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Ver.251201

Superoxide Anion Assay Kit

BC2208-02(100T/96S)

FOR RESEARCH USE ONLY, DO NOT USE IT IN CLINICAL DIAGNOSIS

Product Description

Active oxygen such as superoxide anion in the living body has the functions of immunity and signal transduction. But if it accumulates too much, it will destroy the cell membrane and biomacromolecules, leading to abnormal metabolism of the cells and tissues of the body, and cause many diseases.

The superoxide anion reacts with hydroxylamine hydrochloride to form NO_2^- and the NO_2^- under the action of p-aminobenzenesulfonamide and naphthalene ethylenediamine hydrochloride is produced a red azo compound with a characteristic absorption peak at 530 nm. The content of O_2^- can be calculated according to the A_{530} value.

Kit components

Reagent	Volume	Storage
Extraction Reagent	110mL	2-8°C
Reagent I	10mL	2-8°C
Reagent II	7mL	2-8°C
Reagent III	7mL	2-8°C
Reagent IV	Requirid but not provided	-
Standard	1mL	2-8°C

Solution Preparation

- 1. Reagent IV:** self-prepared chloroform, about 12mL, stored at room temperature; An empty brown 30mL bottle is provided in the kit. Please label the reagent name by yourself.
- 2. Standard:** 10 $\mu\text{mol/mL}$ nitrite.
- 3. Preparation of 0.03125 $\mu\text{mol/mL}$ standard:** Take 100 μL 10 $\mu\text{mol/mL}$ sodium nitrite standard and add 900 μL distilled water to dilute it into 1 $\mu\text{mol/mL}$ standard; Then take 30 μL 1 $\mu\text{mol/mL}$ standard and dilute it with 930 μL distilled water to 0.03125 $\mu\text{mol/mL}$ standard for standard tube determination in the following operating table.

Reagents and Equipment Required but Not Provided

Spectrophotometer/Microplate reader, water-bath, balance, mortar/homogenizer/cell ultrasonic crusher, centriruge, microglass cuvette/96 well flat-bottom plate, chlorororm (>98%, AR), ice and distilled Water.

Operation Procedures

I. Sample Preparation

(The sample size to be tested can be adjusted appropriately, and the specific proportion can be referred to the literature.)

- 1. Plant and animal tissues:** Add Extract solution according to the ratio of tissue mass (g): Extract solution (mL) = 1:5~10 (it is recommended to weigh 0.1g sample and add 1.0mL. Extract solution), after ice bath homogenization, centrifuge at 4°C, 12000rpm for 10min, take supernatant and placed on the ice for test.
- 2. Bacteria or cell:** The ratio of bacteria/cell amount (10^6): the volume of Extract solution (mL) is 500-1000:1(it is suggested to take about 5 million bacteria/cells and add 1 mL of Extract solution). Bacteria/cell is split by ultrasonic (placed on ice. 200 W, work time 3s, interval 10s, Total 3min). Centrifuge at 12000rpm for 10 minutes at 4°C, take the supernatant and placed on the ice for test.
- 3. Serum or culture medium:** detect directly. (If the solution is turbid, centrifuge to take the supernatant and then measure)

II. Determination procedure:

1. Preheat spectrophotometer/microplate reader for 30 min, adjust the wavelength to 530 nm and set the counter to zero with distilled water.
2. Operation table: (Add the following reagents in turn to the 1.5mL EP tube)

Reagent name (μL)	Blank tube (A _B)	Test tube (A _T)	Standard tube (A _S)
Standard	-	-	100
Sample	-	100	-
Distilled water	100	-	-
Reagent I	80	80	80
Mix and react for 20 minutes at 37°C			
Reagent II	60	60	60
Reagent II	60	60	60
Mix and read for 20 minutes at 37°C			
Reagent IV	100	100	100
Mix well, centrifuge at 8000 rpm for 5 min at 5°C, carefully suck 200 μL of the upper water phase into micro glass cuvette/96 well flat-bottom plate, adjust zero with distilled water, measure the absorbance value at 530 nm, calculate the $\Delta A_S = A_S - A_B$, the $\Delta A_T = A_T - A_B$. Only one blank tube is needed for each experiment. The blank tube and standard tube only need to be measured 1-2 times.			

Calculation

1. Calculated according to the mass of the sample Superoxide anion content (μmol/g mass)

$$= 2 \times \Delta A_T \div (\Delta A_S \div C_S) \times V_{st} \div W$$

$$= 0.0625 \times \Delta A_T \div \Delta A_S \div W$$

$$\text{Rate of superoxide anion production } (\mu\text{mol/min/g mass}) = 2 \times \Delta A_T \div (\Delta A_S \div C_S) \times V_{st} \div W \div T$$

$$= 0.003125 \times \Delta A_T \div \Delta A_S \div W$$

2. Calculated by protein concentration

$$\text{Superoxide anion content } (\mu\text{mol /mg prot}) = 2 \times \Delta A_T \div (\Delta A_S \div C_S) \times V_s = (V_s \times C_{pr})$$

$$= 0.0625 \times \Delta A_T \div \Delta A_S \div C_{pr}$$

$$\text{Rate of superoxide anion production } (\mu\text{mol/min/mg prot}) = 2 \times \Delta A_T \div (\Delta A_S \div C_S) \times V_s \div (V_s \times C_{pr}) \div T$$

$$= 0.003125 \times \Delta A_T \div \Delta A_S \div C_{pr}$$

3. Calculated according to the volume of serum or culture medium

$$\text{Superoxide anion content } (\mu\text{mol /mL}) = 2 \times \Delta A_T \div (\Delta A_S \div C_S) = 0.0625 \times \Delta A_T \div \Delta A_S$$

$$\text{Rate of superoxide anion production } (\mu\text{mol/min/mL}) = 2 \times \Delta A_T \div (\Delta A_S \div C_S) \div T$$

$$= 0.003125 \times \Delta A_T \div \Delta A_S$$

4. Calculated by the number of bacteria or cells

$$\text{Superoxide anion content } (\mu\text{mol /}10^6\text{ cells}) = 2 \times \Delta A_T \div (\Delta A_S \div C_S) \times V_{st} \div N$$

$$= 0.0625 \times \Delta A_T \div \Delta A_S \div N$$

$$\text{Rate of superoxide anion production } (\mu\text{mol /min/}10^6\text{ cells}) = 2 \times \Delta A_T \div (\Delta A_S \div C_S) \div V_{st} \div N \div T$$

$$= 0.003125 \times \Delta A_T \div \Delta A_S \div N$$

Cs: Standard tube concentration, 0.03125 μ mol/m

Vs: sample volume added, 0.1 mL

Vst: volume used in the extraction process, 1mL

Cpr: sample protein concentration, mg/mL

W: Sample mass, g

N: Number of cells/bacteria, measured in 10^6

T: React time, 20 min

2: Two molecules of O₂ react to produce molecule of NO₂.

NOTE:

1. If the $\Delta A < 0.01$ it is recommended to increase the sample size or extend the reaction time of the first step after the determination; If the $\Delta A > 1.2$, it is recommended to dilute the sample for determination, and pay attention to the simultaneous modification of the calculation formula
2. 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.
3. Reagent IV has certain toxicity. Please take protective measures when operating. As the reagent has certain toxicity, please take protective measures during operation