ABSbio™ Total Antioxidant Capacity Detection Kit (K389-200)

Quantitative Colorimetric Total Antioxidant Capacity Assay

Introduction

Oxidative damage to living organisms has been associated with several disease states as well as aging. Antioxidants protect the cells from damages by reactive oxygen species which are produced in oxidation reactions in the cell. Antioxidants are also widely used as dietary supplements and in industry as preservatives in food.

The ABSbio™ Antioxidant Detection Kit (ABTS method) provides a simple, sensitive, one-step colorimetric assay to detect total antioxidant capacity in biological samples, food and drink (juice, coffee, wine & beer), agricultural and pharmaceutical products. In this assay, antioxidant reduce oxidized blue-green complex to produce a colorless form product (ABTS). The intensity of color, measured at 660 nm, is directly proportional to the total antioxidant capacity activity in the sample. The kit is supplied with sufficient reagents for 200 tests in 96-well plate assay, linear detection range of 1-1000 µM Trolox equivalents. It could easily be modified for use in cuvette assay, 384-well assay and high-throughput assay.

Kit Components (200 tests)

Reagent A:	40 mL	Reagent B:	4 mL	Trolox Standard (50 mM):	0.1 mL

Storage and Handling: Shipping on room temperature. Store standard at -20°C, other component at 4°C. Shelf Life: 6 months after receipt. Warm up Reagents to room temperature before use.

Protocol

1. Sample preparation

Serum, plasma, urine, or cell culture supernatant can be measured directly by a series of dilutions of the sample to ensure the readings are within the standard curve range. Homogenize Cell (2×10^6) or tissue (20 mg) sample in 200 µL cold PBS. Centrifuge to collect the supernatant. It is recommended with all sample types to assay immediately or aliquot and store the samples at -80°C.
Transfer 10 µL sample into the 96-well clear flat bottom plate in duplicate. If sample is colored, 10 µL sample blank control needs to run parallel.

2. Standard Preparation

Transfer 5 µL 50 mM Trolox standard into 245 µL dH_2O to generate a 1 mM Trolox standard, then following the table to generate 1000, 600, 300, 100 and 0 µM standards.

Trolox std(µL)	dH_2O(µL)	final trolox concentration µM
50	0	1000
30	20	600
15	35	300
5	45	100
0	50	0

Transfer 10 µL of appropriate standards into the 96-well plate in duplicate. The blank control containing dH_2O only.

3. Reaction

Prepare enough working reagent by mixing 200 µL Reagent A and 20 µL Reagent B for each reaction (samples & standards). Transfer 200 µL prepared working reagent into each reaction well. Colored sample needs run sample blank control by directly add 200 µL Reagent A to sample blank control well.

Tap plate to mix well. Incubate 10 min. at room temperature, protected from light.

4. Measurement

Read the optical density at 660 nm (650-690 nm).

5. Calculation

Average the duplicate OD660 nm reading for standard and sample. Subtract the average OD of the standards from the average OD of the blank and plot the result (ΔOD) versus the Trolox concentration of the standards. Determine the slope by linear regression and calculate the total antioxidant capacity of samples.

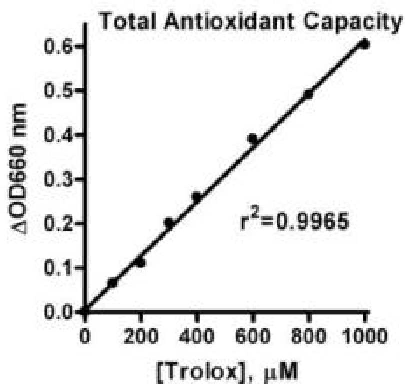
[Total antioxidant capacity]**= $(OD_{blank} - OD_{sample})/\text{Slope} \times n$ (µM** Trolox equivalent**)**

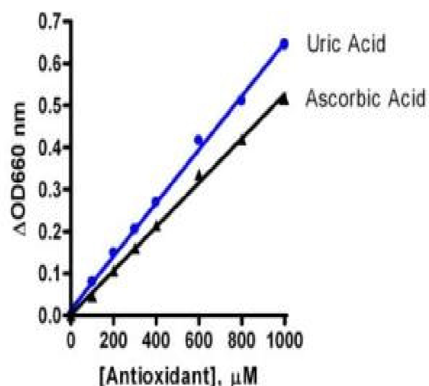
OD_{sample} and OD_{blank} are related optical density of the sample and dH_2O. *n* is the sample dilution factor.

Note: For colored samples, correct for any sample interference by subtracting the sample blank reading from the sample reading.

If calculated Total antioxidant capacity is higher than 1000 µM Trolox equivalents, dilute sample in dH_2O and repeat assay. Multiply the results by the dilution factor.

Typical Standard Curve





Trolox standard in 96 wells-plate assay.
Always run your own standard curves for calculation of results.

Sample total antioxidant capacity

Sensitivity and Limit of Detection

The Limit of Detection was determined as 1 μM, and linear detection range up to 1000 μM Trolox in 96-well plate colorimetric assay. Sensitivity was determined as 0.3 μM. Samples with values above 1000 μM Trolox equivalent should be dilute with dH_2O, re-assayed, and multiply results by dilution factor.

Interferences

Culture media with phenol red, BSA, Triton x-100, Tween 20 were tested in the assay for interference. No significant change in the measured total antioxidant capacity level was observed. **To ensure accurate determination of total antioxidant capacity in the colored samples, we recommend run sample blank.**

References

Craft, BD, et al. 2012, Comprehensive Reviews in Food Science and Food Safety. 11:148-173
Erel, O. 2004, Clinical Biochemistry 37: 277–285
Re, R. et al. 1999, Free Radical Biology & Medicine, 26:1231–1237