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DPPH Free Radical Scavenging Capacity Assay Kit

BC4755(100T/48S)

FOR RESEARCH USE ONLY, DO NOT USE IT IN CLINICAL DIAGNOSIS

Product Description

DPPH free radical is a very stable nitrogen-centered free radical. It is one of the important indicators of the samples 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. Kinetic determination of lactate dehydrogenase according to the following reaction.

Kit components

Reagent	Volume	Storage
Extraction Reagent	80mL	2-8°C
Reagent I	Self-provided reagent	-
Reagent II	Powder x1	2-8°C
Reagent III	Powder x1	2-8°C

Reagent Preparation

Extract Solution: Liquid 80mL×1. Storage at 2-8°C.

Reagent I: self-prepared anhydrous ethanol, about 25 mL, stored at room temperature; a 30mL brown empty bottle is provided in the kit, which is only used for packaging. Please mark the name of the reagent yourself. **Reagent II:** Powder×1. Storage at 2-8°C. Add 4.05 mL of reagent I before use to shake and dissolve. Unused reagents can be stored at -20°C for 1 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 2-8°C for a week; The working fluid needs to be temporarily prepared before use.

Reagent III: Powder (VC)×1, Storage at 2-8°C. Add 1mL extract solution before use to prepare 10mg/mL positive control tube. The unused reagent can be stored at 2-8°C for 2 weeks.

Reagents and Equipment Required but Not Provided

Spectrophotometer/microplate reader, water bath, centrifuge, micro glass cuvette/96well flat-bottom plate, motor/grinder, absolute ethanol, oven, 30-50 mesh sieve and distilled water

Operation Procedures

I. Sample Preparation

1. 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 10min, take the supernatant, and place it on ice for testing

2. Tissue

Dry fresh samples in a 60°C 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°C water bath. Centrifuge at 10,000 rpm at room temperature 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).

I. Determination Procedure

1. Preheat spectrophotometer/ microplate reader for 30 min, adjust the wavelength to 515 nm. The spectrophotometer needs to be zeroed with ethanol.
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	-
Reagent III	-	-	-	10
Extract solution	10	-	-	-
Reagent I	-	-	190	-
Working solution	190	190	-	190

After fully mixing, leave it in dark for 30 min at room temperature. Determine the absorbance at 515 nm. The absorbance values of blank tube, control tube, positive control tube and test tube are recorded as A_B , A_C , A_P and A_T respectively. The blank tube only need to be measured 1-2 times.

Calculation

I. Calculation of DPPH free radical scavenging rate

1. Formula for the Free Radical Scavenging Rate of the Positive Control:

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

2. Formula for the Free Radical Scavenging Rate of Sample:

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

II. Calculation of DPPH free radical scavenging ability (optional)

1. Curve drawing : Select the linear part of the S-shaped curve (with Vitamin C concentration as x and $(A_B - A_P)$ as the y value), and established the standard curve $y = kx + b$
2. Unit definition: The DPPH scavenging ability of the sample was expressed as the Vitamin C concentration (mg/mL) required to achieve the same absorbance change value $(A_B - (A_T - A_C))$.
3. Sample weight : **DPPH free radical scavenging ability (mg/mL):** $X \times V_E \div W \times F$
4. Liquid volume

$$\text{DPPH free radical scavenging ability (mg/mL)} = X \times (V_{\text{Total solution}} \div V_{\text{Liquid}}) \times V_{\text{Liquid}} \div V_{\text{Liquid}} \times F = 10 \times X \times F$$

V_E : The volume of the extraction liquid added to the sample, mL W :

Sample mass, g

$V_{\text{Total solution}}$: The total volume of liquid extraction, 0.9 mL + 0.1 mL = 1 mL V_{Liquid} :

Volume of liquid sample, 0.1 mL

F : Dilution factor

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.