Hydroxyl Radical Scavenging Capacity Assay Kit

Note: Take two or three different samples for prediction before test.

Operation Equipment: Spectrophotometer/microplate reader

Catalog Number: BC1325

Size:100T/96S

Components:

Extract Reagent: Liquid 110 mL×1. Storage at 4℃.

Reagent I: Liquid 8 mL×1. Storage at 4℃, avoid light.

Reagent II: Liquid 15 mL×1. Storage at 4℃.

Reagent III: Liquid 15 mL×1. Storage at 4℃.

Reagent IV: Liquid 0.1 mL×1. Storage at 4℃, avoid light. Add 9.84 mL of distilled water before use, mix thoroughly. You also can prepare when the Reagent will be used in proportion, store at 4℃ for one week.

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, constant temperature water bath, micro glass cuvette/96 well flat-bottom plate, centrifuge, mortar/homogenizer 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 and other liquid samples can be measured directly.
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 the counter to zero with distilled water.
2. Sample list: add the following reagents to 1.5/0.5 mL EP tube.

	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 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)÷ (A_B -A_C)×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. 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 flat-bottom plates to calculate: Hydroxyl Radical Scavenging Rate D% =(A_T-A_C) ÷ (A_B -A_C) ×100%=(0.77-0.222)÷(0.884-0.222)×100%=82.78%.
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, with 96-well flat-bottom plates to calculate: Hydroxyl Radical Scavenging Rate D% =(A_T-A_C) ÷ (A_B -A_C) ×100%=(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 flat-bottom plates 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] Hu J, Wang Q, Wang Y, et al. Polydopamine-Based Surface Modification of Hemoglobin Particles for Stability Enhancement of Oxygen Carriers[J]. Journal of Colloid and Interface Science, 2020.

[2] Liang R, Zhao J, Li B, et al. Implantable and degradable antioxidant poly (ε-caprolactone)-lignin nanofiber membrane for effective osteoarthritis treatment[J]. Biomaterials, 2020, 230: 119601.

[3] Yang Y, Liu M, Wang K, et al. Chemical and cytological evaluation of honeybee pollen antioxidant ability[J]. Journal of Food Science, 2020, 85(3): 824-833.

Related Products:

BC1300/BC1305 Ceruloplasmin (CP) Assay Kit

BC1310/BC1315 Total antioxidant capacity (T-AOC) Assay Kit

BC1370/BC1375 Total Sulfhydryl Assay Kit