



ISO 13485:2016 ISO 9001:2015

Ver. 240802

Superoxide Dismutase (SOD) Assay Kit

BC3301-01 (50 Tests/24 Samples)

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

Product Description

Superoxide dismutase is a kind of metalloenzyme widely found in organism. It is an important oxygen radical scavenger and can catalytic disproportionation of 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 darker the blue color of the reaction solution, the lower the activity of SOD. The lighter the blue color of the reaction solution, the higher the activity of SOD.

Kit components

Reagent	Volume	Storage
Extraction Reagent	60 mL \times 1	2-8°C
Reagent I	15 mL \times 1	2-8°C
Reagent II	160 μL \times 1	2-8°C
Reagent III	11 mL \times 1	2-8°C
Reagent IV	0.5 mL \times 1	2-8°C

Solution Preparation

1. **Reagent II:** Mix by pipetting after centrifugation.
2. **Reagent II working solution:** Mix Reagent II: distilled water=30 μL : 270 μL (300 μL , about 5 S) according to sample number before use.
3. **Reagent IV working solution:** Mix Reagent IV: distilled water=60 μL : 240 μL (300 μL , about 10T) according to sample number before use.

Reagents and Equipment Required but Not Provided

Spectrophotometer, table centrifuge, transferpettor, mortar/homogenizer/cell ultrasonic crusher, 1mL glass cuvette, ice and distilled water.

Protocol

I. Sample preparation

Bacteria or cells: collecting bacteria or cells into the centrifuge tube, discard supernatant after centrifugation. According to the proportion of bacteria or cells (10^4): extraction solution volume (mL) of 500-1000:1 to extract. It is suggested that 5 million of bacteria or cells with 1mL of Extraction reagent. Splitting the bacteria or cells with ultrasonication (place on ice, ultrasonic power 200W, working time 3second, interval 10second, repeat for 30 times). Centrifuge at 8000 $\times g$ for 10 minutes at 4°C to remove insoluble materials and take the supernatant on ice before testing.

Tissue: according to the proportion of tissue weight (g): Extraction solution volume (mL) of 1:5-10 to extract. It is suggested that 0.1 g of tissue with 1 mL of Extraction reagent and fully homogenized on ice bath. Centrifuge at 8000 ×g for 10 minutes at 4°C to remove insoluble materials and take the supernatant on ice before testing.

Serum (plasma) sample: detect sample directly. Centrifuge before detect if there are precipitation.

II. Determination procedure:

1. Preheat the spectrophotometer for 30 minutes, adjust wavelength to 560 nm and set zero with distilled water.
2. Keep Reagent I, Reagent III, Reagent IV working solution for more than 5 minutes at 37°C.
3. Add reagents with the following list.

Reagent(μL)	Test tube (T)	Control tube (C)	Blank tube (B1)	Blank tube (B2)
Sample	90	90	-	-
Reagent I	240	240	240	240
Reagent II working solution	60	-	60	-
Reagent III	180	180	180	180
Distilled water	400	460	490	550
Reagent IV working solution	30	30	30	30

Mix thoroughly and the mixture is incubated at 37°C for 30 minutes. Add the mixture into 1mL glass cuvette and detect the absorbance value of each tube at 560 nm. $\Delta A_T = A_T - A_C$, $\Delta A_B = A_{B1} - A_{B2}$.

If there is precipitation at the bottom, mix thoroughly and then measure. (Blank tube only need to be measured once or twice, and each test tube needs one control tube)

Calculation

1. Inhibition percentage

$$\text{Inhibition percentage (P)} = [\Delta A_B - \Delta A_T] \div \Delta A_B \times 100\%$$

$$\text{Inhibition Factor (I)} = [\Delta A_B - \Delta A_T] \div \Delta A_B$$

The inhibition percentage should be in 30%~70% (the value close to 50 % will have a more accurate result). If the calculated inhibition percentage is less than 30% or more than 70%, it is usually necessary to adjust the sample addition amount and re-determine. If the percentage of inhibition is too high, the sample should be diluted properly. If the percentage of inhibition is too low, the sample should be reprepared with a higher concentration and reduce the distilled water volume at the same time.

2. Unit definition:

One unit of enzyme activity is defined as the amount of enzyme catalyzes the inhibition of 50% in the reaction system of the above xanthine oxidase.

3. Calculation

A. Serum (plasma) sample

$$\begin{aligned}\text{SOD activity (U/mL)} &= [P \div (1-I) \times V_{rv}] \div 1\text{mL} \div V_s \times F \\ &= 11.11 \times P \div (1-I) \times F\end{aligned}$$

B. Tissue, bacteria or cultured cells

a) Protein concentration:

$$\begin{aligned}\text{SOD activity (U/mL prot)} &= [P \div (1-I) \times V_{rv}] \div 1\text{mL} \div (V_s \times C_{pr}) \times F \\ &= 11.11 \times P \div (1-I) \div C_{pr} \times F\end{aligned}$$

b) Sample weight

$$\begin{aligned}\text{SOD activity (U/g weight)} &= [P \div (1-I) \times V_{rv}] \div 1\text{mL} \div (W \times V_s \div V_{sv}) \times F \\ &= 11.11 \times P \div (1-I) \div W \times F\end{aligned}$$

c) Bacteria or cell amount

$$\begin{aligned}\text{SOD activity (U/10}^4\text{ cell)} &= [P \div (1-I) \times V_{rv}] \div 1\text{mL} \div (N \times V_s \div V_{sv}) \times F \\ &= 11.11 \times P \div (1-I) \div N \times F\end{aligned}$$

Vrv: Total reaction volume, 1 mL
Vs: Sample volume, 0.09 mL
Vsv: Extraction volume, 1 mL
Cpr: Sample protein concentration, mg/mL
W: Sample weight, g
N: Total number of bacteria and cells, count by 10^4
P: Inhibition percentage, %
F: Sample dilution multiple
1mL: the volume of reaction.

Note:

- The Sample and Reagent II working solution should be placed on ice when using.
- When there are many samples, the working solution (including Reagent I, Reagent II working solution, Reagent III and distilled water) can be configured according to the table. Reagent IV working solution must be added finally.
- After the reaction completed, there may be precipitation formed, which can be determined after mixing