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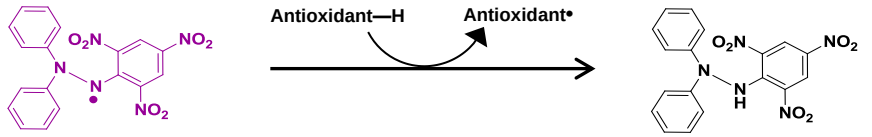
DPPH Antioxidant Assay Kit (Colorimetric)

02/21

(Catalog # K2078-100; 100 Reactions; Store at 4 °C)

I. 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. **BioVision's DPPH Antioxidant Assay Kit** 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**).



DPPH Radical (Blue)
λ_{abs} = 517 nm

DPPH-H (Pale Yellow Color)
↓ λ_{abs} = 517 nm

II. Application:

- Rapid quantification of antioxidant activity in beverages, foods, plant extracts, biological fluids i.e. human serum, etc.

III. Sample Types:

- Food and beverages (fruits, berries, tea, etc.)
- Plant and herbal extracts
- Biological fluids (i.e. serum)

IV. Kit Contents:

Components	K2078-100	Cap Code	Part Number
DPPH Assay Buffer	50 ml	WM	K2078-100-1
DPPH	1 vial	Amber	K2078-100-2
Trolox Standard	1 vial	Yellow	K2078-100-3

V. User Supplied Reagents and Equipment:

- Reagent-grade (anhydrous) methanol
- DMSO
- Proteinase K (BioVision Cat# 9211)
- 96-well clear plate with flat bottom
- Multi-well microplate reader

VI. Storage Conditions and Reagent Preparation:

Store the kit at 4 °C, protected from light. Read the entire protocol before performing the assay procedure.

- **DPPH Assay Buffer:** Ready to use. Store at 4 °C. Bring to room temperature (RT) for the assay.
- **DPPH:** Reconstitute the vial in 825 µl anhydrous methanol to prepare 8 mM DPPH 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 retighten 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 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.

VII. DPPH Radical Scavenging Activity Assay Protocol:

1. 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 (BioVision Cat# 9211) 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.
  Note: 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 (BioVision Cat# M4332 or equivalent) 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.
  Notes: i. Sample volume and the dilution factor may vary based on the sample type.
  ii. For Unknown Samples, we suggest testing several concentrations to ensure the readings are within the Standard Curve range.
  iii. 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**.

2. **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 nmoles/well of Trolox Standard. Adjust the volume to 100 µl/well with DPPH Assay Buffer.
3. **DPPH Reaction Mix Preparation:** Prepare 600 µM DPPH working solution by diluting the 8 mM DPPH stock with DPPH Assay Buffer. Prepare enough volumes of 600 µM DPPH working solution for the number of assays to be performed. For each well, prepare 100 µl of 600 µM DPPH working solution. For example, for 10 wells, mix 75 µl of 8 mM DPPH stock with 925 µl of DPPH Assay Buffer. Add 100 µl of 600 µM DPPH working solution to the sample and Standard wells only. 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. Incubate the plate at RT for 10 min, protected from light. **Note: Do not add DPPH working solution to the Assay Buffer Control and Sample Background Control wells.**
4. **Measurement:** Measure the absorbance of all wells at 517 nm in end point mode at RT, protected from light.
5. **Calculations:** Subtract the **Assay Buffer Control** reading from all Standards readings and calculate the % inhibition as shown below.

A. % inhibition of Standard $= ((\mathbf{A0} - \mathbf{AT})/\mathbf{A0}) * \mathbf{100}$

Where,

A_0: Abs $_{\text{(0 nmol Trolox Standard)}}$ - Abs $_{\text{(Assay Buffer Control)}}$
A_T: Abs $_{\text{(5-30 nmol Trolox Standard)}}$ - Abs $_{\text{(Assay Buffer Control)}}$

Draw the Trolox Standard Curve by plotting the % inhibition on the y-axis vs Trolox concentration (µg/ml) on the x-axis.

B. % inhibition of Sample $= (\frac{\mathbf{Ac-As}}{\mathbf{Ac}}) * \mathbf{100}$

Where,

A_c: Abs $_{\text{(0 nmol Trolox Standard)}}$ - Abs $_{\text{(Assay Buffer Control)}}$
A_S: Abs $_{\text{(sample)}}$ - Abs $_{\text{(Sample Background Control)}}$

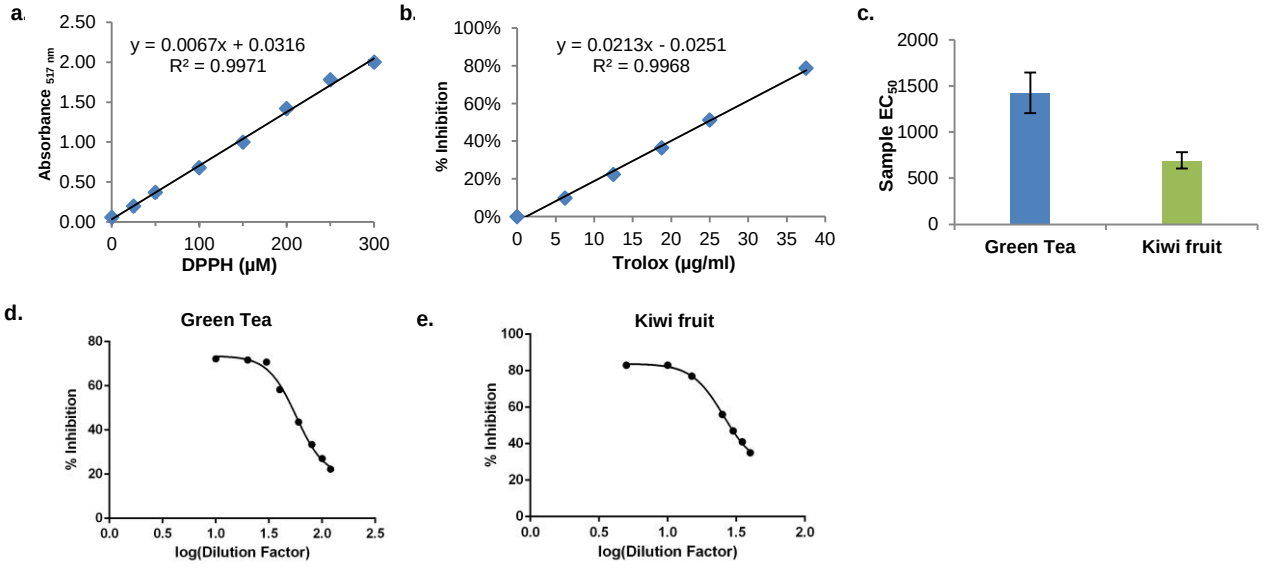
Note: For diluted samples, calculate the % inhibition for every dilution. Plot the log Dilution Factor vs % Inhibition, fitted into logistic regression.

$$\mathbf{EC50\ (sample\ (\mu g/ml))} = \mathbf{(Trolox\ EC50) * D}$$

Where, D is the dilution factor of sample at 50% inhibition

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

$$\mathbf{TEAC = EC50\ (Trolox)/EC50(sample)}$$



Figures: a. Linear relationship between DPPH concentrations and Absorbance. **b.** Trolox Standard Curve (linear range of inhibition changes after Trolox treatment under 300 µM DPPH concentration). **c.** DPPH assay using green tea and kiwi fruit samples. **d, e.** Logistic fitting including the dilution factor for calculating EC_{50}.

VIII. Related Products:

Total Antioxidant Capacity (TAC) Colorimetric Assay Kit (K274)
Ferric Reducing Antioxidant Power Assay Kit (C) (K515)
Phenolic Compounds Assay Kit (Colorimetric) (K527)
Catalase Activity Colorimetric/Fluorometric Assay Kit (K773)

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