ABTS Antioxidant Assay Kit

Product Datasheet | SKU BA0266

Description

The ABTS cation radical is a green species that returns to its colourless 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. This assay measures the change in absorbance that occurs when the ABTS radical is reduced and decolorised 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.

Specifications

Detection Method	Colorimetric (OD 650 nm)
Detection Range	Up to 800 µM Trolox equivalents
Sample Type	Food, beverage and urine samples (aqueous and ethanol extracts)
Species Reactivity	All
Assay Time	Approximately 35 minutes (5-minute reagent incubation plus 30-minute read)
Kit Size	100 Assays
Equipment Required	Microplate reader
Storage	-20°C
Shelf Life	6 months
Shipping	Room Temperature

Kit Components

Component	Quantity	Storage
ABTS Reagent	20 mL	-20°C
3% H2O2	20 µL	-20°C
Trolox Standard	100 µL	-20°C
HRP	100 µL	-20°C

Ordering Information

Assay Genie Cat. No.	Pack Size
BA0266	100 Assays

Assay Procedure

Note: This is a sample protocol. Protocols are batch/lot specific - always follow the instructions supplied with your kit.

1. Thaw all components to room temperature and vortex briefly to mix prior to assay, avoiding detergents and reducing agents (DTT, 2-mercaptoethanol, glutathione, etc.) in samples.
2. Prepare 1000 µL of 800 µM Premix by mixing 16 µL of the Trolox Standard (50 mM) and 984 µL distilled water (or the solvent the sample is prepared in), then dilute to give standards of 800, 480, 240 and 0 µM and transfer 20 µL of each into separate wells of a clear flat-bottom 96-well plate.
3. Transfer 20 µL of each sample into separate wells; dilute urine samples 5- to 20-fold and neutralise acid or base extracts before assay.
4. Prepare 20 mM H2O2 fresh by mixing 2 µL 3% H2O2 with 86 µL distilled water.
5. Prepare Working Reagent for all wells by mixing, for each well, 190 µL ABTS Reagent, 1 µL 20 mM H2O2 and 1 µL HRP (mix ABTS Reagent and H2O2 first, then add HRP), and incubate at room temperature for 5 minutes.

6. Add 180 µL Working Reagent to all sample and standard wells, tap the plate briefly but thoroughly to mix, and read OD at 650 nm at 30 minutes or longer if desired (less than 2 hours).

Standard Preparation

No	Premix + H2O/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

Calculation

Subtract the standard OD values from ODWATER (Standard #4) and plot delta-OD-STANDARD against Trolox standard concentrations to obtain a standard curve slope (µM-1). For samples: [Trolox] = (ODWATER - ODSAMPLE) / Slope (µM-1) x n (µM), where n is the dilution factor. If the calculated concentration is higher than 800 µM, dilute the sample, repeat the assay and multiply the result by the dilution factor.

Notes

- Avoid the use of detergents and reducing agents (DTT, 2-mercaptoethanol, glutathione, etc.) during sample preparation.
- For plant or food extracts, homogenise in aqueous or ethanol solution; neutralise acid or base extracts and centrifuge turbid samples, using the clear supernatant for the assay.
- For unknown samples, it is prudent to test several dilutions to determine an optimal sample dilution factor.
- 20 mM H2O2 is unstable and should be prepared fresh each time the assay is run.
- Normal precautions for laboratory reagents should be exercised while using the reagents.
- Reagents are for research use only. Refer to the MSDS for detailed safety information.