QuantiChrom™ DPPH Antioxidant Capacity Assay Kit (DAOC-120)

Quantitative Colorimetric Kinetic Antioxidant Capacity Determination

DESCRIPTION

An *ANTIOXIDANT* is a molecule capable of slowing or preventing the oxidation of other molecules. Antioxidants, such as the small molecules glutathione and vitamins or the macromolecules catalase and glutathione peroxidase, protect the cells from damage by reactive oxygen species. Antioxidants are widely used as dietary supplements and in industry as preservatives in food, cosmetics, rubber and gasoline.

Simple, direct and high-throughput assays for antioxidant capacity find wide applications in research, food industry and drug discovery. BioAssay Systems' improved antioxidant capacity assay is based on the reduction of DPPH by antioxidant molecules in a sample, which results in a drop in absorbance at 517 nm. The antioxidant capacity can be quantified and expressed using a Trolox standard.

KEY FEATURES

Sensitive and accurate. Detects 11 µM to 300 µM Trolox equivalents in 96-well plate.

Stable reagent. DPPH in this kit is included as a powder form that can be aliquoted to test as many or few wells as desired, or repeated testing over separate days, or weeks.

Simple and high-throughput. This room temperature, homogenous, "mix-incubate-measure" assay can be run in as little as 10 minutes. Can be readily automated to assay thousands of samples per day.

APPLICATIONS

For quantitative determination of antioxidant capacity in food, beverages and biological samples.

KIT CONTENTS (100 TESTS IN 96-WELL PLATES)

Reagent: 110 mg **Assay Buffer:** 4 mL

Standard: 100 µL

Storage conditions. The kit is shipped at room temperature. Store all components at 4°C to -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. Also exercise caution when handling methanol; it can be difficult to pipette, is highly flammable and poisonous when ingested. Consult local officials on proper methanol waste storage and disposal.

PROCEDURES

Prior to assay, bring all kit components to room temperature.

Standard Preparation: Prepare 300 µM Trolox Premix by mixing 6 µL provided standard and 994 µL 70% methanol. Further dilute standard in 70% methanol as follows.

No	300 µM Premix	Vol (µL)	Standard (µM)
1	150 µL + 0 µL	150	300
2	120 µL + 30 µL	150	240
3	90 µL + 60 µL	150	180
4	60 µL + 90 µL	150	120
5	45 µL + 105 µL	150	90
6	30 µL + 120 µL	150	60
7	15 µL + 135 µL	150	30
8	0 µL + 150 µL	150	0

Add 100 µL of each standard to separate wells of a clear 96 well plate.

Sample Preparation: Samples perform best if extracted in 70% methanol, but 50-70% methanol will work. If extracting with acid or base, neutralize samples prior to assay. If a sample contains less than 50% methanol, add methanol to a final 70% v/v.

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

Transfer 100 µL sample to two separate wells, one serving as sample and one serving as sample blank.

Reagent Preparation: The provided reagents are sufficient for 120 wells of assay. To prepare working reagent (WR), reconstitute the Reagent with 8.8 mL methanol in a 15-mL tube. Vortex 30 seconds. Add 3.8 mL Assay Buffer. Vortex until all powder is dissolved.

Alternatively, for testing fewer wells, prepare sufficient WR for each well: mix 0.88 mg Reagent, 73 µL methanol and 31 µL Assay Buffer. Note: WR should be used within 3 hours.

Reaction Preparation: Transfer 100 µL WR to standard and sample wells. To sample blank wells, add 100 µL 70% methanol. Tap plate to mix.

Read OD_{517nm} at 10 minutes.

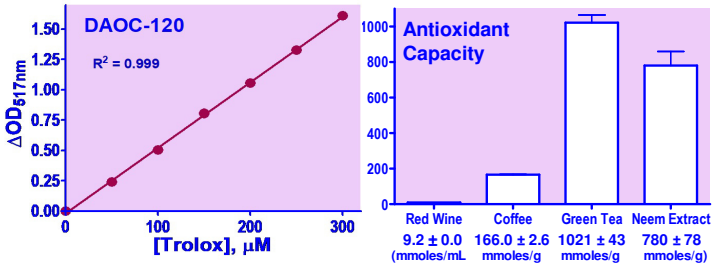
CALCULATION

Subtract the standard OD values from OD_{WATER} (#8) and plot the ΔOD_{517nm} against Trolox standard concentrations. For samples, calculate the $\Delta OD_{517nm} = OD_{WATER} - (OD_{SAMPLE} - OD_{SAMPLE\ BLANK})$ and determine the Trolox equivalent (µM) from the standard curve.

Note: If sample Trolox equivalent exceeds 300 µM *dilute samples in 70% methanol and repeat the assay.*

MATERIALS REQUIRED, BUT NOT PROVIDED

100% methanol, pipetting devices and accessories (e.g. multi-channel pipettor), clear flat-bottom 96-well plates (e.g. VWR cat# 82050-760), centrifuge tubes and plate reader. Also any materials needed for sample extraction.



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

LITERATURE

1. Niki, E. (2011). Antioxidant capacity: which capacity and how to assess it?. *Journal of Berry Research*, *1*(4), 169-176.

2. Karadag, A., et al. (2009). Review of methods to determine antioxidant capacities. *Food analytical methods*, *2*, 41-60.

3. Sun, T., & Tanumihardjo, S. A. (2007). An integrated approach to evaluate food antioxidant capacity. *Journal of Food Science*, *72*(9), R159-R165.