

ab289847 – DPPH Assay Kit (Colorimetric)

For the quantification of antioxidant in food, beverages, plant extracts and biological fluids.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:

<http://www.abcam.com/ab289847>

Introduction

Antioxidant capacity is considered as an important factor in many human diseases and health impairments. Epidemiological evidence suggests that a diet of antioxidant-rich foods such as vegetables, fruits and berries can reduce the risk of various diseases including cardiovascular disease and various cancers. A rapid, simple and inexpensive way to evaluate the antioxidant capacity of samples employs 2,2-diphenyl-1-picrylhydrazyl radical (DPPH), which is a stable free radical that acts as a scavenger for hydrogen radical. This DPPH Assay Kit (Colorimetric) (ab289847) is a high-throughput adaptable, microplate-based assay that allows the rapid quantification of antioxidant capacity of many samples including foods, beverages, biological fluids (e.g. human serum), etc. In this assay, DPPH free radical, which is deep blue in color abstracts a hydrogen atom in a one-electron reaction to form 2,2-diphenyl-1-picrylhydrazine (DPPH-H), which is pale yellow in color. The color change is associated with a decrease in absorbance at 517 nm. The decrease in absorbance is proportional to the antioxidant capacity of the sample. The synthetic antioxidant, Trolox is included in the kit and is used to standardize the sample antioxidant capacity relative to Trolox (Trolox Equivalents Antioxidant Capacity, TEAC).

Storage and Stability

All components in this kit are shipped on blue ice and are suitable for storage at 4°C, unless reconstituted. Upon receipt, immediately store kit at 4°C in the dark. Individual components may be stored at alternative temperatures as shown in the table below. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Materials Supplied

Item	Quantity	Storage Condition
DPPH Assay Buffer	50 mL	4°C
DPPH Probe	1 vial	4°C
Trolox Standard	1 vial	4°C or Ambient

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Reagent-grade (anhydrous) methanol
- DMSO
- Proteinase K (Cat. No. ab286007)
- 96-well clear plate with flat bottom
- Multi-well microplate reader

Reagent Preparation

DPPH Assay Buffer: Ready to use. Store at 4°C. Bring to room temperature (RT) for the assay.

DPPH Probe: Reconstitute the vial in 825 µL anhydrous methanol to prepare 8 mM DPPH Probe stock solution. Vortex for 2 min until completely dissolved. Divide into aliquots and store at 4°C, protected from light. Prior to use, vortex well and keep on ice until use. After use, promptly re-tighten the cap to minimize the evaporation of methanol.

Trolox Standard: Reconstitute the vial in 20 µL DMSO by pipetting up and down several times and vortexing. Add 980 µL of DPPH Assay Buffer to the same vial and mix well to generate 1 mM Trolox
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Standard stock solution. Store the reconstituted Trolox Standard stock solution at 4°C. The reconstituted Trolox Standard stock solution is stable for 1 month when stored at 4°C.

Sample Preparation

- For each sample type prepare a set of dilutions to build EC dose response curve.

Serum:

Add 15 µL of 20 mg/mL Proteinase K (Cat. No. ab286007) to 285 µL of serum in a microfuge tube and vortex. Incubate the tube at 37°C for 45 min. Increase the temperature to 90°C and incubate for another 10 min to denature the Proteinase K. Cool down the tube on ice and centrifuge at 12000 x g for 60 min and 4°C. Add 20 µL of serum supernatant (deproteinized serum) per well for the assay and adjust the volume to 100 µL/well with DPPH Assay Buffer.

Fruits, vegetables, other foods and plants: We recommend using 500 µL of DPPH Assay Buffer for extraction (see Note) of 100 mg of sample mass. Vortex thoroughly and centrifuge at 10000 x g for 10 min and use the supernatant for the assay. Prepare several dilutions using DPPH Assay Buffer. Add 2-100 µL of diluted supernatant per well for the assay and adjust the volume to 100 µL/well with DPPH Assay Buffer.

ΔNote: Extraction volume and method may vary based upon the sample type. Soft samples (such as fruits and berries) may be minced or pulverized and added to a microfuge tube. More rigid samples may be homogenized or sonicated.

Beverages including juices, wines, teas and other liquid samples: Filter the samples using 0.2 µm syringe filters prior to performing the assay. Filtered samples can be used directly for the assay with appropriate dilutions. Add 2-100 µL of the diluted samples per well for the assay and adjust the volume to 100 µL/well with DPPH Assay Buffer.

ΔNotes:

- a) Sample volume and the dilution factor may vary based on the sample type.
- b) For Unknown Samples, we suggest testing several concentrations to ensure the readings are within the Standard Curve range.
- c) For Samples with high background, prepare a parallel well containing 2-100 µL of diluted samples labeled as Sample Background Control well. Adjust the volume to 200 µL/well with DPPH Assay Buffer. Add 200 µL of DPPH Assay Buffer to a well labeled as Assay Buffer Control.

Trolox Standard Curve Preparation

Add 0, 5, 10, 15, 20, 30 µL of the 1 mM Trolox Standard stock solution into a series of wells to generate 0, 5, 10, 15, 20, 30 nmol/well of Trolox Standard. Adjust the volume to 100 µL/well with DPPH Assay Buffer.

DPPH Reaction Mix Preparation

1. Prepare 600 µM DPPH Probe working solution by diluting the 8 mM DPPH Probe stock with DPPH Assay Buffer. Prepare enough volumes of 600 µM DPPH Probe working solution for the number of assays to be performed.
2. For each well, prepare 100 µL of 600 µM DPPH Probe working solution. For example, for 10 wells, mix 75 µL of 8 mM DPPH Probe stock with 925 µL of DPPH Assay Buffer.
3. Add 100 µL of 600 µM DPPH Probe working solution to the sample and Standard wells only.
4. Gently pipette up and down to mix the contents of the well. The final reaction volume should be 200 µL for all wells including sample, Standards, Sample Background Control and Assay Buffer Control.
5. Incubate the plate at RT for 10 min, protected from light.

ΔNote: Do not add DPPH Probe working solution to the Assay Buffer Control and Sample Background Control wells.

Measurement

Measure the absorbance of all wells at 517 nm in end point mode at RT, protected from light.

Calculation

Subtract the Assay Buffer Control reading from all Standards readings and calculate the % inhibition as shown below.

$$\% \text{ inhibition of Standard} = ((A0 - AT)/A0) \times 100$$

Where:

$$A0 = \text{Abs}_{(0 \text{ nmol Trolox Standard})} - \text{Abs}_{(\text{Assay Buffer Control})}$$

$$AT = \text{Abs}_{(5-30 \text{ nmol Trolox Standard})} - \text{Abs}_{(\text{Assay Buffer Control})}$$

Draw the Trolox Standard Curve by plotting the % inhibition on the y-axis vs Trolox concentration ($\mu\text{g/ml}$) on the x-axis.

$$\% \text{ inhibition of Sample} = ((AC - AS) / AC) \times 100$$

Where:

$$AC = \text{Abs}_{(0 \text{ nmol Trolox Standard})} - \text{Abs}_{(\text{Assay Buffer Control})}$$

$$AS = \text{Abs}_{(\text{sample})} - \text{Abs}_{(\text{Sample Background Control})}$$

ΔNote: For diluted samples, calculate the % inhibition for every dilution. Plot the log Dilution Factor vs % Inhibition, fitted into logistic regression.

$$\text{EC50 (sample } \mu\text{g/mL)} = (\text{Trolox EC50}) \times D$$

Where:

D = the dilution factor of sample at 50% inhibition

Calculate the total Trolox Equivalents Antioxidant Capacity (TEAC) of samples using the following equation:

$$\text{TEAC} = \text{EC50 (Trolox)} / \text{EC50 (Sample)}$$

Technical Support

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