

Ver.22.12

# **Superoxide Anion Activity Assay Kit**

BC2208-01 (50 Tests/48 Samples)

**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. Accumulation at high levels 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  $\text{NO}^{2-}$ , and the  $\text{NO}^{2-}$  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  $\text{O}^{2-}$  can be calculated according to the  $A_{530}$  value.

## Kit components

Reagent	Volume	Storage
Extract Solution	100mL × 1	4°C
Reagent I	32mL × 1	4°C
Reagent II	25mL × 1	4°C, in dark
Reagent III	25mL × 1	4°C, in dark
Reagent IV	Chloroform. To be arranged by the end user.	
Standard 10 μmol/mL $\text{NaNO}_2$	1mL × 1	4°C

## Reagents and Equipment Required but Not Provided

Constant temperature water bath, spectrophotometer, 1mL glass cuvette, chloroform and distilled water.

## Protocol

### I. Sample Preparation

**Plant and animal tissues:** Weigh about 0.1 g of sample, add 1 mL of Extract solution and fully grind. Centrifuge at 12000 rpm and 4°C for 20 minutes, then take 20 μL of supernatant to determine protein content, and the other supernatants as samples to be tested.

**Serum or culture medium:** Use directly.

### II. Assay procedure

- Preheat the spectrophotometer/microplate reader for 30 minutes, adjust wavelength to 530 nm and set zero with distilled water.
- Prepared standard solution: Take a proper amount of sodium nitrite standard solution, first dilute it 8 times to 0.625 μmol/mL, then dilute it to 0.3125, 0.15625, 0.078, 0.039, 0.0195, 0.009765, 0.0049, 0.00244, 0.0012, 0.0006 μmol/mL gradient standard solution, and use 0.3125, 0.15625, 0.078, 0.039, 0.0195, 0.0097625, 0.00244, 0.0006 μmol/mL standard tube as standard curve
- Perform the assay as given in the table below.

Reagent	Blank Tube (A <sub>b</sub> )	Standard tube (A <sub>s</sub> )	Test tube(A <sub>t</sub> )
Standard	-	0.2mL	-
Sample	-	-	0.2mL
Extract Solution	0.5mL	0.3mL	0.3mL
Reagent I	0.4mL	0.4mL	0.4mL
Mix thoroughly and incubate at 37°C for 20 minutes.			
Reagent II	0.3mL	0.3mL	0.3mL
Reagent III	0.3mL	0.3mL	0.3mL
Mix thoroughly and incubate at 37°C for 20 minutes.			
Reagent IV	0.5mL	0.5mL	0.5mL
Mix well, centrifuge at 8000 rpm for 5 min at 25°C, carefully pipette 1 mL of the upper water phase into 1 mL glass cuvette, 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.			

## Calculations

1. According to concentration of standard solution and absorbance to create the standard curve, take standard solution as X-axis,  $\Delta A_S$  as Y-axis. Take  $\Delta A_T$  into the equation to obtain x (mg/mL).

2. Calculation of superoxide anion content

Take  $\Delta A$  sample into the equation to get x value ( $\mu$  mol/mL)

(1) Calculated according to the fresh weight of the sample

The content of superoxide anion ( $\mu$ mol/g fresh weight)

$$= 2x \times V_S \div (V_S \div V_E \times W) = 2x \div W.$$

The production rate of superoxide anion ( $\mu$ mol/min/g fresh weight)

$$= 2x \times V_S \div (V_S \div V_E \times W) \div T = 0.1x \div W.$$

(2) Calculated by protein concentration

Superoxide anion content ( $\mu$  mol/mg prot)  $= 2x \times V_S \div (V_S \times C_{pr}) = 2x \div C_{pr}.$

The production rate of superoxide anion ( $\mu$ mol/min/mg prot)

$$= 2x \times V_S \div (V_S \times C_{pr}) \div T = 0.1x \div C_{pr}.$$

(3) Calculated according to the volume of serum or culture medium

Superoxide anion content ( $\mu\text{mol/mL}$ ) =  $2x$

The production rate of superoxide anion ( $\mu\text{ mol/min/mL}$ ) =  $2x \div T = 0.1x$

Vs: sample volume added, 0.2 mL;

Vst: volume used in the extraction process, 1 mL;

Cpr: sample protein concentration, mg/mL;

W: Fresh weight of sample, g;

T: React time, 20 minutes.

### Note

- Dilute sample with extract solution if OD>1.0. The sample shall be diluted properly and determined. Pay attention to multiply the dilution times in the calculation formula
- 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.
- Reagent IV has certain toxicity. Please take protective measures when operating.