Total Antioxidant Capacity (TAC) Colorimetric 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^{2+} ion is converted to Cu^{+} by both small molecule and protein. The Protein Mask prevents Cu^{2+} 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:

Component	K274-100 100 assays	Cap Color	Part Number
Cu^{2+} 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 µmol)	1 vial	Yellow	K274-100-4

III. Reconstitution of Reagents:

1. **Cu^{2+} 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 ddH_2O 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 nmol per well (or 1 µM) of Trolox.
3. **Preparation of working solutions:** Dilute one part Cu^{2+} reagent with 49 parts of Assay diluent. Dilute enough working solution for the number of assays. Each well requires 100 µl of Cu^{2+} working solution.
4. **Assay procedure:**
   1) Add 100 µl Cu^{2+} 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:

Sample antioxidant capacity = Sa/Sv = nmol/µl or mM Trolox equivalent

Where:

Sa is the sample amount (in nmol) read from the standard curve
Sv is the undiluted sample volume added to the wells.

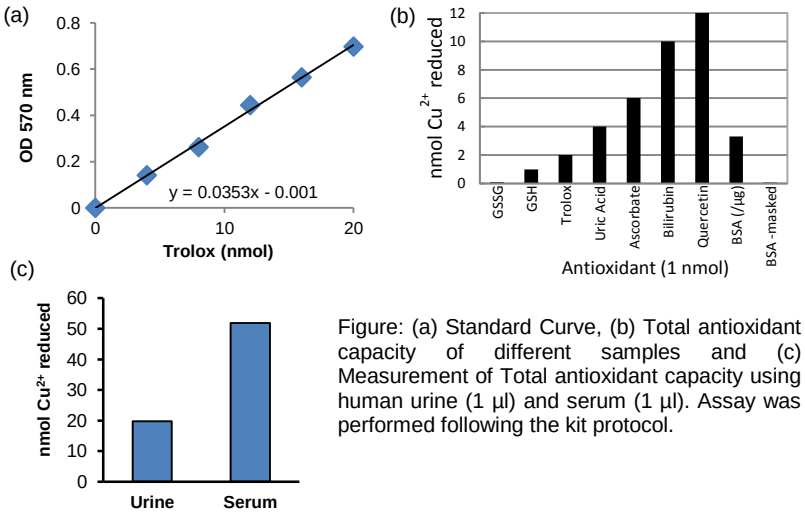

Figure: (a) Standard Curve, (b) Total antioxidant capacity of different samples and (c) Measurement of Total antioxidant capacity using human urine (1 µl) and serum (1 µl). Assay was performed following the kit protocol.

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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