

# OxisResearch™

A Division of OXIS Health Products, Inc.

## BIOXYTECH® AOP-490™

Colorimetric, Quantitative Assay for Total Antioxidant Potential (Aqueous Samples)

For Research Use Only. Not For Use In Diagnostic Procedures.

Catalog Number 21052

## INTRODUCTION

### The Analyte

Antioxidants fall into two major groups that either prevent the initiation of oxidation (by chemical or enzymatic reduction of hydroperoxides or the chelation of transition metals) or compete with the peroxy radical formation of lipid peroxidation of polyunsaturated fatty acids (4). Three basic antioxidant classifications can be found in biological systems: (a) enzymes (superoxide dismutase, glutathione peroxidase and catalase); (b) large molecules (albumin, ceruloplasmin and ferritin); and (c) small molecules (ascorbic acid,  $\alpha$ -tocopherol,  $\beta$ -carotene, plasma ubiquinol, uric acid, methionine, bilirubin and glutathione). These antioxidants can be found either as water-soluble or fat-soluble molecules (1).

Individual antioxidant moieties play specific roles in combating oxidative stress and measurement of single antioxidants may be beneficial to some studies. However, individual results may not be indicative of the overall effect of multiple antioxidants working in concert with one another. Therefore, an important index in oxidative stress studies may be measurement of the total antioxidant potential of the biological system.

### Principles of the Procedure

The BIOXYTECH® AOP-490™ Assay<sup>1</sup> is based upon the reduction of  $\text{Cu}^{++}$  to  $\text{Cu}^+$  by the combined action of all antioxidants present in the sample (6). A chromogenic reagent, Bathocuproine (2,9-dimethyl-4,7-diphenyl-1,10-phenanthroline), selectively forms a 2:1 complex with  $\text{Cu}^+$  which has a maximum absorbance at 490 nm (7).

A standard of known uric acid (a water soluble antioxidant) concentration is used to create a calibration curve. The results of the assay may be expressed either as "mM Uric Acid Equivalents" directly from the plot of absorbance change versus mM uric acid concentration, or as " $\mu\text{M}$  Copper Reducing Equivalents." The conversion between the two units is based upon the reduction of  $2189 \mu\text{M}$   $\text{Cu}^{++}$  to  $\text{Cu}^+$  by 1 mM uric acid. Figure 1 shows the results of a study investigating the correlation of the concentration of several antioxidants in human serum with values obtained from AOP-490.

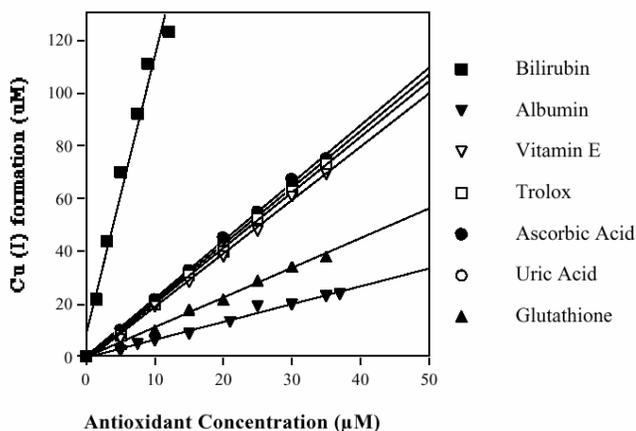


Figure 1. Correlation between antioxidant concentration and Cu reduction (II to I) using AOP-490.

<sup>1</sup>US Patent 6,613,577

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## REAGENTS

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### Materials Provided (for 100 tests)

- |                                     |        |
|-------------------------------------|--------|
| • R1 (Containing Bathocuproine)     | 60 mL  |
| • R2 (Containing Cu <sup>++</sup> ) | 5 mL   |
| • Uric Acid Standard                | 1 Vial |
| • Stop Solution                     | 5 mL   |
| • 96 well microplate                | 1      |

### Materials Required But Not Provided

- Deionized water.
- Adjustable pipettes (50-500 µL) with disposable tips.
- Microplate reader capable of absorbance measurements at 490 nm; preferably temperature controlled to 25°C (or room temperature).

### Warnings and Precautions

Use established laboratory precautions when handling or disposing any chemical contained in this product. Refer to the Material Safety Data Sheet for risk, hazard, and safety information. If any of the components come in contact with skin or eyes, rinse immediately with plenty of water. Seek medical advice.

### Reagent Storage and Handling

Store reagent bottles tightly sealed at 2-8°C.  
Unopened reagents are stable until the indicated expiration date.

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## PROCEDURE

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### Reagent Preparation

All reagents are ready to use.

### Standard Preparation

The Uric Acid Standard is provided as a lyophilized solid. Reconstitute the standard with 1.5 mL deionized water. Avoid air turbulence in the vial while making this addition by pipetting slowly and on the internal sides of the vial. Vortex for 30 to 60 seconds to dissolve the standard. The uric acid concentration of this solution is 2.0 mM. Reconstituted standards are stable at least one year at -70°C.

Perform serial dilutions of the 2.0 mM uric acid standard to make six standard levels:

- |           |                                                           |
|-----------|-----------------------------------------------------------|
| 2.0 mM:   | No change to solution prepared above required.            |
| 1.0 mM:   | Add 500 µL of 2.0 mM standard to 500 µL deionized water.  |
| 0.5 mM:   | Add 500 µL of 1.0 mM standard to 500 µL deionized water.  |
| 0.25 mM:  | Add 500 µL of 0.5 mM standard to 500 µL deionized water.  |
| 0.125 mM: | Add 500 µL of 0.25 mM standard to 500 µL deionized water. |
| 0.0 mM:   | Only 500 µL of deionized water.                           |

### Sample Preparation

All samples should be stored at -70°C until use. Samples with a concentration above 2 mM Uric Acid Equivalents must be diluted with R1 prior to assay. An approximately 1/3 – 1/4 dilution is recommended for urine samples.

### Tissue Lysates

If not tested immediately, tissues should be snap frozen and stored at -70°C until ready for use in assay. Homogenize in ice-cold, phosphate-buffered saline (PBS) and centrifuge at 3000g for 10-15 minutes at

4°C. Pour off and aliquot clear supernatant for protein determination and use in AOP-490 assay. Samples that exceed 2mM uric acid equivalents may be diluted with dilution buffer prior to running the assay.

### Cell Cultures

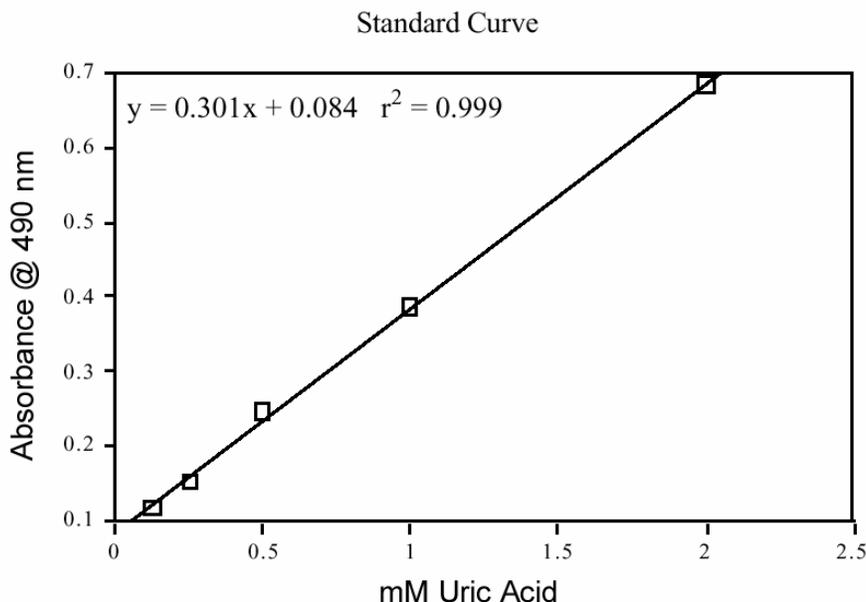
Cells should be washed 2-3 times with ice-cold, phosphate-buffered saline (PBS) prior to being sonicated in PBS. Centrifuge at 3000g for 10-15 minutes at 4°C. Pour off and aliquot clear supernatant for protein determination and use in AOP-490 assay. Samples that exceed 2mM uric acid equivalents may be diluted with dilution buffer prior to running the assay.

### Assay

1. Label a test tube for each replicate of the standards and samples to be assayed.
2. Dilute each standard or sample 1/40 with R1 (for example, 15 µL plus 585 µL R1).
3. Add 200 µL of each into a well on the microplate.
4. Read the plate at 490 nm.
5. Add 50 µL of R2 to each well and mix.
6. Incubate for 3 minutes at room temperature.
7. Add 50 µL Stop Solution and mix well.
8. Read the plate at 490 nm.

### Calculations

Calculate the net absorbance by subtracting the initial absorbance reading from Step 4 from the final absorbance reading from Step 8. Plot the net absorbance at 490 nm for each level of standard versus the uric acid concentration. A sample calibration curve is:



Determine the Uric Acid Equivalents for each sample from the curve and sample net absorbance by solving for "x". If you intend to report results in µM Copper Reducing Equivalents, multiply the result by 2189.

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## TECHNICAL SUPPORT

An OXIS Health Products, Inc. Technical Support Representative can be reached by telephone (800) 547-3686, (503) 283-3911, or e-Mail ([techsupport@oxis.com](mailto:techsupport@oxis.com)) Monday through Friday, 8:00 AM to 5:00 PM Pacific Time.

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