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/microplate reader

Catalog Number: BC1325

Size:100T/96S

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 110 mL×1	2-8℃
Reagent I	Solution 8 mL×1	2-8℃
Reagent II	Solution 15 mL×1	2-8℃
Reagent III	Solution 15 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/microplate reader, micro glass cuvette/96 well plate, balance, low temperature 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/microplate reader for 30 min, adjust the wavelength to 536 nm, set spectrophotometer counter to zero with distilled water.
2. Operation table: add the following reagents to 1.5/0.5 mL EP tube.

Reagent	Blank Tube (A_B)	Control Tube (A_C)	Test Tube (A_T)
Reagent I (μL)	50	50	50
Reagent II (μL)	100	100	100
Reagent III (μL)	100	100	100
Mix immediately to prevent excessive color.			
Sample (μL)	-	-	50
Reagent IV (μL)	-	50	50
H_2O (μL)	100	50	-
Mix thoroughly, place at 37℃ for 60 min. Centrifuge at 10000 rpm for 10 min, Take 200 μL supernatant micro glass cuvette/96 well flat-bottom plate. Measure the absorbance value of 536 nm at once. Denote the absorbance values of blank tube, control tube and test tube record as AB, AC and AT. Test the control tube and blank tube only once or twice.			

III. Calculations

Hydroxyl Radical Scavenging rate D% = $(A_T-A_C)\div(A_B-A_C)\times100\%$

Note:

1. In order to compare the hydroxyl radical scavenging ability of different samples, an equal amount of samples must be added to the same batch of samples. Liquid samples such as serum, tissue homogenate, and juice must be added to the same volume, and extracts (or drugs) must be prepared at 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 = 0.05:0.1:0.1. Prepare when the solution will be used.

Examples:

1. Add 0.1g liver to 1mL extract solution and grind thoroughly, take supernatant, follow the determination procedure to operate, with 96 well plate to calculate: Hydroxyl Radical Scavenging Rate D% $=(A_T-A_C)\div(A_B-A_C)\times100\%=(0.77-0.222)\div(0.884-0.222)\times100\%=82.78\%$.
2. Add 0.1g barnyard grass (Echinochloa crus-galli (L.) Beauv.) to 1mL extract solution and grind thoroughly, take supernatant, follow the determination procedure to operate, with 96 well plate to calculate: Hydroxyl Radical Scavenging Rate D% $=(A_T-A_C)\div(A_B-A_C)\times100\%=(0.698-0.222)$

÷(0.884-0.222)×100%=71.9%.

3. Take 0.1g rabbit serum, follow the determination procedure to operate, with 96 well plate to calculate: Hydroxyl Radical Scavenging Rate D% =(A_T-A_C) ÷ (A_B -A_C) ×100% = (0.553-0.222) ÷ (0.884-0.222) ×100%=50%.

Recent Product citations:

[1] Tong MQ, Lu CT, Huang LT, Yang JJ, Yang ST, Chen HB, Xue PP, Luo LZ, Yao Q, Xu HL, Zhao YZ. Polyphenol-driven facile assembly of a nanosized acid fibroblast growth factor-containing coacervate accelerates the healing of diabetic wounds. Acta Biomater. 2023 Feb;157:467-486. doi: 10.1016/j.actbio.2022.11.054. Epub 2022 Nov 30. PMID: 36460288.

[2] Chen H, Liu Y, Zhang J, Jiang Y, Li D. Pectin extracted from dragon fruit Peel: An exploration as a natural emulsifier. Int J Biol Macromol. 2022 Nov 30;221:976-985. doi: 10.1016/j.ijbiomac.2022.09.069. Epub 2022 Sep 11. PMID: 36103906.

[3] Hu H, Yang J, Zhong Y, Wang J, Cai J, Luo C, Jin Z, Gao M, He M, Zheng L. Polydopamine-Pd nanozymes as potent ROS scavengers in combination with near-infrared irradiation for osteoarthritis treatment. iScience. 2023 Apr 18;26(5):106605. doi: 10.1016/j.isci.2023.106605. PMID: 37182095; PMCID: PMC10172781.

[4] Zhu X, Guo R, Su X, Shang K, Tan C, Ma J, Zhang Y, Lin D, Ma Y, Zhou M, Yang J, Wu Q, Sun J, Wang Z, Guo Y, Su R, Cui X, Han J, Lü Y, Yue C. Immune-enhancing activity of polysaccharides and flavonoids derived from Phellinus igniarius YASH1. Front Pharmacol. 2023 Apr 25;14:1124607. doi: 10.3389/fphar.2023.1124607. PMID: 37180713; PMCID: PMC10166811.

[5] Zhi D, Xu S, Zhang L, Li Y, Zhu H, Zhao C, Wang D. Shenqi formula delayed Alzheimer's disease-like symptoms by skn-1 pathway in Caernorhabditis elegans. J Ethnopharmacol. 2023 Nov 15;316:116741. doi: 10.1016/j.jep.2023.116741. Epub 2023 Jun 6. PMID: 37290734.

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
BC1370/BC1375	Total Sulfhydryl Assay Kit

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