Mirco DPPH Free Radical Scavenging Capacity Assay Kit

Cat #: orb3148351 (manual)

Product Features

Product name: Mirco DPPH Free Radical Scavenging Capacity Assay Kit

Catalog number: orb3148351

Sample Types: Plant Tissues, Red Wine, and other Liquid samples

Storage: Stored at 4°C for 6 months, protected from light.

Assay Principle

DPPH radical is a very stable nitrogen-centered free radical and is one of the important indicators of sample antioxidant capacity. It is widely used in the research of antioxidant foods, health supplements, and pharmaceuticals. Mirco DPPH Free Radical Scavenging Capacity Assay Kit provides a simple, convenient, and rapid method for measuring DPPH radical scavenging activity, suitable for plant tissues, red wine, and other liquid samples.The principle is based on the fact that DPPH radicals possess an unpaired electron, giving their ethanol solution a purple color with strong absorption at 515 nm. In the presence of antioxidants, DPPH radicals are scavenged, causing the solution color to fade and the absorbance at 515 nm to decrease. Within a certain range, the change in absorbance is proportional to the degree of radical scavenging.

Kit Components

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	70 mL	70 mL×2	4°C
Reagent I	Powder×1 vial	Powder×1 vial	4°C, protected from light
Working Reagent I	Empty Bottle×1	Empty Bottle×1	RT
Vitamin C (Positive Control)	Powder×1 vial	Powder×1 vial	4°C, protected from light

Note: Before formal testing, it is recommended to select 2-3 samples with large expected differences for pre-experiment.

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 515 nm
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips
- Drying oven, vortex mixer, low-temperature centrifuge, 30-50 mesh sieve
- Deionized water, anhydrous ethanol
- Homogenizer (for tissue samples)

Reagent Preparation

Extraction Buffer: Ready to use as supplied. Pre-cool before use. Store at 4℃.

Working Reagent Ⅰ: Prepare before use. For the 48 T kit, dissolve the Reagent Ⅰ thoroughly in 30 mL anhydrous ethanol and transfer it to the Working Reagent I bottle. For the 96 T kit, dissolve the Reagent Ⅰ thoroughly in 60 mL anhydrous ethanol and transfer it to the Working Reagent I bottle. Unused reagent should be aliquoted and stored at -20℃, protected from light to one month. Avoid repeated freeze-thaw cycles.

Notes: Reagent I is present in a small amount and is not visible to the naked eye. Please do not pour it out; once dissolved, it will form a purple solution.

Vitamin C Working Reagent (Positive Control): Prepare before use. Add 1 mL of Extraction Buffer and mix thoroughly to prepare a 10 mg/mL Vitamin C solution. Store at 4℃, protected from light to two weeks. This is used as positive control.

Preparation of Positive Control: If a linear relationship is required, it is recommended to 10 times dilute the 10 mg/mL Vitamin C

solution with Extraction Buffer to 1 mg/mL, then prepare working solutions of 0.25, 0.125, 0.0625, 0.03125, and 0.015625 mg/mL Vitamin C for use. Refer to the table below for dilutions.

Num.	Vitamin C Volume (μL)	Extraction Buffer Volume (μL)	Concentration (mg/mL)
1	125 μL of 1 mg/mL	375	0.25
2	200 μL of 0.25 mg/mL	200	0.125
3	200 μL of 0.125 mg/mL	200	0.0625
4	200 μL of 0.0625 mg/mL	200	0.03125
5	200 μL of 0.03125 mg/mL	200	0.015625
Blank Well	0	200	0

Notes: Diluted Solution is unstable and must be used within 4 h.

Sample Preparation

Note: We recommend that you use fresh samples. If not assayed immediately, samples can be stored at -80℃ for one month.

1. Preparation of Plant Samples: Place fresh samples in an oven at 60℃ until they reach a constant weight, then grind them using a mortar (or use a grinder) and sieve through a 30-50 mesh screen. Weigh out approximately 0.05 g of the sample, add 1 mL of Extraction Buffer, and homogenize on ice. Centrifuge at 10,000 g for 10 min at 4℃, collect the supernatant, and keep it on ice for subsequent analysis.

2. Liquid Samples such as Red Wine, Juice, etc.: Pipette 100 μL of the sample solution into 900 μL of Extraction Buffer, mix thoroughly by vortexing. Centrifuge at 10,000 g for 10 min at 4 ℃, collect the supernatant, and keep it on ice for subsequent analysis.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 515 nm, visible spectrophotometer was returned to zero with anhydrous ethanol.
2. Operation table (The following operations are performed in a 96-well plate or micro glass cuvette.):

Reagent	Test Well (μL)	Control Well (μL)	Positive Control Well (μL)
Sample Supernatant	10	10	0
Different Concentrations of Vitamin C Solution	0	0	10
Anhydrous Ethanol	0	190	0
Working ReagentI	190	0	190
Mix thoroughly and incubate at room temperature in the dark for 20 min. Then, measure the absorbance at 515 nm. Record these measurements as ATest, AControl, APositive Control, and ABlank, respectively.			

Note: For each test well, a corresponding control well should be set up; the positive control well and the blank well need to be measured only 1-2 times. The ability of different samples to scavenge DPPH radicals may vary significantly. It is recommended to conduct a preliminary experiment with 2-3 samples that are expected to show large differences before performing the full experiment. If the scavenging rate is less than 5%, the sample amount can be appropriately increased, for example, by increasing the weight of the dried sample or the volume of the liquid sample. If the scavenging rate exceeds 90%, the sample can be further diluted with Extraction Buffer, and the calculation result should be multiplied by the dilution factor, or the amount of sample used for extraction can be reduced. This translation maintains the technical details and context of the original instructions, ensuring clarity for experimental procedures.

Data Analysis

Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

1. Formula for calculating the radical scavenging rate of the positive control:

DPPH Radical Scavenging Rate (DVC%)=$[(A_{Blank}-A_{Positive\ Control})\div A_{Blank}]\times 100\%$

2. Formula for calculating the radical scavenging rate of the sample:

DPPH Radical Scavenging Rate (D%)=$[[A_{Blank}-(A_{Test}-A_{Positive\ Control})]\div A_{Blank}]\times 100\%$

Precautions

1. Avoid using reagents that turn red or near red under acidic conditions as much as possible, as they may interfere with the test results of this kit.
2. Do not add detergents such as Tween, Triton, and NP-40, or reducing agents like DTT (dithiothreitol) and mercaptoethanol that can affect oxidation-reduction reactions to the samples.
3. If the liquid sample is alkaline, it should be diluted to acidity using Extraction Buffer before testing.
4. The ability of different samples to scavenge DPPH radicals may vary significantly. If comparing the DPPH radical scavenging ability of different samples, it is recommended to use equal amounts of samples from the same batch for testing.
5. Samples are recommended to be extracted and tested on the same day.

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Typical Data

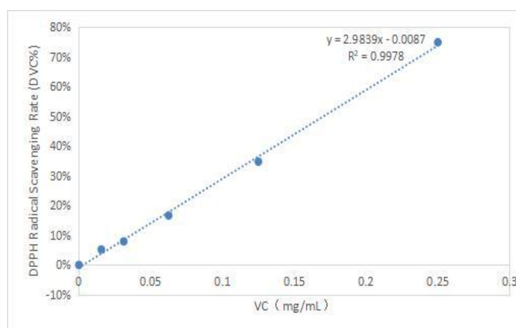

Figure 1. Positive control curve

(This curve is for reference only.)

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes. For your safety and health, please wear a lab coat and disposable gloves.

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