



ISO 13485:2016 ISO 9001:2015

Ver.240105

Superoxide Dismutase (SOD) Activity Assay Kit

BC1101-02 (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 H_2O_2 and O_2 . SOD is not only the superoxide anion scavenging enzyme, but also the main H_2O_2 producing enzyme, which plays an important role in the biological antioxidant system.

Superoxide anion (O_2^-) is produced by the xanthine and xanthine oxidase reaction system. O_2^- can reduce blue tetrazole to form blue formazan, which has absorbance in 560 nm. SOD can remove O_2^- and inhibit the formation of methionine. The blue colour has a absorption maxima at 560nm. SOD activity is inversely proportional to the absorbance.

Kit components

Reagent	Volume	Storage
Extraction Solution	110mL \times 1	4°C
Reagent I	5mL \times 1	4°C
Reagent II	100 μ L \times 1	4°C
Dilute 10 times in distilled water before use.		
Reagent III	4mL \times 1	4°C
Reagent IV	0.25mL \times 1	4°C
Dilute 5 times in distilled water before use.		

Reagents and Equipment Required but Not Provided

Constant temperature water bath, cooling centrifuge, Spectrophotometer/microplate reader, micro glass cuvette/96 well flat-bottom plate and distilled water.

Protocol

I. Sample preparation

Tissue: Add 1mL Extract 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 Extract 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 20 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/microplate reader for 30 min, adjust wavelength to 560nm and set zero with distilled water.
- Keep Reagent I, Reagent III, Reagent V in water bath for more than 5 minutes at 37°C(mammal) or 25°C (other species).
- Perform the subsequent assay in a 1.5mL micro centrifuge tube. Add the reagents in the order as mentioned in the list below.

Note: Calculate number of samples and the total volume of Reagent II required for the assay. Dilute Reagent II 10 times with distilled water before use. Prepare just enough for the assay. Do not store diluted Reagent II.

Dilute Reagent IV, 5 times with distilled water. Prepare enough for the assay. Do not store and reuse diluted Reagent IV

Reagent	Test tube (T)	Control tube (C)	Blank tube (B1)	Blank tube (B2)
Sample (supernatant/serum)	20μL	20μL	-	-
Reagent I	45μL	45μL	45μL	45μL
Diluted Reagent II	20μL	-	20μL	-
Reagent III	35μL	35μL	35μL	35μL
Distilled water	70μL	90μL	90μL	110μL
Diluted Reagent IV	10μL	10μL	10μL	10μL

- Mix thoroughly and incubate at room temperature for 30 minutes.
- Add the mixture to a 96 well flat bottom plate or a micro glass cuvette and measure absorption at 560nm.
- The absorbance $\Delta A_T = A_T - A_C$
 $\Delta A_B = A_{B1} - A_{B2}$.

Calculation

1. Calculation of inhibition percentage:

Inhibition percentage (P) = $[(\Delta A_B - \Delta A_T) / \Delta A_B] \times 100\%$

Inhibition Factor (I) = $[(\Delta A_B - \Delta A_T) / \Delta A_B]$

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 catalyzes an inhibition of 50% in the reaction system for every milligram of protein.

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

$$= [10 \times P \div (1-I) \div C_{pr}] \times F$$

b) Sample weight:

Unit definition: One unit of enzyme is defined as the amount of enzyme which catalyzes an inhibition of 50% in the reaction system for every gram of sample.

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

$$= [10 \times P \div (1-I) \div W] \times F$$

c) Cells

Unit definition: One unit of enzyme is defined as the amount of enzyme which catalyzes an inhibition of 50% in the reaction system for every 10^4 cells.

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

$$= [0.02 \times P \div (1-I)] \times F$$

d) Liquids (Serum/Plasma)

Unit definition: One unit of enzyme is defined as the amount of enzyme which catalyzes an inhibition of 50% in the reaction system for every millilitre of liquid.

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

$$= [10 \times P \div (1-I)] \times F$$

V_{rv} : Total reaction volume, 0.2 mL
V_s : Sample volume, 0.02 mL
V_{sv} : Extraction volume, 1 mL
C_{pr} : 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.