

Superoxide anion scavenging ability Assay Kit

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

Operation Equipment: Spectrophotometer/Microplate reader

Catalog Number: BC1415

Size: 100T/96S

Components:

Extract solution: 110 mL×1, store at 2-8°C.

Reagent I: 1.3 mL×1, store at 2-8°C.

Reagent II : Powder×2, store at 2-8°C. Add 5.34 mL of distilled water and mix it to prepare before use. Unused reagents can be stored for 4 weeks at 2-8°C.

Reagent III: 6mL×1, store at 2-8°C.

Reagent IV: 6mL×1, store at 2-8°C.

Reagent V: 6mL×1, store at 2-8°C.

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 oxygen anions was negatively correlated with the absorbance at 530 nm.

Reagents and Equipment Required but Not Provided:

Spectrophotometer/microplate reader, desk centrifuge, Constant temperature incubator/water bath, pipette, micro glass cuvette/96 well plate, 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 $10000 \times g$ for 10 minutes at 4°C 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°C , centrifuge for 10 min, take the supernatant and put it on ice for testing.

II. Determination procedure:

1. Preheat spectrophotometer/microplate reader for 30 minutes, adjust wavelength to 530 nm, spectrophotometer set zero with distilled water.
2. Add reagents with the following list:

Reagent (μL)	Blank tube(b)	Test tube(t)
Reagent I	10	10
Reagent II	40	40
Mix well and react at 25°C for 1 min		
distilled water	25	
Sample		25
Reagent III	50	50
Mix well and react at 37°C for 30 min		
Reagent VI	50	50
Reagent V	50	50
Mix well, react at 37°C for 20min, pipette 200μL into micro glass cuvette/96 well plate, measure the absorbance values of the blank tube and the test tube at 530nm, 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. Take 0.1 g of boxwood leaf tissue, add 1 mL of extract solution, homogenize in ice bath, 10000 g, centrifuge for 10 min at 4°C; take the supernatant and place on ice for testing. Use 96 well plate to follow the determination steps, calculate Ab=0.762, At=0.311, Ac=0.088, calculate according to the formula:

Superoxide anion scavenging rate=(0.762-0.311)/0.762×100%=59.18%

2. Draw 25 μl goat serum, use 96 well plate to follow the determination steps, calculate Ab=0.762, At=0.342, calculate according to the formula:

Superoxide anion scavenging rate=(0.762-0.342)/0.762×100%=55.12%

Related products

BC1090/BC1095	Xanthine Oxidase(XOD) Activity Assay Kit
BC0690/BC0695	Glucose Oxidase (GOD) Activity Assay Kit
BC1270/BC1275	Protein Carbonyl Content Assay Kit
BC1280/BC1285	Diamine Oxidase(DAO) Activity Assay Kit