



DPPH Free Radical Scavenging Capacity Kit

(Cat/No.:BC175 Size:48T)

1. Product Introduction

DPPH (1,1 - Diphenyl - 2 - picrylhydrazyl radical) is 1,1 - diphenyl - 2 - picrylhydrazyl radical. It is widely used for the quantitative determination of the antioxidant capacity of biological samples and foods.

This method is based on the characteristic that DPPH radical has a single electron and has a strong absorption at 517nm, and its alcohol solution is purple. When a free radical scavenger is present, due to the pairing with its single electron, the absorption gradually disappears, and the lighter the color, that is, the lower the A value. Then, the DPPH scavenging capacity in the sample can be quantitatively analyzed.

2. Composition and configuration: (The kit is valid for 3 months)

Reagent Name	Specification	Storage Condition	Remarks
Reagent 1	1 bottle of working solution powder	4°C, protected from light	Shake the bottle before use to make the powder fall to the bottom, then add 40mL of absolute ethanol and dissolve it thoroughly for later use (prepare half an hour in advance). The unused reagent should be stored at 4°C protected from light.
Reagent 2	1 vial of standard powder	4°C	Used for making the standard curve

1. Required Instruments and Supplies

Visible spectrophotometer, 1mL colorimetric cuvette (light path 1cm), centrifuge, pipette, absolute methanol, absolute ethanol, distilled water.



2. Operation Procedures

Please read the sixth point "Precautions" in carefully before operation to understand the relevant points.

1. Sample Pretreatment

① Tissue Samples:

Weigh about 0.1g of tissue, cut it into small pieces, add 1mL of 80% methanol solution (or add it according to the ratio of sample weight in grams to the volume of 80% methanol solution in milliliters as 1:10 or 1:5), homogenize in an ice - water bath at 12,000 rpm for 10 minutes, centrifuge, take the supernatant and place it on ice for testing.

② Liquid Samples: Directly detect. If the sample is turbid, centrifuge and take the supernatant for determination.

2. Operation Steps (Operate in EP tubes)

	Control Tube	Test Tube	Blank Tube
Sample (μL)	400	400	-
80% Methanol (μL)	600	-	400
Working Solution (μL)	-	600	600
Mix well, keep it in the dark at room temperature (25°C) for 30 minutes, centrifuge at 4,000 rpm for 5 minutes. At a wavelength of 517nm, pipette 800 μL in to a colorimetric cuvette, zero with 80% methanol, and measure the absorbance value of each tube.			

(Note: If $A_{\text{test}} - A_{\text{control}}$ is close to or less than 0.05 during the preliminary test, the sample needs to be diluted before detection, and the dilution factor should be substituted into the formula for calculation; if there are many samples, it is recommended to measure them in batches; only 1 - 2 blank tubes are required.)

3. Result Calculation

1. Preparation of Standard Curve:

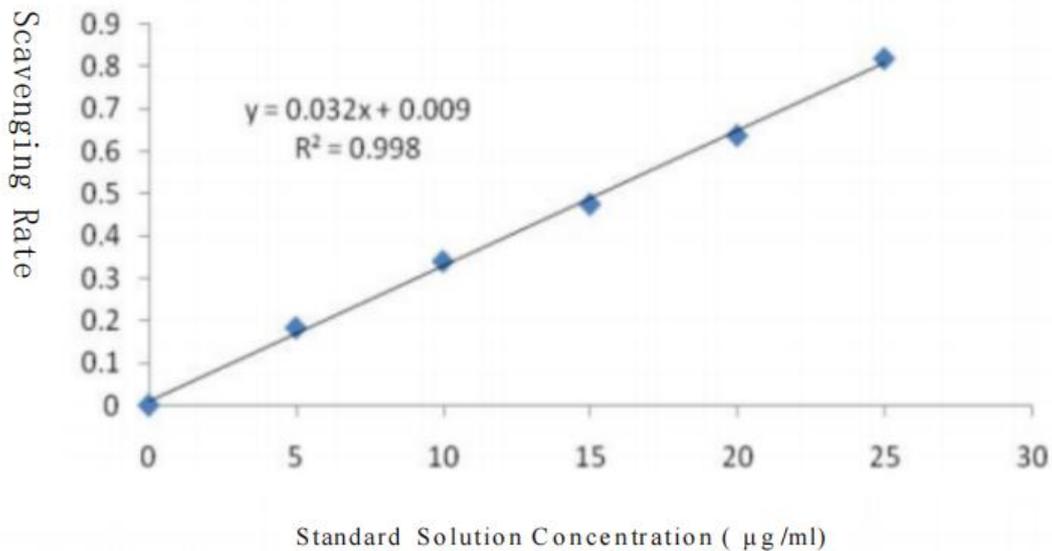
(Dissolve one vial of standard powder in 2mL of 80% methanol to prepare 0.5mg/mL (Trolox) standard working solution, then dilute it with 80% methanol to 5 $\mu\text{g/mL}$, 10 $\mu\text{g/mL}$, 15 $\mu\text{g/mL}$, 20 $\mu\text{g/mL}$, and 25 $\mu\text{g/mL}$ respectively, and operate according to the following table.)

Standard Solution Concentration ($\mu\text{g/mL}$)	0	5	10	15	20	25



80% Methanol	400	-	-	-	-	-
Standard Solution (μ L)	-	400	400	400	400	400
Working Solution (μ L)	600	600	600	600	600	600
Mix well, keep it in the dark at room temperature (25 ° C) for 30 minutes. At a wavelength of 517nm, with a 1cm light path, zero with 80% methanol, and measure the absorbance A of each tube.						

DPPH Free Radical Scavenging Rate Standard Curve



2. Calculation of DPPH Free Radical Scavenging Rate of Samples

DPPH free radical scavenging rate of samples (%) = $(1 - (A_{\text{test}} - A_{\text{control}}) \div A_{\text{blank}}) \times 100\%$

DPPH free radical scavenging rate of standard curve (%) = $(1 - A(S) \div A(S_0)) \times 100\%$ (where S represents each concentration standard tube, and S₀ represents the tube with a standard concentration of 0)

3. Definition

The DPPH free radical scavenging capacity of the sample is expressed by the amount equivalent to the antioxidant Trolox calculated from the standard curve.

4. Calculation Formulas

1)

DPPH Free Radical Scavenging Capacity in the Tissue
(μg Trolox/g fresh weight)

$$= \frac{\text{concentration equivalent to Trolox obtained from the standard curve} \times \text{Total volume (mL)} \times \text{Dilution factor}}{\text{Fresh weight of sample (g)}}$$

2)



DPPH Free Radical Scavenging Capacity in the Liquid Sample
($\mu\text{g Trolox/mL}$) =
concentration equivalent to Trolox obtained from the standard curve \times Dilution factor

4. Precautions

1. A preliminary test must be carried out before sample testing. On the one hand, it can help understand the situation of this batch of samples (or the required dilution factor). Otherwise, if the scavenging rate is too high

($\geq 90\%$), the calculation result will be on the low side. On the other hand, it can also help get familiar with the experimental process and avoid the waste of samples and reagents.

2. The standard concentration of the standard curve can be adjusted according to needs, and the upper limit should not be greater than $25 \mu\text{g/mL}$.

3. Since ethanol is volatile, it is not recommended to use a microplate reader for reading.

4. The operation should be carried out in a well-ventilated environment to avoid methanol poisoning caused by inhaling excessive amounts of methanol.

5. This kit is only for scientific research purposes.