QuantiChrom™ ABTS Antioxidant Assay Kit (DABA-100)

Quantitative Colorimetric Antioxidant Determination

DESCRIPTION

2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\bullet+}$) is a green cation radical that returns to its colorless state when reduced. The ABTS assay is widely used to measure antioxidant capacity, which is the ability of redox molecules in foods and biological systems to scavenge free radicals. BioAssay Systems' ABTS antioxidant assay measures the change in absorbance that occurs when the ABTS radical is reduced and decolorized from green to clear. The decrease in absorbance at 650 nm is directly proportional to the antioxidant concentration and is compared to a Trolox antioxidant standard curve.

KEY FEATURES

Fast and convenient. 5-minute reagent incubation period allows for quick sample testing.

Adaptable. Compatible with aqueous and ethanol extracted samples, and stable signal allows for extended run-time length (up to 2 hours).

High-throughput. Homogeneous "mix-incubate-measure" type assay. Can be readily automated to assay thousands of samples per day.

APPLICATIONS

For quantitative determination of antioxidant capacity in food, beverage, and urine samples.

KIT CONTENTS (100 TESTS IN 96-WELL PLATES)

ABTS Reagent:	20 mL	**3% H_2O_2:**	20 µL
Trolox Standard:	100 µL	**HRP:**	100 µL

Storage conditions. The kit is shipped at room temperature. Store all components at -20°C upon receiving. Shelf life: 6 months after receipt.

Precautions: reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

PROCEDURES

Sample Preparation: Avoid the use of detergents and reducing agents (DTT, 2-mercaptoethanol, glutathione, etc).

Plant or food extracts: Homogenize sample in aqueous or ethanol solution. If extraction is in acid or base, sample should be neutralized prior to assay. If a sample contains particulates or turbidity, centrifuge and use the clear supernatant for the assay.

Urine: Dilute urine samples 5 to 20-fold, depending on hydration level.

For unknown samples, it is prudent to test several dilutions to determine an optimal sample dilution factor, *n*.

Reagent Preparation: Thaw all components to room temperature and vortex briefly to mix prior to assay.

Procedure using 96-well plate

Standards. Prepare 1000 µL 800 µM Premix by mixing 16 µL of the Trolox Standard (50 mM) and 984 µL dH_2O, or the solution the sample is prepared in (e.g. ethanol). Dilute standards in 1.5-mL centrifuge tubes as described in the table. Transfer 20 µL standards into separate wells of a clear flat bottom 96-well plate.

No	Premix + H_2O/Ethanol	Trolox (µM)
1	100 µL + 0 µL	800
2	60 µL + 40 µL	480
3	30 µL + 70 µL	240
4	0 µL + 100 µL	0

Transfer 20 µL of each sample into separate wells.

Prepare 20mM H_2O_2 by mixing 2 µL 3% H_2O_2 and 86 µL dH_2O. *Note: 20 mM H_2O_2 is unstable. Prepare fresh each time assay is run.*

Prepare enough Working Reagent (WR) for all standard and sample wells by mixing, for each well, 190 µL ABTS Reagent, 1 µL 20 mM H_2O_2, 1 µL HRP. First, mix ABTS Reagent and H_2O_2, then add HRP. Mix briefly and incubate at room temperature for 5 minutes.

Add 180 µL WR to all sample and standard wells. Tap plate briefly, but thoroughly to mix. If some turbidity is observed, tap plate until it dissolves.

Read OD at 650 nm at 30 minutes, or longer, if desired (< 2 hours).

CALCULATION

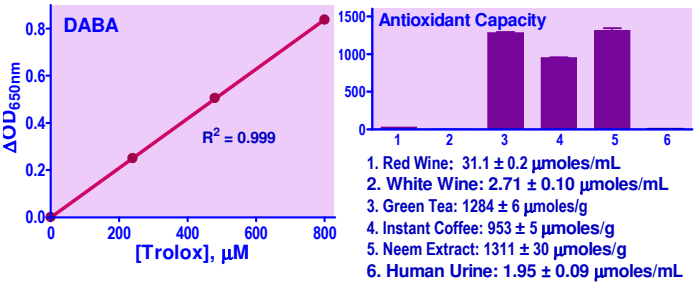
Subtract the standard OD values from OD_{WATER} (Standard #4) and plot the $\Delta OD_{STANDARD}$ against Trolox standard concentrations to obtain a standard curve slope (μM^{-1}). For samples, calculate the Trolox equivalent antioxidant capacity:

$$[\text{Trolox}] = \frac{OD_{WATER} - OD_{SAMPLE}}{\text{Slope } (\mu M^{-1})} \times n \quad (\mu M)$$

Where n represents the dilution factor. Note: if the calculated concentration is higher than 800 µM, dilute the sample and repeat assay. Multiply the result by the dilution factor.

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipetting devices and accessories (e.g. multi-channel pipettor), clear flat-bottom 96-well plates (e.g. VWR cat# 82050-760), centrifuge tubes, plate reader, and materials needed for sample extraction.



Left: Trolox standard curve; Right: Samples were tested using the standard protocol.

LITERATURE

1. García-Alonso, et al. (2004). Evaluation of the antioxidant properties of fruits. *Food chemistry*, 84(1), 13-18.
2. Gupta, D. (2015). Methods for determination of antioxidant capacity: A review. *International Journal of Pharmaceutical Sciences and Research*, 6(2), 546.
3. Niki, E. (2010). Assessment of antioxidant capacity in vitro and in vivo. *Free Radical Biology & Medicine*, 49(4), 503-515.