Ver.251201

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 blue-green 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℃
Reagent I	40mL	2-8℃
Reagent II	Powder×1	2-8℃
Reagent III	20μL	2-8℃
Reagent IV	1.5 mL	-20℃
Reagent V	Powder×1	2-8℃

Reagent Preparation

1. **Reagent II:** Add 1 mL of distilled water before use, and fully dissolve it; the reagent that cannot be used up can be packed separately and stored at -20℃ for 4 weeks.
2. **Preparation of Reagent III working solution:** There is a 15mL empty bottle in the kit. Preparation of Reagent III working solution according to the proportion of Reagent III (μL): distilled water(mL) = 1 μL: 12mL. prepare when the solution will be used, put the unused reagent in 4℃ for 4 hour.
3. **Preparation of Reagent IV working solution:** the Reagent IV can be stored in separate packages at -20℃. Before use, prepare the Reagent IV working solution according to the number of sample and the ratio of Reagent V: Reagent I (V: V) = 1: 9. prepare when the solution will be used. The unused reagent can be stored at -20℃ for 2 weeks..
4. **Reagent V:** The powder containing 5 mg vitamin C is placed in the glass bottle in the reagent bottle. 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 4℃ for 2 weeks.
5. **Preparation of ABTS working solution:** Before use, according to the amount required by the test, 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

Constant temperature water bath, spectrophotometer/microplate reader, 1 mL glass cuvette, desktop centrifuge, mortar/grinder, drying box, 30~50 mesh sieve, ice and distilled water.

Operation Procedures

I. Sample Preparation

1. Preparation of plant tissue samples:

Dry the fresh samples in an oven at 60℃ 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 1mL of Extract solution, and then put them in a 40℃ water bath for 30 min; Centrifuge at 10000 rpm for 10 min 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 μLof Extract solution, mix with vortex shaking, centrifuge at room temperature 10000 rpm for 10 min, 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, suchas5mg/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 wavelengthto405nm, 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 10mmol/L vitamin C solution with Extract solution into 0.4, 0.2, 0.1, 0.05, 0.025, 0.0125 mmol/L mg/mL vitamin C solution for use; If a positive control with a clearance rate of about 90% is needed, it is recommended to prepare10mmol/L vitamin C solution with Extract solution into vitamin C solution larger than 1.0 mmol/L for use.
3. Operation table: add the following reagents into 96 well plate or EP tube respectively:

Reagent name (μL)	Blank tube (B)	Test tube (T)	Control tube (C)	Positive control tube (PC)
Supernatant	-	10	10	-
VC solution of different	-	-	-	10
concentration				
Distilled water	10	-	-	-
Reagent IV working solution	20	20	-	20
Reagent IV working solution	170	170	-	170
ABTS working solution	-	-	190	-
After fully mixing, leave it in dark for 6 min at room temperature. Determine the absorbance at 405nm. The absorbance values of blank tube, control tube, positive control tube and test tube are recorded as A_B, A_C, A_{PC} and A_T respectively. The blank tube only needs to be tested 1-2 times				

Calculation

1. Calculation of ABTS Free Radical Scavenging Rate

1. Establishment of standard curve:
The formula of free radical scavenging rate of positive control:
ABTS free radical scavenging rate $D_{VC}\% = [(A_B-A_{PC}) \div A_B] \times 100\%$

2. Calculation formula of free radical scavenging rate of sample:
ABTS free radical scavenging rate $D\% = [A_B- (A_T-A_C)] \div A_B] \times 100\%$

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 pre experiment, and the clearance rate of the same concentration (the same dilution ratio) is compared.
2. It is recommended that samples be taken on the same day and tested on the same