Hydroxyl Radical Scavenging Capacity Assay Kit

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

Operation Equipment: Spectrophotometer

Catalog Number: BC1320

Size:50T/48S

Product Composition: Before use, please carefully check whether the volume of the reagent is consistent with the volume in the bottle. If you have any questions, please contact Solarbio staff in time.

Reagent name	Size	Storage
Extract Solution	Solution 55 mL×1	2-8℃
Reagent I	Solution 10 mL×1	2-8℃
Reagent II	Solution 20 mL×1	2-8℃
Reagent III	Solution 20 mL×1	2-8℃
Reagent IV	Solution 0.16 mL×1	2-8℃

Solution preparation:

1. Reagent IV: Place the reagent in the EP tube inside the reagent bottle. Add 9.84 mL of distilled water before use, mix thoroughly. You also can prepare in proportion when the Reagent will be used. Store at 2-8℃ for 4 weeks.

Product Description

Hydroxyl radical is a kind of free radical produced by human body in the course of metabolism, which is highly toxic and harmful to organisms. It can cause oxidative damage to carbohydrates, amino acids, proteins and nucleic acids in tissues, leading to cell necrosis or mutation. Hydroxyl radical scavenging capacity is one of the important indicators of antioxidant capacity of samples. It has been widely used in the research of antioxidant health products and medicines.

H_2O_2/Fe^{2+} generates hydroxyl radicals through Fenton reaction, and oxidizes Fe^{2+} into Fe^{3+} in the aqueous reagent of phenanthroline-Fe^{2+}, resulting in the decreased absorbance of 536 nm, and the inhibition of the decreased rate of absorbance of 536 nm, reflecting the ability of scavenging hydroxyl radicals of samples.

Reagents and Equipment Required but Not Provided.

Spectrophotometer, 1mL glass cuvette, balance, centrifuge, water bath/constant temperature incubator, adjustable pipette, mortar/homogenizer, ice and distilled water.

Procedure

I. Sample preparation

1. Tissue samples: Add 0.1 g of tissue to 1 mL of Extract solution on ice bath for homogenate, centrifuge at 10000 ×g and 4℃ for 10 min. Take supernatant on ice for test.
2. Serum, juice or other liquid samples can be measured directly. If the solution is turbid, centrifuge and remove the supernatant for measurement.
3. Extract (or drug) can be prepared in a certain concentration, such as 5 mg/mL.

II. Determination procedure

1. Preheat spectrophotometer for 30 min, adjust the wavelength to 536 nm, set the counter to zero with distilled water.
2. Operation table: add the following reagents to 1.5 mL EP tube.

	Blank Tube (A_B)	Control Tube (A_C)	Test Tube (A_T)
Reagent I (mL)	0.15	0.15	0.15
Reagent II (mL)	0.3	0.3	0.3
Reagent III (mL)	0.3	0.3	0.3
Mix thoroughly to prevent excessive color.			
Sample (mL)	-	-	0.15
Reagent IV (mL)	-	0.15	0.15
H_2O (mL)	0.75	0.60	0.45
Mix thoroughly, place at 37℃ for 60 min. Centrifuge at 10000 rpm for 10 min. Take the supernatant for test. Measure the absorbance at 536 nm. Denote the absorbance values of blank tube, control tube and test tube record as A_B, A_C and A_T. Test the control tube and blank tube only once or twice.			

III. Calculations

Hydroxyl Radical Scavenging rate D% = $(A_T-A_C) \div (A_B-A_C) \times 100\%$

Note:

1. In order to compare the hydroxyl radical scavenging capacity of different samples, it is necessary that adding the same amount of samples to the same batch of samples. Add the same volume of liquid samples to serum, tissue homogenate, juice and so on, and prepare the extract (or drug) to the same concentration.
2. When there are too many samples, the working solution can be prepared according to the ratio of Reagent I: Reagent II: Reagent III = 0.15:0.3:0.3, ready for use.

Examples:

1. Add 0.1g liver to 1mL extract solution and grind thoroughly, take supernatant, follow the determination procedure to operate, and calculate: Hydroxyl Radical Scavenging Rate D% =(AT-AC) ÷ (AB -AC) ×100%=(0.889-0.209)÷(0.959-0.209)×100%=90.67%.
2. Add 0.1g barnyardgrass (Echinochloa crus-galli (L.) Beauv.) to 1mL extract solution and grind thoroughly, take supernatant, follow the determination procedure to operate, and calculate: Hydroxyl Radical Scavenging Rate D% =(AT-AC) ÷ (AB -AC) ×100% = (0.796-0.209) ÷

(0.959-0.209)

×100%=78.27%.

3. Take 0.1g rabbit serum, follow the determination procedure to operate, and calculate: Hydroxyl Radical Scavenging Rate D% =(AT-AC) ÷ (AB -AC) ×100% = (0.629-0.209) ÷ (0.959-0.209) ×100%=56%.

Recent Product citations:

[1] Fan Y, Zhang Y, Shi K, Cheng S, Pei D, Shu X. Identification of a group of bisbenzylisoquinoline (BBIQ) compounds as ferroptosis inhibitors. Cell Death Dis. 2022 Nov 26;13(11):1000. doi: 10.1038/s41419-022-05447-8. PMID: 36435804; PMCID: PMC9701226.

[2] Zhang S, He Z, Cheng Y, Xu F, Cheng X, Wu P. Physicochemical characterization and emulsifying properties evaluation of RG-I enriched pectic polysaccharides from Cerasus humilis. Carbohydr Polym. 2021 May 15;260:117824. doi: 10.1016/j.carbpol.2021.117824. Epub 2021 Feb 16. Erratum in: Carbohydr Polym. 2021 Jul 15;264:118007. PMID: 33712165.

[3] Du Y, Zhang S, Sun-Waterhouse D, Zhou T, Xu F, Waterhouse GIN, Wu P. Physicochemical, structural and emulsifying properties of RG-I enriched pectin extracted from unfermented or fermented cherry pomace. Food Chem. 2023 Mar 30;405(Pt B):134985. doi: 10.1016/j.foodchem.2022.134985. Epub 2022 Nov 17. PMID: 36442238.

[4] Lin D, Yan R, Xing M, Liao S, Chen J, Gan Z. Fucoidan treatment alleviates chilling injury in cucumber by regulating ROS homeostasis and energy metabolism. Front Plant Sci. 2022 Dec 23;13:1107687. doi: 10.3389/fpls.2022.1107687. PMID: 36618644; PMCID: PMC9816408.

[5] Zhang L, Qu H, Xie M, Shi T, Shi P, Yu M. Effects of Different Cooking Methods on Phenol Content and Antioxidant Activity in Sprouted Peanut. Molecules. 2023 Jun 10;28(12):4684. doi: 10.3390/molecules28124684. PMID: 37375239; PMCID: PMC10300812.

Reference:

[1] Takeshi Nagai, Reiji Inoue, Hachiro Inoue. et al. Scavenging capacities of pollen extracts from cistus ladaniferus on autoxidation, superoxide radicals, hydroxyl radicals, and DPPH radicals [J]. Nutrition Research, 2002, 22(4): 519-526.

[2] Tsai CH, Stern A, Chiou JF. et al. Rapid and specific detection of hydroxyl radical using an ultraweak chemiluminescence analyzer and a low-level chemiluminescence emitter: application to hydroxyl radical-scavenging ability of aqueous extracts of Food constituents[J]. Journal of Agricultural and Food Chemistry, 2001, 49(5): 2137-2141.

Related Products:

BC1300/BC1305	Ceruloplasmin (CP) Assay Kit
BC1310/BC1315	Total antioxidant capacity (T-AOC) Assay Kit

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