



Ver.250401

## **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 A530 value.

## Kit components

Reagent	Volume	Storage
Extract Solution	100mL × 1	4°C
Reagent I	32mL × 1	4°C
Reagent II	32.5mL × 1	4°C, in dark
Reagent III	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 / microplate reader, micro glass cuvette / 96-well flat bottom plates and distilled water.

## Protocol

### I. Sample Preparation

**Tissue:** Add 1mL Extract Solution to 0.1g tissue. Homogenate in ice and centrifuge at 12000 rpm at 4°C for 20 minutes. Supernatant is used for the assay (Determine protein concentration of the supernatant).

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

### II. Assay procedure

- Preheat the spectrophotometer/microplate reader for 30 min, adjust wavelength to 530 nm and set zero with distilled water.
- Preparation of standards: Dilute the standard provided with the kit 8 times to get a concentration of 0.625 μmol/ml (Working standard). Serially dilute the working standard to get concentration of 0.3125, 0.15625, 0.078, 0.039, 0.0195, 0.009765, 0.0049, 0.00244, 0.0012, 0.0006 μmol/ml respectively. Use these standard dilutions to prepare a standard graph.
- Perform the assay as given in the table below.

Reagent	Blank Tube (B)	Standard tube (S)	Test tube(T)
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 min.			
Reagent II	0.6mL	0.6mL	0.6mL
Mix thoroughly and incubate at 37°C for 20 min.			
Reagent III	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. Prepare a standard graph with standard concentration in x-axis and absorption ( $\Delta A_S$ ) in y-axis. Find out the concentration ( $\mu\text{mol/mL}$ ) of the test sample by extrapolating  $\Delta A_T$  in the standard graph. Unknown sample concentration obtained from the standard graph is referred as 'x' in the following calculations.

2. Protein concentration:

$$\text{Superoxide anion content } (\mu\text{mol/mg protein}) = 2x \div \text{Cpr}$$

$$\text{Rate of superoxide anion production } (\mu\text{mol/min/mg protein}) = 0.1x \div \text{Cpr}$$

3. Sample weight:

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

$$\text{Rate of superoxide anion production } (\mu\text{mol/min/g}) = 0.1x \div W$$

4. Liquid samples (serum)

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

$$\text{Rate of superoxide anion production } (\mu\text{mol/min/g}) = 0.1x$$

Cpr: Sample protein concentration (mg/mL)

W: Sample weight in grams.

## Note

- Dilute sample with Extract Reagent if O.D>1.0. Multiply by the dilution factor while calculation.
- It is recommended to use fresh samples. Stored samples may not give proper results.