Product Information

Total Antioxidant Capacity (TAC) Colorimetric Assay Kit

I. Kit Contents:

Components	K2116-100 100 assays	Cap Color	Part Number
Cu^{2+} Reagent	0.2 ml	Blue	K2116-C-1
Assay Diluent	10 ml	WM	K2116-C-2
Protein Mask	10 ml	NM	K2116-C-3
Trolox Standard (1 µmol)	1 vial	Yellow	K2116-C-4

Add 150 µl of DMSO, and mix well before use.

II. Introduction:

Antioxidants is essential for preventing the formation of free radicals and other toxic oxidizing species. Three types of antioxidants (enzyme, small molecules and proteins) are different in their reducing power. Trolox can standardize antioxidants by measuring the antoxiants with Trolox equibanletns. Detection of the combined nonenzymatic antioxidant capacity of biological fluids and other samples gives an indication of the total 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. This Total Antioxidant Capacity (TAC) Colorimetric Assay Kit can measure both small molecule antioxidants and proteins in combination or small molecules alone with Protein Mask. Cu^{2+} ion is converted to Cu^{2+} by both small molecule and protein. The Protein Mask prevents Cu^{2+} reduction by protein, allowing analysis of only the small molecule antioxidants. The reduced Cu^{+} ion is chelated with a colorimetric probe offering a broad absorbance peak around 570 nm, proportional to the total antioxidant capacity.

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 ddH_2O. 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.

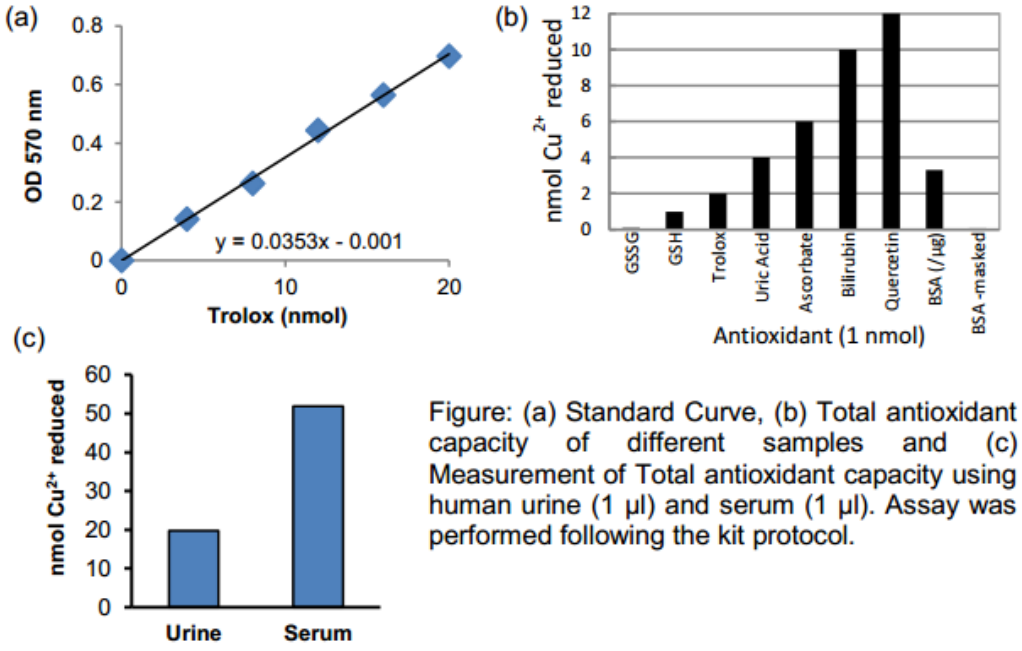

Figure: (a) Standard Curve, (b) Total antioxidant capacity of different samples and (c) Measurement of Total antioxidant capacity using human urine (1 µl) and serum (1 µl). Assay was performed following the kit protocol.

Frequently Asked Questions:

1. Why do we get different results from the same sample at different times?

This can occur due to 3 main possibilities:

1) Different lots may give different readings. However, when using calibration curve together with the sample testing, the sample concentration should be in the same range.

2) Sample may change during storage, especially Vitamin C.

3) When using the same lot, the same sample reading should be similar, otherwise maybe there was some experimental error.

2. Is the theory of TAC assay kit the same as the DPPH radical method? We are using DPPH reagent (final conc 250 µM in toluene) and want to know is there any advantage for using your TAC assay kit.

The two assays are different from one another. Our TAC assay uses Cu++ to Cu+ as a mechanistic tool. It is easy to reduce so some molecules such as uric acid will respond quickly to it. Some of the disadvantages of the DPPH are:

1) This method quantifies DPPH after exposure to sample-The standard curve gives negative results.

2) Reaction kinetics between DPPH and anti oxidants are not linear. So the anti oxidant capacity is rather arbitrary.

3) The time to steady state using DPPH varies with different anti oxidants. So it may get conflicting relative capacity depending on the reaction time.

3. Does EDTA in blood affects the use of this kit on plasma?

The presence of EDTA should have no problem on the function of this kit.

4. Is the protein mask necessary with urine sample, since the level of protein in urine compared to serum is minimal?

If the total antioxidant capacity is desired, I would not recommend the use of the protein mask. If only the levels of the small molecule antioxidants are required, please use the protein mask.

5. Which sample is better between serum and plasma? Why?

Both human serum and plasma have an antioxidant capacity of 0.5 - 2mM. So both samples would be equally good for detection.

6. To draw the 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." It's not serial dilution. That is ,they first make 32mM solution ,then they dilute 32mM solution to 16mM. then they use 16mM solution to make 8mM solution and so on. Do you think which method is better?

The standards we recommend are not serial dilutions. Please use them as recommended.

7. Can this kit be used with samples like bacteria, plants, drosophila, yeast etc?

We have optimized the kit with mammalian samples. However, theoretically these kits should work with samples from multiple species/sources. Since the optimal conditions depend on the sample type, the protocol has to be be adapted to fit the samples for efficient results. Please refer to this kits citations to see what kind of samples have been used with this kit other than mammalian samples.

8. Can we use frozen samples with this assay?

Fresh samples are always preferred over frozen samples. However, frozen samples can also be used, provided, they were frozen right after isolation, were not freeze thawed multiple time (for which we recommend aliquoting the samples before freezing) and have been frozen for relatively short periods.

9. Can we use a different wavelength than recommended for the final analysis?

It is always recommended to use the exact recommended wavelength for the most efficient results. However, most plate readers have flexibility in their band width of detection in increments of +/- 10 nm. Depending on this flexibility range, you can deviate from the recommended wavelengths within limits.

10. What is the exact volume of sample required for this assay?

There is no specific volume we can recommend for the amount any sample to be used since it is completely sample concentration and quality based. You have to do a pilot expt with multiple sample volumes to determine the optimal volume which gives a reading within the linear range of the standard curve. Please refer to the citations for this product to see what other clients have used with similar sample types.

11. What is the shelf life of this kit?

This kit is good for 12 months from the date of shipment in the unopened form when stored at the appropriate temperature and appropriate conditions. After opening and reconstitution, some of the components in this kit are good for 2 months at -20℃. Please refer to the data sheet for storage information and shelf life of each of the components.

12. Why are my standard curve values lower than those shown on the data sheet?

There are multiple factors which influence the signals like the incubation times, room temperature, handling etc. In general, to increase the value of the standards, you can increase the incubation time. As long as the standard curve is linear, it should be fine to use, since all of your samples will also be measured under the same conditions on this curve.

13. How do I normalize my samples against protein concentration?

You can use a protein quantitation assay on the supernatants you get from cell/tissue lysates or with any other liquid sample in the assay buffer.

14. Can we use an alternate buffer for sample preparation (cell lysis, sample dilutions etc)?

Our assay buffers are optimized for the reactions they are designed for. They not only contain some detergents for efficient lysis of your cells/tissue, but also contain some proprietary components required for the further reactions. Therefore, we highly recommend using the buffers provided in the kit for the best results.

15. Should I make a standard curve for every expt I do, or is one curve/kit enough?

Yes, It is recommended to do the standards every time you do the expt. There is always a chance that something was done differently that day and we do not want any conditions to differ between standards and samples.

For research use only! Not to be used in humans.

Our promise

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For more details, please visit http://www.apexbt.com/ or contact our technical team.

Tel: +1-(832)696-8203
Fax: +1-832-641-3177
Email: sales@apexbt.com