

Technical Bulletin

DPPH Antioxidant Capacity Assay Kit

Catalogue Number MAK609

Product Description

Antioxidants are molecules capable of slowing or preventing the oxidation of other molecules. Endogenous and dietary antioxidants (e.g., glutathione, vitamins) and antioxidant enzymes (e.g., catalase, glutathione peroxidase) protect cells from reactive oxygen species. Antioxidant capacity measurements are widely used in research, food, beverage, and nutraceutical testing.

The DPPH Antioxidant Capacity Assay Kit provides a simple, direct, colorimetric method to quantify total antioxidant capacity based on reduction of the stable radical DPPH, resulting in a decrease in absorbance at 517 nm. Results are expressed relative to a Trolox standard.

This assay detects approximately 11–300 μM Trolox equivalents in 96-well plates. It is suitable for a variety of sample types, including food, beverages, and biological samples, and can be performed at room temperature in a homogeneous mix-incubate-measure format.

Components

The kit is sufficient for 120 colorimetric assays in 96-well plates.

- | | |
|--|-------------------|
| • Reagent
Catalogue Number MAK609A | 110 mg |
| • Assay Buffer
Catalogue Number MAK609B | 4 mL |
| • Standard
Catalogue Number MAK609C | 100 μL |

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (e.g., multichannel pipettor).
- Spectrophotometric plate reader capable of reading 517 nm.
- 96-well clear plates for colorimetric assays (Catalogue number M2936 or equivalent).
 - Cell culture or tissue culture-treated plates are not recommended.
- 1.5 mL microcentrifuge tubes.
- 100% Methanol or 70% methanol.

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped at ambient temperature. Store kit at -20°C upon receipt.

Preparation Instructions

Briefly centrifuge small vials prior to opening. Equilibrate all components to room temperature prior to use. To maintain reagent integrity, avoid repeated freeze/thaw cycles.

Working Reagent

The reagents provided are sufficient for 120 wells of assay. To prepare the working reagent, 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. WR should be used within 3 hours.

Note: 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.

Procedure

All samples and standards should be run in at least duplicates. Assays are performed at room temperature. Prior to assay, bring the working reagents and microplate to room temperature.

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.

Preparation of Standards

Prepare 300 μM Trolox Premix by mixing 6 μL provided standard and 994 μL 70% methanol. Further dilute standard in 70% methanol as follows in Table 1.

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

Table 1.
Standard Preparation

Std #	Standard Premix (μL)	70% methanol (μL)	Conc. (μM)
1	150	0	300
2	120	30	240
3	90	60	180
4	60	90	120
5	45	105	90
6	30	120	60
7	15	135	30
8	0	150	0

Assay Reaction

1. For each sample, prepare two wells: one sample well and one sample blank well. Add 100 μL sample to each of two separate wells.
2. Reaction setup: Add 100 μL Working Reagent to each standard and sample well. For sample blank wells, add 100 μL of 70% methanol. Gently tap the plate to mix.
3. Incubate 10 minutes at room temperature.
4. Measure absorbance at 517 nm (A517).

Results

Calculations

Subtract the standard OD values from OD_{WATER} (#8) and plot the $\Delta\text{OD}_{517\text{nm}}$ against the standard concentrations.

For samples, calculate:

$$\Delta\text{OD}_{517\text{nm}} = \text{OD}_{\text{WATER}} - (\text{OD}_{\text{SAMPLE}} - \text{OD}_{\text{SAMPLEBLANK}})$$

Determine the Trolox equivalent (μM) from the standard curve.

If a sample exceeds the upper limit of the standard curve (300 μM), dilute in 70% methanol and repeat the assay.

Figure 1.
Exemplary standard curve

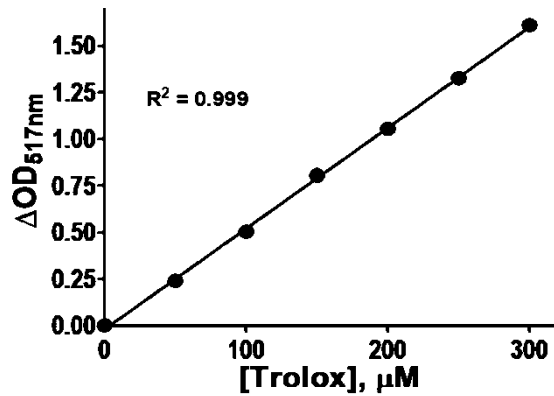
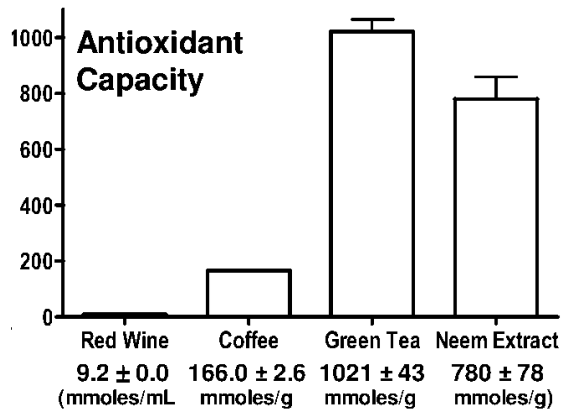


Figure 2.
Various samples tested



References

1. Niki, E. (2011). Antioxidant capacity: which capacity and how to assess it? *Journal of 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.

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mak609pis Rev 02/26

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