DPPH Free Radical Scavenging Capacity Assay Kit

Note: Take two or three different samples for prediction before test.
Operation Equipment: Spectrophotometer/ Microplate reader
Catalog Number: NA0244
Size:100T/48S

Components:
Extract solution: Liquid 80mL×1. Storage at 4℃.
Reagent I: Absolute ethanol 30mL×1. **Self-provided reagent**
Reagent Ⅱ: Powder×1. Storage at 4℃. (0.6 mL EP tube placed in 8 mL reagent bottle). Add 4.05 mL of reagent I before use to shake and dissolve. Unused reagents can be stored at -20℃ for one month. It is recommended to store them separately;
According to the required amount of the test sample to prepare **Working solution**: reagent II: reagent I (V: V) =4:21. The unused working solution can be stored at 4℃ for a week; The working fluid needs to be temporarily prepared before use .
Reagent III: Powder×1, Storage at 4℃. Add 1mL extract solution before use to prepare 10mg/mL positive control tube.

Product Description

DPPH free radical is a very stable nitrogen-centered free radical. It is one of the important indicators of the sample's antioxidant capacity and is widely used in the research of antioxidant foods, health products and pharmaceuticals.

The DPPH radical has a single electron, and its alcohol solution is purple, with strong absorption at 515 nm. When an antioxidant present, DPPH free radicals are cleared, the solution color becomes lighter, and the absorbance at 515 nm decreases. Within a certain range, the change in absorbance is directly proportional to the degree of free radical removal. In this kit, the ability of the sample to remove DPPH free radicals is reflected by the degree of decrease in absorbance.

Reagents and Equipment Required but Not Provided.
Spectrophotometer/ microplate reader, water bath, centrifuge, micro glass cuvette/ 96 well flat-bottom plate, motor/ grinder, absolute ethanol, oven, 30-50 mesh sieve and distilled water.

Procedure
I. Sample preparation
1. Tissue samples: dry fresh samples in a 60℃ oven to constant weight, grind them in a mortar (or grinder), and pass a 30-50 mesh sieve. Weigh about 0.05 g sample, add 1 mL of extraction solution, and leaching for 30 min at 40℃ water bath. Centrifuge at 10,000 rpm at room temperature for 10 min. Take the supernatant

and place it on ice for testing.

2. Serum, juice or other liquid samples：Pipette 100 μL of the sample solution into 900 μL of extraction solution, vortex and mix well, centrifuge at room temperature at 10,000 rpm for 10 min, take the supernatant, and place it on ice for testing.

3. Extract (or drug) can be prepared in a certain concentration, such as 5 mg/mL.

Note: The ability of different samples to remove DPPH free radicals may vary greatly. In order to ensure the accuracy of the experimental results, the samples should be adjusted appropriately according to the results of the pre-experiment (if the removal rate is greater than 90%, it is recommended to dilute the extracted samples with the extraction solution ; The removal rate is less than 5%, it is recommended to increase the quality of the dried sample or the volume of the liquid sample for extraction).

II. Determination procedure

1. Preheat spectrophotometer/ microplate reader for 30 min, adjust the wavelength to 515 nm, set the counter to zero with distilled water.

2. Preparation of positive control tube: If a linear relationship is required, it is recommended that the 10 mg/mL vitamin C solution be prepared into 0.3, 0.25, 0.125, 0.0625, 0.03125, 0.015625 mg/mL with extract solution for use; if 100% clearance rate is required, it is recommended to prepare 0.3 mg/mL vitamin C solution (or greater) with extraction solution.

3. Sample list: add the following reagents to 1.5 mL EP tube.

reagent（μL）	Blank Tube (A_B)	Test Tube (A_T)	Control Tube (A_C)	Positive control tube (A_P)
Supernatant	-	10	10	-
positive control solution	-	-	-	10
Extract solution	10	-	-	-
Reagent I	-	-	190	-
Working solution	190	190	-	190
混匀后室温避光静置 30 min，于 515 nm 处的吸光度。空白管、阳性对照管、对照管和测定管的吸光值分别记为 A空白、A 阳性对照、A 对照和 A测定。空白管只需测 1-2 次。				

III. Calculations

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

DPPH free radical scavenging rate $D_{VC}\% = [(A_B - A_P) \div A_B] \times 100\%$

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

DPPH free radical scavenging rate $D_{VC}\% = [[(A_B - (A_T - A_C)] \div A_B] \times 100\%$

Note:

1. The DPPH free radical scavenging ability of different samples may vary greatly. If you want to compare the DPPH free radical scavenging ability of different samples, it is recommended to add the same amount of samples to the same batch of samples: Add liquid samples such as red wine, tissue homogenate, and juice to

the same volume; The extract (or drug) is formulated to the same concentration.

During the comparison, the sample is adjusted appropriately according to the results of the pre-experiment, and the scavenging rate of the same concentration (same dilution factor) is compared.

2. Samples are recommended to be tested on the day of extraction.

Experimental Examples:

1. Take 0.05g of Leonurus artemisia leaves and add 1mL extract for sample processing, centrifuge by the 96 well plate to take the supernatant and operate according to the determination steps. It is measured that Ab=1.229, A c=0.078, A t=0.300, according to the calculation formula:

DPPH Free Radical Scavenging Capacity D%=[[Ab-(At-A c)]÷A b]×100%=81.9%.

2. Take 100μL of red wine and add 900μL of extraction solution for sample processing, centrifuge by the 96 well plate to take the supernatant and operate according to the determination steps. Ab=1.229, Ac=0.051, At=0.898, according to the calculation formula:

DPPH Free Radical Scavenging Capacity D%=[[Ab-(At-Ac)]÷Ab]×100%=31.1%.

Related Products:

NA0768/NA0527 Total Antioxidant Capacity(T-AOC) Assay Kit

NA0767/NA0526 Hydroxyl Free Radical Scavenging Capacity Assay Kit