DATA SHEET

Total Antioxidant Capacity (TAC) Assay Kit (Catalog #K274-100; 100 assays; Store kit at 4°C)

I. Introduction:

Antioxidants play an important role in preventing the formation of and scavenging of free radicals and other potentially toxic oxidizing species. There are three categories of antioxidant species: enzyme systems (GSH reductase, catalase, peroxidase, etc.), small molecules (ascorbate, uric acid, GSH, vitamin E, etc.) and proteins (albumin, transferrin, etc.). Different antioxidants vary in their reducing power. Trolox is used to standardize antioxidants, with all other antioxidants being measured in Trolox equivalents. Measurement of the combined nonenzymatic antioxidant capacity of biological fluids and other samples provides an indication of the overall capability to counteract reactive oxygen species (ROS), resist oxidative damage and combat oxidative stress-related diseases. In some cases, the antioxidant contribution of proteins is desired whereas in other cases only the contribution of the small molecule antioxidants is needed. BioVision developed the TAC Assay Kit, which can measure either the combination of both small molecule antioxidants and proteins or small molecules alone in the presence of our proprietary Protein Mask. Cu++ ion is converted to Cu+ by both small molecule and protein. The Protein Mask prevents Cu++ reduction by protein, enabling the analysis of only the small molecule antioxidants. The reduced Cu+ ion is chelated with a colorimetric probe giving a broad absorbance peak around 570 nm, proportional to the total antioxidant capacity.

II. Kit Contents:

Components	K274-100	Cap Code	Part Number
Cu^{++} Reagent	0.2 ml	Blue	K274-100-1
Assay Diluent	10 ml	WM	K274-100-2
Protein Mask	10 ml	NM	K274-100-3
Trolox Standard (1 µmole)	Lyophilized	Yellow	K274-100-4

III. Reconstitution of Reagents:

1. Cu++ Reagent, Assay Diluent, Protein Mask: Ready to use as supplied and may be kept at room temperature.
2. Trolox Standard: Dissolve the lyophilized Trolox standard in 20 µl of pure DMSO by vertxing, then add 980 µl of distilled water and mix well, generating a 1 mM solution.

Following reconstitution, aliquot and store at -20°C. The reconstituted standard is stable for 4 months when stored at -20°C.

IV. Measurement of Antioxidants:

1. Trolox standard curve: Add 0, 4, 8, 12, 16, 20 µl of the Trolox standard to individual wells. Adjust the total volume to 100 µl with ddH2O to give 0, 4, 8, 12, 16, 20 nmol of Trolox standard.

2. Preparation of sample: The kit has been tested with serum, urine, culture media, food and drinks. No sample purification from these sources is necessary. If only small molecule TAC is desired, samples should be diluted 1:1 with protein mask. Sample volumes between 0 - 100 µl can be assayed per well and should be done in duplicate. For serum samples, we suggest to assay 0.01-0.1 µl without Protein Mask, or 1-10 µl with protein Mask. All well volumes should be adjusted to 100 µl with ddH2O. The absorbance of samples should be in the linear range of the standard curve (0-20 nmol/well). If they fall outside of this range, they should be rediluted and rerun. The detection limit of the assay is approximately 0.1 nmole per well (or 1 µM) of Trolox.

3. Preparation of working solutions: Dilute one part Cu++ reagent with 49 parts of Assay diluent. Dilute enough working solution for the number of assays. Each well requires 100 µl of Cu++ working solution.

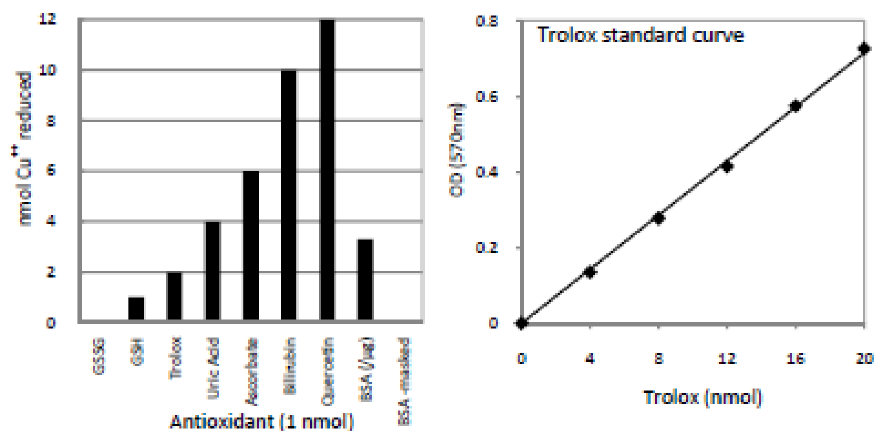
4. Assay procedure:
1) Add 100 µl Cu++ working solution to all standard and sample wells.
2) Cover the plate and incubate at room temperature for 1.5 hours.
3) Read the absorbance at 570 nm using the plate reader.

5. Calculations
1) Plot standard curve: Plot absorbance at 570 nm as a function of Trolox concentration.
2) Determine sample antioxidant Trolox equivalent concentrations:

$$\text{Sample antioxidant capacity} = \frac{[(Sample\ absorbance - blank\ absorbance) X (ul\ of\ sample)]}{[Slope\ of\ std\ curve]}$$

OR

$$Sa/Sv = \text{nmol/µl or mM Trolox equivalent}$$



V. Related Products:

Ascorbic Acid Assay Kit (K661-100)
Glutathione Assay Kits (K251-100, K261-100, K264-100)
Uric Acid Assay Kit (K608-100)
Cholesterol & HDL/LDL Assay Kits (K603-100, K613-100)
Lactate Assay Kit (K607-100)
Glucose Assay Kit (K606-100)
Ethanol Assay Kit (K620-100)
NADH/NADPH Assay Kit (K337-100, K347-100)

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GENERAL TROUBLESHOOTING GUIDE

Problems	Cause	Solution
Assay not working	• Use of ice-cold assay buffer • Omission of a step in the protocol • Plate read at incorrect wavelength • Use of a different 96-well plate	• Assay buffer must be at room temperature • Refer and follow the data sheet precisely • Check the wavelength in the data sheet and the filter settings of the instrument • Fluorescence: Black plates (clear bottoms) ; Luminescence: White plates ; Colorimeters: Clear plates
Samples with erratic readings	• Use of an incompatible sample type • Samples prepared in a different buffer • Cell/ tissue samples were not completely homogenized • Samples used after multiple free-thaw cycles • Presence of interfering substance in the sample • Use of old or inappropriately stored samples	• Refer data sheet for details about incompatible samples • Use the assay buffer provided in the kit or refer data sheet for instructions • Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope • Aliquot and freeze samples if needed to use multiple times • Troubleshoot if needed • Use fresh samples or store at correct temperatures until use
Lower/ Higher readings in Samples and Standards	• Improperly thawed components • Use of expired kit or improperly stored reagents • Allowing the reagents to sit for extended times on ice • Incorrect incubation times or temperatures • Incorrect volumes used	• Thaw all components completely and mix gently before use • Always check the expiry date and store the components appropriately • Always thaw and prepare fresh reaction mix before use • Refer datasheet & verify correct incubation times and temperatures • Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	• Use of partially thawed components • Pipetting errors in the reaction mix • Air bubbles formed in well • Calculation errors • Substituting reagents from older kits/ lots	• Thaw and resuspend all components before preparing the reaction mix • Prepare a master reaction mix whenever possible • Pipette gently against the wall of the tubes • Recheck calculations after referring the data sheet • Use fresh components from the same kit
Unanticipated results	• Measured at incorrect wavelength • Samples contain interfering substances • Use of incompatible sample type • Sample readings above/below the linear range	• Check the equipment and the filter setting • Troubleshoot if it interferes with the kit • Refer data sheet to check if sample is compatible with the kit or optimization is needed • Concentrate/ Dilute sample so as to be in the linear range
Note# The most probable list of causes is under each problem section. Causes/ Solutions may overlap with other problems.		

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