Cat. No. 307

RadiClean®

DPPH Free-radical scavenging capacity Assay Kit Product Information

Quick Facts

1. Storage conditions before opening:
   4 ℃, protected from light.
2. Measure the absorbance at **517** nm.
3. Prepare the **DPPH working solution** by mixing Reagent **A** and **10.0 mL** of **B**.
4. Prepare the **Trolox Std. solution** (200 μg/mL) by mixing Reagent **D** and **10.0 mL** of **B**.
5. Volumes for working, sample & Buffer (Reagent **C**) solutions：**100**, **20** & **80** **μL.**

Introduction

Antioxidant capacity is considered as a crucial factor in many human diseases and health impairment. Recent evidences suggest that a diet of antioxidant-rich food, such as vegetables, fruits, barriers, and teas can reduce the risk of various cardiovascular diseases and cancers.

We provided a rapid, simple, and cost-effective product, **ReQxi,** to evaluate the antioxidant capacity of aqueous and/or alcoholic solutions, such as plant extracts, beverages, serums, etc. As shown in Fig. 1, 2,2-diphenyl-1-picrylhydrazyl (DPPH), a stable compound with free radicals, acts as a scavenger for antioxidant hydrogen radicals. After the absorption of one hydrogen radical, the purple-colored DPPH molecule turns into a colorless DPPH-H. The color change, with an absorbance decrease in 517 nm, is proportional to the antioxidant capacity of the sample solution. Using Trolox as a standard compound, which is included in this assay kit, the antioxidant capacity can be calculated as TEAC (Trolox Equivalent Antioxidant Capacity) value.

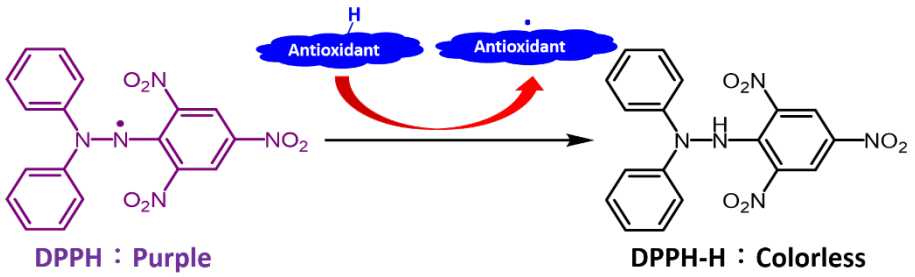


Fig. 1 The principle of DPPH assay.

Kit Contents, Shipping, and Storage Information

	Description	100 tests	Shipping and Storage
Reagent A	DPPH	15ml ORANGE centrifuge tube (in black bag) X1	Shipping at room temperature. Store at 4°C with protection from light.
Reagent B	Ethanol	30 mL plastic bottle X1	
Reagent C	Assay buffer	15 mL plastic bottle X1	
Reagent D	Trolox Std.	15 mL BLUE centrifuge tube (in black bag) X1	

Protocol

1. Prepare the DPPH working solution：

1.1 Warn the assay kit to room temperature (take >30 mins).

1.2 Add 5 mL of Ethanol (Reagent B) to the orange DPPH tube (Reagent A, in black bag). Dissolve the contents completely by vortexing (using sonication will be helpful). Make up to a final volume of 10.00 mL with Ethanol (Reagent B).

<**Note**>Undissolved DPPH completely may result in the variation of data.

<**Note**>Prepare the DPPH working solution fresh on each day. If this working solution cannot be used up, please store it at -20°C and protect it from light. Use it up as soon as possible. Do not compare two measurements that were taken on different dates.

2. Prepare the Trolox Std. solution：

2.1 Warn the assay kit to room temperature (take >30 mins).

2.2 Add 5 mL of Ethanol (Reagent B) to the blue Trolox Std. tube (Reagent D). Dissolve the contents completely by vortexing. Make up to a final volume of 10.00 mL with Ethanol (Reagent B). The final concentration will be 200 µg/mL.

2.3 Serially dilute Trolox Std. as the following table:

Concentration (µg/mL)	Volume of 200 µg/mL Trolox Std solution (Reagent D) (µL)	Volume of Ethanol (Reagent B) (µL)
200	1000	0
160	800	200
120	600	400
80	400	600
0	0	1000

<**Note**> Prepare the Trolox Std. solution fresh on each day. If this Trolox Std. solution cannot be used up, please store it at -20°C and protect it from light. Use it up as soon as possible. Do not compare two measurements that were taken on different dates.

3. Assay procedure：

(µL)	Sample	Trolox Std.	Blank1	Blank2	Blank3
Sample solution	20	--	--	--	--
Sample solvent	--	--	20	20	--
Ethanol (Reagent B)	--	--	--	100	120
Trolox Std.	--	20	--	--	--
Assay Buffer (Reagent C)	80	80	80	80	80
DPPH working solution	100	100	100	--	--

Blank 1 refers to the coloring without antioxidant; Blank 2 refers to the sample solvent blank ; Blank 3 refers to the ethanol blank.

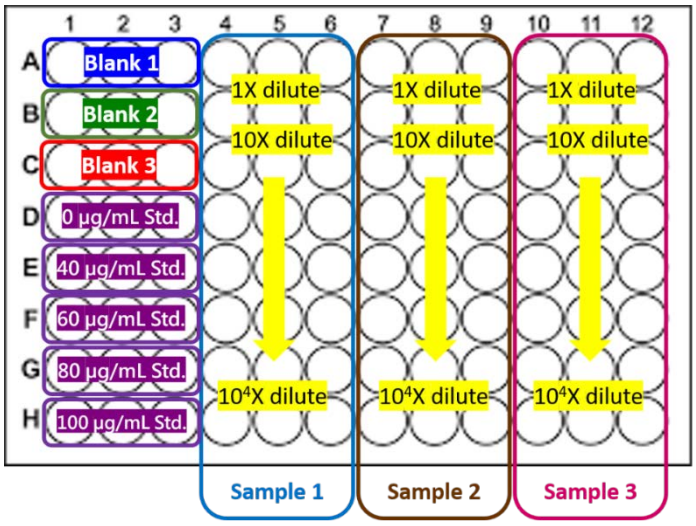


Fig. 2 Example of 96-wells plate format.

3.1 Add solutions to 96-well plate：

3.1.1. Add **20** μl of 0, 40, 60, and 80 μg/ml of Trolox Std. solutions to each well.

3.1.2. Add **20** μl of the sample solution with different concentrations to each well.

<**Note**>In the preliminary study of determination of the sample IC_{50} (50% of DPPH radicals are scavenged), the dilution range of the sample should be as large as 1~10,000. After that, a measurement with a narrow concentration range should be done to get a more accurate result.

3.1.3. Add **20** μl of the solvent that was used for sample dilution to the wells of Blank 1 and Blank 2. Add **20** μl of ethanol to the wells of Blank 3.

<**Note**>Because of the volatilization of organic solvent, move to step 3.1.4 as soon as possible.

3.1.4. Add 80 μl of Assay Buffer to each well.

3.1.5. Add 100 μl of ethanol (Reagent B) to the wells of Blank 2 and Blank 3 and mix gently by pipetting.

3.1.6. Add 100 μl of DPPH working solution to the wells of Trolox Std, samples, and Blank 1, and mix gently by pipetting.

3.1.7. Incubate the 96-well plate at room temperature for **30** mins **in a dark place**.

3.1.8. Measure the absorbances at 517 nm.

3.2 Calculation：

3.2.1. Inhibition ratio of Trolox：

$$\text{Inhibition ratio of Trolox (\%)} = (A_{TB} - A_T)/A_{TB} \times 100$$

A_{TB}：Absorbance of 0 μg/ml Trolox Standard solution - Blank 3

A_T：Absorbance of 40, 60, 80 & 100 μg/ml Trolox Standard solution - Blank 3

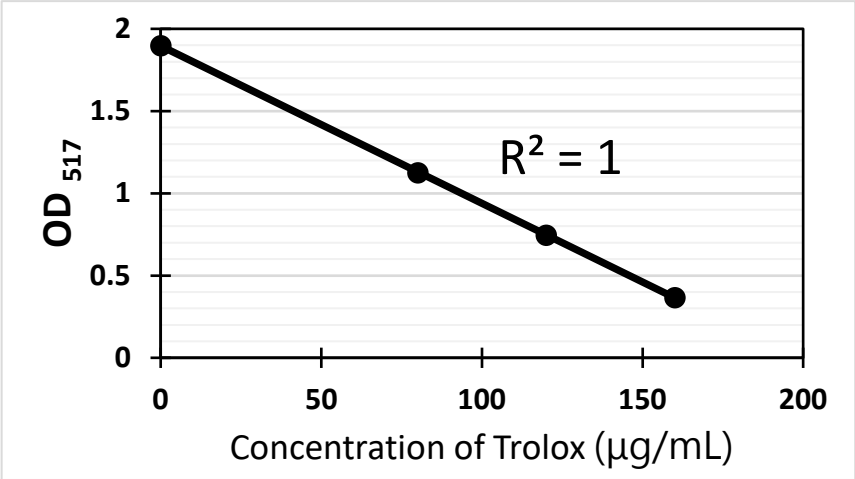
3.2.2. Inhibition ratio of sample：

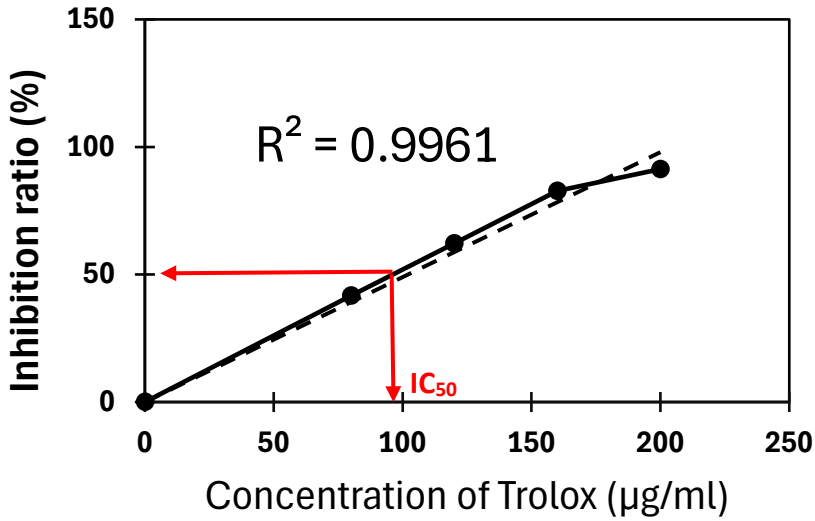
$$\text{Inhibition ratio of Sample (\%)} = (A_{SB} - A_S)/A_{SB} \times 100$$

A_{SB}：Blank 1 - Blank 2

A_S：Absorbance of Sample solutions - Blank 2

3.2.3. Polt the inhibition ratio (y-axis) against the concentration (x-axis) and draw a regression line (y=ax+b). Calculate the IC_{50} of Sample and Trolox, respectively.





3.2.4. Calculation of the Trolox equivalent antioxidant capacity (TEAC)：

TEAC = IC_{50}(Trolox)/ IC_{50} (sample)

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