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Northwest
Life Science Specialties, LLC

Premier Products for Superior Life Science Research

NWLSS™ Antioxidant Reductive Capacity Assay

(For aqueous samples)

Product NWK-ARC01

For Research Use Only

Simple colorimetric test for determining the antioxidant capacity of aqueous samples in terms of their reductive capacity.

nwkarc01rev051506

Table of Contents

Interpretation of Data (continued):
Serum sample Antioxidant Reductive Capacity data was also compared to the resistance of serum lipids in these samples to oxidation. The results of this study show the two parameters are highly correlated (R=0.769 with p+0.0001). The higher the Antioxidant Reductive Capacity in copper reducing equivalents, the more protected are the serum lipids to oxidation.

References:

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Statement of Limited Warranty:
Northwest Life Science Specialties, LLC (NWLSS) makes no guarantee of any kind, expressed or implied, that extends beyond the description of the material in this kit, except that they will meet our specifications at the time of delivery. Customer's remedy and NWLSS' sole liability is limited to, at NWLSS' option, refund of the purchase price, or the replacement of material not meeting our specification. By acceptance of our product, customer assumes all liability and will indemnify and hold NWLSS harmless for the consequence of this product's use or misuse by the customer, its employees, or others. Refund or replacement is conditioned of customer notifying NWLSS within twenty-one (21) days of the receipt of product. Failure to give notice within 21 days shall constitute a waiver by the customer of all claims hereunder with respect to said product.

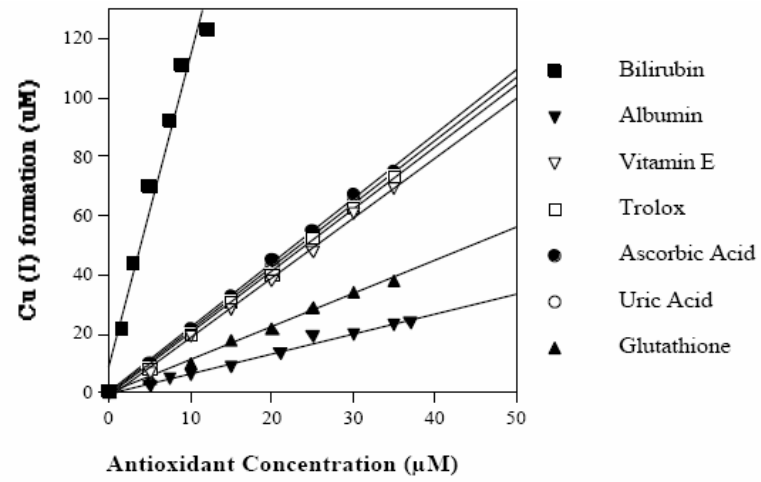
Data Analysis (continued):
The net difference between absorbance readings for each sample is then compared to the standard curve obtained.

Reporting in uric acid equivalents:
For example, an average OD difference measurement of 0.25 would correspond to an equivalent uric acid concentration of 0.55 mM.

Reporting as Copper Reducing Equivalents:
The equivalent uric acid concentration can be converted to copper reducing capacity by multiplying by 2189 μM Cu++/mM uric acid which is the amount of Cu^{++} *that can be reduced by 1 mM uric acid.*

Interpretation of Data:
Following are the results of a study comparing the concentration of serum antioxidants to the value obtained for Antioxidant Reductive Capacity in copper reducing equivalents using this method are presented in Figure 1.

Fig.1: *Reductive capacity of various pure antioxidants as shown by generation of* Cu+ .



Multivariate analysis of these results yields a correlation coefficient of R=0.804 with a very high significance (p= 0.0003).

Introduction:
Oxidative stress is the condition in which a lack of balance exists between the oxidant stimuli and the various antioxidants (1). Oxidative stress appears to be involved in the pathogenesis of several diseases, including artherosclerosis (2), chronic inflammatory diseases (3), and cancer (4). Organisms possess several antioxidant systems that are very important for the prevention of oxidative stress (5) including both intra-cellular and extra-cellular components that are either enzymatic or non-enzymatic in nature. Lipid soluble antioxidants (most importantly vitamin E) and water-soluble antioxidants (uric acid, vitamin C, bilirubin, thiols and gluthathione) are a few of the antioxidants involved in these processes. Given the large number of antioxidant pathways, and their importance in regulation of an organism's redox status, it is important to be able to quantitatively measure the total antioxidant capacity or antioxidant power within biological specimens (6-11).

Intended Use:
The NWLSS™ Antioxidant Reductive Capacity assay is intended for the quantitative measurement of antioxidant status in serum, plasma, cell culture, food extracts and other aqueous biological samples.

Assay Principle:
The test is based on the ability of sample antioxidants to reduce Cu^{++} to Cu^{+}. Cu^{+} reacts with bathocuproine (BC) to form a complex with maximal absorbance at 480-490 nm. Measurement at 490 nm before and after addition of BC generates a net absorbance proportional to the sample's reductive capacity. Net absorbance values obtained for samples are compared with a standard curve generated using uric acid. Alternatively, researchers may select a different standard of their choosing for use in the assay.

Specifications:

Format:	96 well colorimetric	
Number of tests:	Triplicate:	24
	Duplicate:	40
Specificity:	Reductive Capacity in animal and plant aqueous samples.	
Sensitivity:	22 μM Uric Acid Equivalents using standard assay protocol. 0.1 μM Uric Acid Equivalent sensitivity is possible by increasing the sample to dilution buffer ratio from 1:39 to 1:3 (Standard Assay Protocol Step 1).	

Kit Contents:

Assay Dilution Buffer: (containing bathocuprione disulfonic acid)	60 mL
Cu^{++} solution:	5 mL
Stop Solution containing EDTA:	5 mL
Uric Acid Standard (lyophilized – under vacuum):	2 mL

Required Materials not Provided

Disposable plastic tubes

96 well microtiter plate or strips with frame

Pipettes

Distilled, deionized (dd) water

Required Instrumentation:

Microtiter plate reader with 490 nm capability.

Warnings, Limitations, Precautions:

Individual components may be harmful if swallowed, inhaled or absorbed through the skin. Contact should be minimized through the use of gloves and standard good laboratory practices. If contact with skin or eyes occurs, rinse the site immediately with water and consult a physician.

Storage Instructions

The reagents supplied in this assay are stable until the expiration date noted on the product packaging when stored refrigerated at 4-8ºC. The reconstituted standard is stable up to one year if stored at –80°C.

Assay Preparation:

1. Determine the number of wells required to assay standards, samples and controls for the appropriate replicate number. It is recommended that testing be performed in duplicate or triplicate.

2. Create an assay template with corresponding standards, controls and samples shown for reference during the assay.

3. Bring samples and reagents to room temperature (18-25ºC) before use.

Reagent Preparation:

Lyophilized Standard:

Reconstitute the lyophilized Uric Acid Standard by slowly adding 1.5 mL dd water. Vortex for 30-60 seconds to fully dissolve the 2 mM Standard.

Standard Curve Preparation:

1. Label microtubes 2-6 and add 500 µL deionized water to each.

2. Transfer 500 µL of reconstituted 2 mM Standard to tube 2 and mix well. Serially transfer 500 µL through tube 6 to create a standard range of 2 mM to 0.063 mM.

Std Tube # :	***1***	***2***	***3***	***4***	***5***	***6***
Conc. (mM):	2.0	1.0	0.50	0.25	0.125	0.063

Sample Handling/Preparation:

This kit is not recommended for use with EDTA plasma samples due to its dependency on copper ions and due to the chelating properties of EDTA. Samples should be fresh or collected and immediately stored at –80°C. Samples that exceed 2 mM uric acid equivalents may be diluted with water prior to running the assay. For greater sensitivity the amount of sample in Step 1 may be increased relative to Assay Dilution Buffer up to a 1/4 dilution or 1:3 ratio.

Standard Assay Protocol:

1. Allow samples and standards to equilibrate to room temperature (RT) then dilute 1:40 with the Assay Dilution Buffer provided (20 µL sample or standard to 780 µL buffer allows triplicate sample assay).

2. Transfer 200 µL of diluted samples or standards to each well.

3. Read the plate at 490 nm.

4. Add 50 µL of Cu^{++} solution to each well and incubate 3 minutes at RT.

5. Add 50 µL of stop solution.

6. Read the plate a second time at 490 nm.

Data Analysis:

Plot the difference between the two absorbance readings versus the concentration for each standard to construct a standard curve.