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ABTS Free Radical Scavenging Capacity Assay Kit

BC4775(100T/48S)

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

Product Description

ABTS method can be used to determine the antioxidant capacity of hydrophilic and lipophilic substances, and it is the most widely used indirect detection method. ABTS is oxidized to form a stable bluegreen cation ABTS radical with the largest absorption peak at 405 nm or 734 nm. After the tested substance is added into ABTS free radical solution, the antioxidant component can react with ABTS free radical to make the reaction system fade, and the absorbance of 405 nm decreases. In a certain range, the change of absorbance is directly proportional to the degree of free radical removal. In this kit, the sample's ability to scavenge ABTS radicals is reflected by measuring the degree of absorbance decrease.

Note: It is recommended to select 2-3 samples with large expected differences for pre experiment before test. If the sample absorbance value is not within the measurement range, it is recommended to dilute or increase the sample size for testing.

Kit Components

Reagent	Volume	Storage
Extraction Reagent	80mL	2-8°C
Reagent I	40mL	2-8°C
Reagent II	Powder×1	2-8°C
Reagent III	Liquid 20μL	2-8°C
Reagent IV	Liquid 1.5 mL	-20°C
Reagent V	Powder×1	2-8°C

Reagent Preparation

- 1. Reagent II:** Add 1 mL distilled water before use, and fully dissolve it; the remaining reagents can be packed separately and stored at -20°C for 4 weeks.
- 2. Preparation of Reagent III working solution:** The liquid is placed in the EP tube in the Reagent III reagent bottle. Before use, according to the amount of sample according to the reagent three (μL): distilled water (mL) = 1 μL: 12mL (pay attention to the unit) the ratio of reagent three working solution, ready to use now, as much as possible, as much as possible, try to be used up within 4h.
- 3. Preparation of Reagent IV working solution:** The Reagent IV can be stored in separate packages at - 20°C. Before use, prepare the Reagent IV working solution according to the number of sample and the ratio of Reagent IV: Reagent I (V: V) = 1: 9. prepare when the solution will be used. The unused reagent can be stored at -20°C for 2 weeks.
- 4. Reagent V:** 5 mg vitamin C. Add 2.8 mL of Extract solution before use, fully shake and dissolve; prepare 10 mmol/L vitamin C solution for positive control. Stored at 2-8°C for 2 weeks.
- 5. Preparation of ABTS working solution:** Before use, according to the sample amount, ABTS working solution is prepared in the proportion of Reagent I: Reagent II: Reagent III working solution (V: V: V) = 76:5:4, which is prepared when the solution will be used and stored in dark room temperature. It must be used within 30 minutes.

Reagents and Equipment Required but Not Provided

Spectrophotometer/microplate reader, desk centrifuge, transferpettor, micro glass cuvette/96-well flat bottom plates, mortar/homogenizer/cell ultrasonic crusher ice and distilled water.
the working reagent if the blank absorbance is less than 1.0 at 340 nm

Operation Procedures

I. Sample Preparation

1. Preparation of plant tissue samples: dry the fresh samples in an oven at 60°C to constant weight, grind them in a mortar (or grinder), and pass through a 30~50 mesh sieve; weigh about 0.05 g of samples, add 1 mL of Extract solution, and then put them in a 40°C water bath for 30 minutes; Centrifuge at 10000 rpm for 10 minutes at room temperature, take the supernatant, and put it on ice for testing.

2. Red wine, fruit juice and other liquid samples: take 100 μL of sample solution and add 900 μL of Extract solution, mix with vortex shaking, centrifuge at room temperature 10000 rpm for 10 minutes, take the supernatant and place it on ice for testing.

3. Extraction or drug: it can be prepared into a certain concentration with the Extract solution, such as 5 mg/mL.

Note: the ability of different samples to scavenge ABTS free radicals may vary greatly. In order to ensure the accuracy of the experimental results, samples should be adjusted appropriately according to the pre experimental results (for example, if the scavenging rate is greater than 90%, it is recommended to dilute the extracted samples with Extract solution; if the scavenging rate is less than 5%, it is recommended to increase the mass of dried samples or the volume of liquid samples for extraction).

II. Determination procedure

1. Preheat the spectrophotometer/microplate reader for more than 30 minutes, adjust the wavelength to 405 nm, The spectrophotometer needs to be zeroed with distilled water.
2. Preparation of positive control: if a linear relationship is needed, it is recommended to prepare 10 mmol/L vitamin C solution with Extract solution into 0.4, 0.2, 0.1, 0.05, 0.025, 0.0125 mmol/L vitamin C solution for use; If a positive control with a clearance rate of about 90% is needed, it is recommended to
3. prepare 10 mmol/L vitamin C solution with Extract solution into vitamin C solution larger than 1.0 mmol/L for use

Number	Concentration before (mmol/mL)	$V_{\text{vitamin C}}$ (μL)	$V_{\text{Extract solution}}$ (μL)	Concentration after (mmol/mL)
1	10	100	900	1
2	1	80	120	0.4
3	0.4	100	100	0.2
4	0.2	100	100	0.1
5	0.1	100	100	0.05
6	0.05	100	100	0.025
7	0.025	100	100	0.0125

Note: In the following experiment, each positive control tube requires 50 μL vitamin C solution (Do not directly measure the absorbance at this step).

4. Operation table: add the following reagents into 96 well plate or EP tube respectively.

Reagent (μL)	Blank tube(B)	Test tube(T)	Control tube(C)	Positive control tube (PC)
Supernatant	-	10	10	-
VC solution of different concentration	-	-	-	10
Distilled water	10	-	-	-
Reagent IV working solution	20	20	-	20
ABTS working solution	170	170	-	170
Reagent I	-	-	190	-

After fully mixing, leave it in **dark** for 6 min at room temperature. Determine the absorbance at 405 nm. The absorbance values of blank tube, control tube, positive control tube and test tube are recorded as A_B , A_T , A_C and A_{PC} respectively. The standard curve and blank tube only need to be measured 1-2 times.

Note: The graph of vitamin C concentration - A positive control yields an S-shaped curve.

I. Calculation of ABTS Free Radical Scavenging Rate

1. Calculation of ABTS free radical scavenging rate

(1) Formula for the ABTS free radical scavenging rate of the positive control: ABTS free radical scavenging rate $DVC\% = [(A_B - A_P) \div A_B] \times 100\%$

(2) Formula for the ABTS free radical scavenging rate of sample:

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

2. Calculation of the ABTS 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 establish the standard curve $y = kx + b$.

(2) Unit Definition: The ABTS scavenging ability of the sample was expressed as the Vitamin C concentration (μmol/mL) required to achieve the same absorbance change value $(A_B - (A_T - A_C))$.

(3) Sample weight:

$$ABTS \text{ free radical scavenging capacity } (\mu\text{mol/g mass}) = x \times V_E \div W \times F$$

(4) Liquid volume

$$ABTS \text{ free radical scavenging ability } (\mu\text{mol/mL}) = x \times (V_{\text{Total solution}} \div V_{\text{liquid}}) \times V_{\text{liquid}} \div V_{\text{liquid}} \times F \\ = 10 \times x \times F$$

V_E : the volume of the extraction solution added to the sample, mL

W: sample mass, g;

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

V_{liquid} : volume of liquid sample, 0.1mL

F: dilution factor.

Note:

1. The ability of different samples to scavenge ABTS free radicals may vary greatly. If we want to compare the scavenging ability of different samples, it is recommended to add the same amount of samples to the same batch of samples, Red wine, tissue homogenate, juice and other liquid samples need to be added with the same volume, and the extract (or drug) is prepared in the same concentration. During the comparison, the samples are adjusted according to the results of the preexperiment, and the clearance rate of the same concentration (the same dilution ratio) is compared.
2. If the measured value exceeds the linear range of the positive control line, the sample volume needs to be increased or diluted.
3. It is recommended that the samples be extracted and tested on the same day.