}$  of the diluted Uric Acid Standards or samples to the 96-well microtiter plate.
2. Add 180  $\mu\text{L}$  of the 1X Reaction Buffer to each well using either a multichannel pipette or a plate reader liquid handling system. Mix thoroughly.
3. Obtain an initial absorbance by reading the plate at 490 nm.
4. To initiate the reaction, add 50  $\mu\text{L}$  of the 1X Copper Ion Reagent into each well. Incubate 5 minutes on an orbital shaker.
5. Add 50  $\mu\text{L}$  of 1X Stop Solution to each well to terminate the reaction.
6. Read the plate again at 490 nm.

## **Example of Results**

The following figures demonstrate typical OxiSelect™ 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.

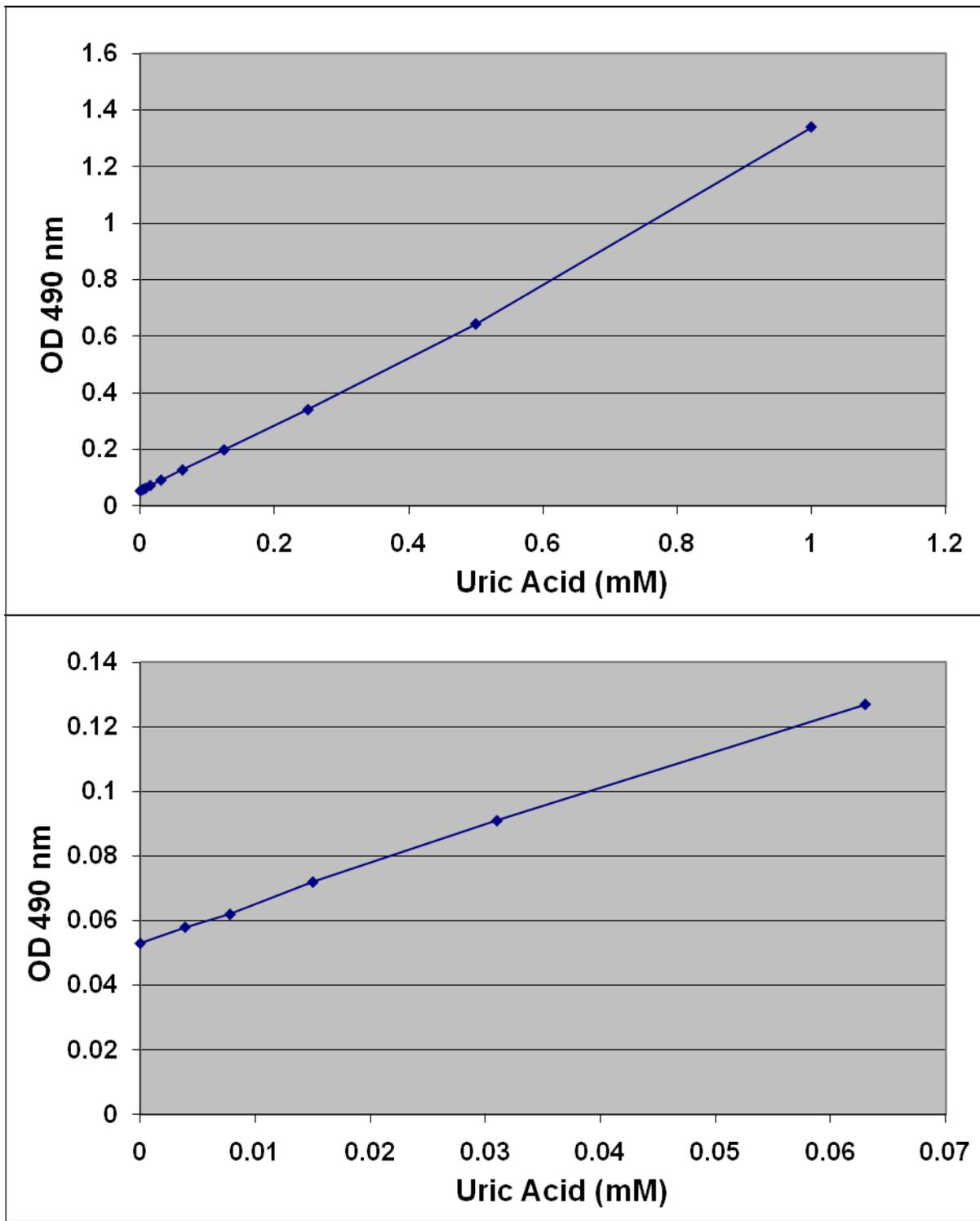


Figure 2: TAC Assay Standard Curve.

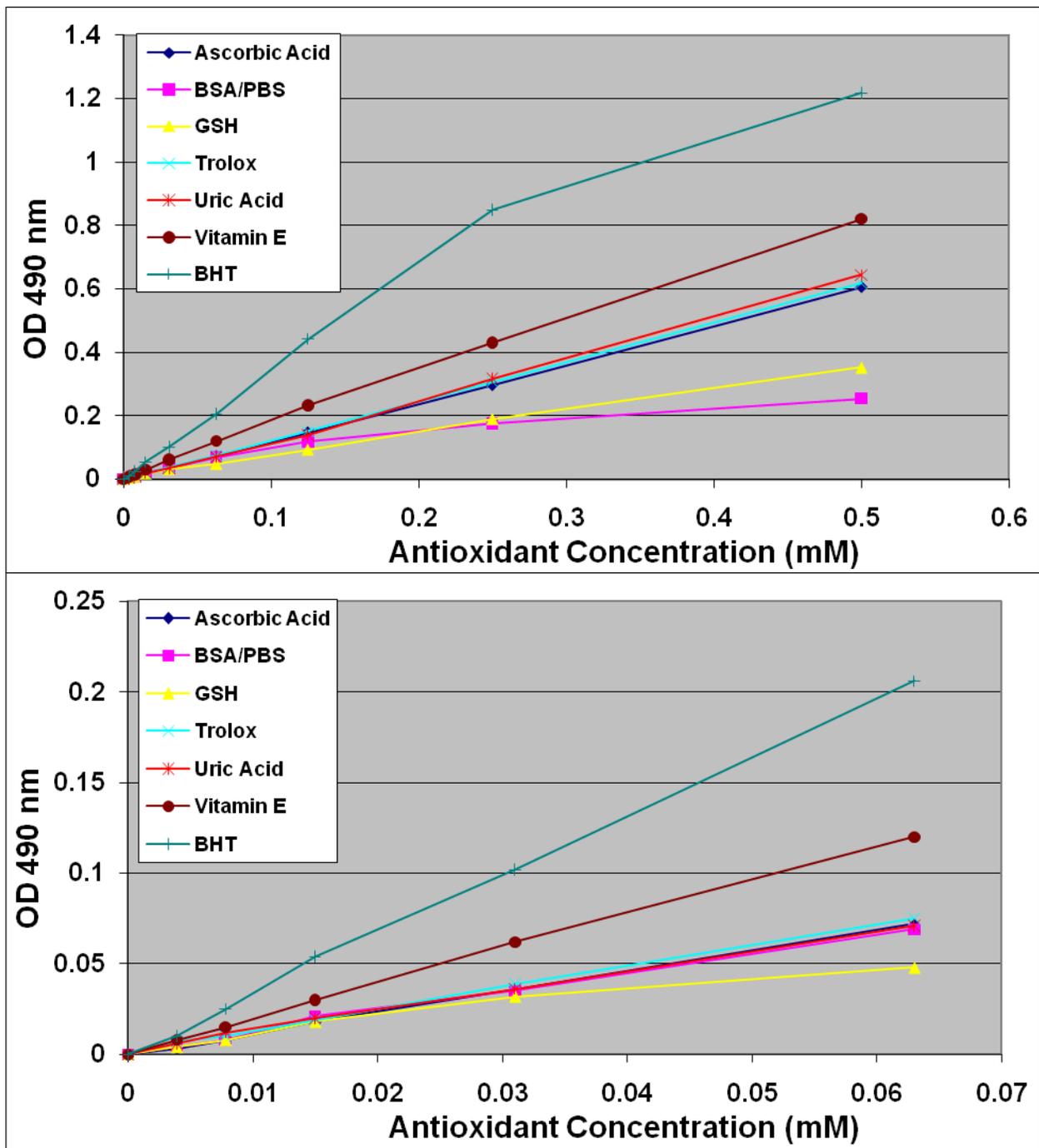


Figure 3: TAC Assay of various antioxidants.

## Calculation of Results

1. Calculate the net absorbance by subtracting the initial absorbance readings for samples and standards (Step 3) from the final readings taken for each (Step 6).
2. Plot the net absorbance against the uric acid concentration for the uric acid standard curve.
3. Calculate the antioxidant capacity of unknown samples by comparing the net OD 490 nm values of samples to the uric acid standard curve.

To determine the “mM uric acid equivalents” (UAE) for samples, extrapolate the uric acid concentration from the sample’s analogous uric acid OD 490 nm value (For example, based on the sample standard curve above, an OD 490 nm value of 1.0 corresponds to 0.75 mM uric acid equivalents (UAE)).

To determine “ $\mu$ M Copper Reducing Equivalents” (CRE) for samples, multiply the uric acid equivalence (UAE) concentration by 2189  $\mu$ M  $\text{Cu}^{++}$ / mM uric acid. **1 mM of uric acid = 2189  $\mu$ M Copper Reducing Equivalents.** (For example, 0.75 mM UAE x 2189 = 1642 Copper Reducing Equivalents (CRE)). CRE sample values are proportional to the sample’s Total Antioxidant Capacity or Total Antioxidant Power.

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