Superoxide anion scavenging ability 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: BC1410

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 60 mL×1	2-8℃
Reagent I	Solution 3 mL×1	2-8℃
Reagent II	Powder×2	2-8℃
Reagent III	Solution 15 mL×1	2-8℃
Reagent IV	Solution 15 mL×1	2-8℃
Reagent V	Solution 15 mL×1	2-8℃

Solution preparation:

Reagent II: Dissolve each bottle thoroughly in 6mL of distilled water before use. Store at 2-8 ℃ for 4 weeks.

Product Description：

Reactive oxygen species such as superoxide anion in the organism have the functions of immunity and signal transduction, but if they accumulate too much, they will cause damage to cell membranes and biological macromolecules, resulting in abnormal metabolism of cells and tissues in the body, thereby causing a variety of diseases.

The AP-TEMED system produces superoxide anion, which reacts with hydroxylamine hydrochloride to form NO^{2-}, which reacts with p-aminobenzenesulfonamide and α-naphthylamine to form red azo compounds, with a characteristic absorption peak at 530nm. The scavenging ability of the sample towards superoxide anions is negatively correlated with the absorbance value at 530nm.

Reagents and Equipment Required but Not Provided：

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

Procedure

I. Sample preparation:

1. Tissue sample:

According to the proportion of tissue weight (g): Extract solution (mL) of 1:5-10 to extract. It is

suggested that 0.1 g of tissue with 1 mL of Extract solution and fully homogenized on ice bath. Centrifuge at 10000 ×g for 10 minutes at 4℃ to remove insoluble materials, and take the supernatant on ice before testing.

2. Serum: detect directly. If there is turbidity, take the supernatant after centrifugation for testing.
3. Cell/bacteria samples: collect cells/bacteria into centrifuge tubes, discard the supernatant, add 1 mL of
Extraction solution per 5 million cells, disrupt the cells by ultrasonic (power 200w, ultrasonic for 3s, 10s
interval, repeat 30 times), then 10000g, 4℃ , centrifuge for 10 min, take the supernatant and put it on ice for testing.

II. Determination procedure：

(1) Preheat spectrophotometer for 30 minutes, adjust wavelength to 530 nm, set zero with distilled water.

(2) Add reagents with the following list:

Reagent（μL）	Blank tube(b)	Test tube(t)
Reagent I	50	50
Reagent II	200	200
Mix well and react at 25°C for 1 min		
Distilled water	125	-
Sample	-	125
Reagent III	250	250
Mix well and react at 37°C for 30 min		
Reagent VI	250	250
Reagent V	250	250
Mix well, react at 37°C for 20 min, pipette 1mL into a 1mL glass cuvette, measure the absorbance values of the blank tube and the test tube at 530 nm, and record them as Ab and At. The blank tube only needs to be done 1-2 times.		

III. Calculations：

Superoxide anion scavenging rate (%) = (Ab-At)/Ab×100%

Note:

1. After the sample prepared, measure it immediately. Do not store the sample at low temperature for a long time to avoid affecting the measurement results.

Experimental example

1.Weigh 0.1 g of boxwood leaf tissue, add 1 mL of Extract solution, homogenize in an ice bath, centrifuge at 10,000 g at 4°C for 10 min; take the supernatant and place it on ice for testing. Use a 1 mL glass cuvette, follow the determination steps, calculate Ab= 0.666, At= 0.157, calculate according to the formula:

Superoxide anion scavenging rate = (0.666-0.157) ÷ 0.666 × 100% = 76.4%

2. Draw 125μl of goat serum, use a 1 mL glass cuvette, follow the measurement steps, calculate

Ab=0.666,

At=0.193, calculate according to the formula:

Superoxide anion scavenging rate = (0.666-0.193) ÷ 0.666× 100%=71.0%

Recent Product citations:

[1] Feng C, Xiong Z, Sun X, Zhou H, Wang T, Wang Y, Bai HX, Lei P, Liao W. Beyond antioxidation: Harnessing the CeO2 nanoparticles as a renoprotective contrast agent for in vivo spectral CT angiography. Biomaterials. 2023 Aug;299:122164. doi: 10.1016/j.biomaterials.2023.122164. Epub 2023 May 16. PMID: 37229807.

[2] Zhang X, Sun Y, Yang R, Liu B, Liu Y, Yang J, Liu W. An injectable mitochondria-targeted nanodrug loaded-hydrogel for restoring mitochondrial function and hierarchically attenuating oxidative stress to reduce myocardial ischemia-reperfusion injury. Biomaterials. 2022 Aug;287:121656. doi: 10.1016/j.biomaterials. 2022.121656. Epub 2022 Jun 28. PMID: 35792386.

[3] Li M, Bi D, Yao L, Yi J, Fang W, Wu Y, Xu H, Hu Z, Xu X. Optimization of preparation conditions and in vitro sustained-release evaluation of a novel nanoemulsion encapsulating unsaturated guluronate oligosaccharide. Carbohydr Polym. 2021 Jul 15;264:118047. doi: 10.1016/j.carbpol.2021.118047. Epub 2021 Apr 7. PMID: 33910749.

[4] Bi D, Li M, Yao L, Zhu N, Fang W, Guo W, Wu Y, Xu H, Hu Z, Xu X. Enhancement of the chemical stability of nanoemulsions loaded with curcumin by unsaturated mannuronate oligosaccharide. Food Chem. 2023 Jul 15;414:135670. doi: 10.1016/j.foodchem.2023.135670. Epub 2023 Feb 10. PMID: 36827777.

[5] Ding Y, Li Z, Hu W, Feng X, Chen Y, Yan G, Wang Y, Zhu B, Yao W, Zheng L, He M, Gao M, Zhao J. Carbazate-modified cross-linked dextran microparticles suppress the progression of osteoarthritis by ROS scavenging. Biomater Sci. 2021 Sep 14;9(18):6236-6250. doi: 10.1039/d1bm00743b. Erratum in: Biomater Sci. 2023 Mar 28;11(7):2603-2604. PMID: 34365495.

Related products

BC1320/BC1325	Hydroxyl Radical Scavenging Capacity Assay Kit
BC4750/BC4755	DPPH Free Radical Scavenging Capacity Assay Kit
BC4770/BC4775	ABTS Tree Radical Scavenging Activity Assay Kit
BC1310/BC1315	Total antioxidant capacity (T-AOC) Assay Kit (FRAP method)