

Ver. 250401

# **Superoxide Dismutase (SOD) Activity Assay Kit**

BC3301-02 (100 Tests/48 Samples)

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

## Product Description

Superoxide dismutase is widely found in animals, plants, microorganisms and cultured cells. It catalyzes the superoxide anion to form  $\text{H}_2\text{O}_2$  and  $\text{O}_2$ . SOD is not only the superoxide anion scavenging enzyme, but also the main  $\text{H}_2\text{O}_2$  producing enzyme, which plays an important role in the biological antioxidant system.

Superoxide anion ( $\text{O}_2^-$ ) is produced by the EDTA, Beta-mercaptoethanol and Manganese (II) Chloride reaction system.  $\text{O}_2^-$  in the system oxidises NADPH, thereby reducing absorbance in 340nm. SOD can remove  $\text{O}_2^-$  and inhibit the oxidation of NADPH. SOD activity is proportional to the absorbance.

## Kit components

Reagent	Volume	Storage
Extraction Solution	110mL $\times$ 1	2 - 8°C
Reagent I	50mL $\times$ 1	2 - 8°C
Reagent II	Powder $\times$ 1	2 - 8°C
Reagent IIIA	1mL $\times$ 1	RT
Reagent IIIB	1mL $\times$ 1	RT
Diluent	10mL $\times$ 1	2 - 8°C

## Reagent Preparations

### Preparation of Reagent II

- Dissolve in 6mL Diluent. Prepare aliquots and store at -20°C in dark.

### Reagent III Working Solution

- Mix Reagent IIIA, Reagent IIIB and distilled water in the ratio 1:1:3. Example; to prepare 2mL Working Solution, add 0.4mL Reagent IIIA and 0.4mL Reagent IIIB and 1.2mL distilled water.
- Always prepare fresh according to the number of samples to be assayed.
- Working solution should not be stored for future use.

### Reagent IV [Beta-mercaptoethanol (40mM)]

- Dilute Beta-mercaptoethanol (not provided with the kit) in distilled water to achieve a final concentration of 40mM.
- Take 7 $\mu$ L Beta-mercaptoethanol (14.3M) and make up the volume to 2.5mL with distilled water to achieve final concentration of 40mM.
- Always prepare fresh in a dark tube or bottle.

## Reagents and Equipment Required but Not Provided

UV-Spectrophotometer, table top centrifuge, micro quartz cuvette/96 well UV flat-bottom plate, mortar/ homogenizer/ ultrasonicator, ice and distilled water.

## Protocol

### I. Sample preparation

**Tissue:** Add 1mL Extraction solution to 0.1g tissue. Homogenate in ice and centrifuge at 8000 rpm at 4°C for 10 minutes. Take the supernatant and place it on ice for assay.

**Bacteria or cells:** Add 1mL Extraction solution to 5 million cells. Subject to ultrasonication while keeping the samples in an ice bath (power 200W, sonication 3 seconds, interval 7 seconds for 3 minutes). Centrifuge at 8000 rpm at 4°C for 10 minutes. Take the supernatant and place it on ice for assay. (If the supernatant is not clear, centrifuge for 3 minutes more).

**Serum:** Use directly.

## II. Assay procedure

- Preheat the spectrophotometer for 30 minutes, adjust wavelength to 340nm and set zero with distilled water.
- Perform the subsequent assay in a 1ml quartz cuvette centrifuge tube. Add the reagents in the order as mentioned in the list below.

Reagent	Test tube (T)	Control tube (C)
Sample (supernatant/serum)	20μL	-
Extraction Solution	-	20μL
Reagent I	160μL	160μL
Reagent II	12μL	12μL
Reagent III Working Solution	5μL	5μL

- Mixing and keep at room temperature for 5 minutes
- Record the absorbance at 340nm.
  - This corresponds to  $T_1$  and  $C_1$ .
- Add 25μL Reagent IV and mix well.
- Keep at room temperature for 20 minutes.
- Record the absorbance at 340nm.
  - This corresponds to  $T_2$  and  $C_2$ .
- $\Delta A_T = T_1 - T_2$ ;  $\Delta A_C = C_1 - C_2$ .

## Calculation

### 1. Calculation of inhibition percentage:

$$\text{Inhibition Percentage (P)} = [\Delta A_T / \Delta A_C] \times 100\%$$

$$\text{Inhibition Factor (I)} = [\Delta A_T / \Delta A_C]$$

It is ideal to keep the inhibition percentage of the sample is within the range of 30-70%, and the closer it is to 50%, the more accurate it is. If inhibition percentage is less than 30% or more than 70%, it is usually necessary to adjust sample volume or sample preparation. If inhibition percentage is high, the sample should be diluted properly. If inhibition percentage is low, the sample with high concentration should be prepared again.

### 2. Calculation of SOD activity:

#### a) Protein concentration

Unit definition: One unit of enzyme is defined as the amount of enzyme which inhibits 50% of NADPH oxidation in the reaction system for every milligram of protein.

$$\begin{aligned}\text{SOD (U/mg protein)} &= [P \div (1-I) \times V_{rv}] \div (V_s \times C_{pr}) \times F \\ &= [10 \times P \div (1-I) \div C_{pr}] \times F\end{aligned}$$

#### b) Sample weight:

Unit definition: One unit of enzyme is defined as the amount of enzyme which inhibits 50% of NADPH oxidation in the reaction system for every gram of sample.

$$\begin{aligned}\text{SOD (U/g weight)} &= [P \div (1-I) \times V_{rv}] \div (W \times V_s \div V_{sv}) \times F \\ &= [10 \times P \div (1-I) \div W] \times F\end{aligned}$$

#### c) Cells

Unit definition: One unit of enzyme is defined as the amount of enzyme which inhibits 50% of NADPH oxidation in the reaction system for every  $10^4$  cells.

$$\begin{aligned}\text{SOD (U/}10^4\text{ cells)} &= [P \div (1-I) \times V_{rv}] \div (500 \times V_s \div V_{sv}) \times F \\ &= [0.02 \times P \div (1-I) \div N] \times F\end{aligned}$$

#### d) Liquids (Serum/Plasma)

Unit definition: One unit of enzyme is defined as the amount of enzyme which inhibits 50% of NADPH oxidation in the reaction system for every millilitre of liquid.

$$\begin{aligned}\text{SOD (U/mL)} &= [P \div (1-I) \times V_{rv}] \div V_s \times F \\ &= [10 \times P \div (1-I)] \times F\end{aligned}$$

Vrv : Total reaction volume, 0.2 mL  
Vs : Sample volume, 0.02 mL  
Vsv : Extraction volume, 1 mL  
Cpr : Sample protein concentration, mg/mL  
W : Sample weight, g  
500 : Total number of bacteria and cells, 5 million.  
P : Inhibition percentage, %  
I : Inhibition Factor  
F : Sample dilution multiple

## Note

- The Sample and Reagent II should be placed on ice when using.
- It is recommended not to perform assay with too many samples, as increase in incubation time will result in inaccurate results.
- Precipitates may form at the last step of the assay. This is to be dissolved by mixing before measuring the absorption.