

## Superoxide Dismutase (SOD) Activity Assay Kit with WST-1

**Note:** It is necessary to predict 2-3 large difference samples before the formal determination.

**Operation Equipment:** Spectrophotometer

**Cat No:** BC5160

**Size:** 50T/24S

### Components:

**Extraction reagent:** 30 mL×1. Storage at 2-8°C.

**Reagent I:** 15 mL×1. Storage at 2-8°C.

**Reagent II:** 40 μL×1. Storage at 2-8°C.

**Preparation of Reagent II-working solution:** Reagent II is diluted 50 times with sterile water before use according to the sample number and used up on the same day.

**Reagent III:** 11 mL×1. Storage at 2-8°C.

**Reagent IV:** 0.3 mL ×1. Storage at 2-8°C.

**Preparation of Reagent TV-working solution:** Reagent IV was diluted 10 times with distilled water before use according to the sample number and used up on the same day.

### Product Description:

Superoxide dismutase (SOD, EC 1.15.1.1) 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<sub>2</sub>O<sub>2</sub> and O<sub>2</sub>. SOD is not only the superoxide anion scavenging enzyme, but also the main H<sub>2</sub>O<sub>2</sub> producing enzyme, which plays an important role in the biological antioxidant system.

Superoxide anion (O<sub>2</sub><sup>-</sup>) is produced by xanthine and xanthine oxidase reaction system. O<sub>2</sub><sup>-</sup> can reduce tetrazolium-1(WST-1) to form a water-soluble yellow formazan dye, which has absorbance in 450 nm. SOD can remove O<sub>2</sub><sup>-</sup> and inhibit the formation of the formazan dye. The darker the yellow color of the reaction solution, the lower the SOD activity. The lighter the yellow color of the reaction solution, the higher the activity of SOD.

### Reagents and Equipment Required but Not Provided:

Spectrophotometer, table centrifuge, water bath/constant temperature foster box, transferpettor, 1 mL glass cuvette, mortar/homogenizer, ice and distilled water.

### Operation steps:

#### I. Sample preparation:

1. Bacteria or cells: collect bacteria or cells into the centrifuge tube, discard supernatant after centrifugation. According to the proportion of bacteria or cells (10<sup>4</sup> cells): extraction solution volume (mL) of 500-1000:1 to extract. It is suggested that 5 million of bacteria or cells amount with 1mL of Extraction reagent. Splitting the bacteria or cells with ultrasonication (placed on ice, ultrasonic power 200W, working time 3s, interval 10s, repeat for 30 times). Centrifuge at 8000 ×g for 10 minutes at 4°C to remove insoluble materials, and take the supernatant on ice before testing.
2. 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.

3. 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 450 nm and set zero with distilled water.
2. Keep Reagent I, Reagent III, Reagent IV-working solution in water bath for more than 5 minutes at 37°C(mammals) or 25°C (other species).
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	225	225	225	225
Reagent II-working solution	100	-	100	-
Reagent III	175	175	175	175
Distilled water	360	460	450	550
Reagent IV-working solution	50	50	50	50

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 450 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 tubes need to test once or twice and every test tube need a contrast tube.

## III. Calculation:

1. Inhibition percentage:

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

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 reducing 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

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

### B. Tissue, bacteria or cultured cells

1. Protein concentration:

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

2. Sample weight

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

3. Bacteria or cell amount

$$\text{SOD activity (U/10}^4 \text{ cell)} = [P \div (1-P) \times V_{rv}] \div (500 \times V_s \div V_{sv}) \times F = 0.0222 \times P \div (1-P) \times F$$

$V_{rv}$ : Total reaction volume, 1 mL;

$V_s$ : Sample volume, 0.09 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, %;

$F$ : Sample dilution multiple.

**Note:**

1. The Sample and Reagent II should be placed on ice when using.
2. When there are many samples, the working solution (including Reagent I, II-working solution, III and distilled water) can be configured according to the table. Reagent IV-working solution must be added finally.

**Experimental Examples:**

1. 0.1 g of *Trifolium incarnatum* is added into 1 mL of Extraction reagent for homogenization. After the supernatant is taken and diluted 50 times, the operation is carried out according to the determination steps. The results showed that  $\Delta A_T = A_T - A_C = 0.6408 - 0.0577 = 0.5831$ ,  $\Delta A_B = A_{B1} - A_{B2} = 0.8505 - 0.0542 = 0.7963$ . Inhibition percentage =  $(\Delta A_B - \Delta A_T) \div \Delta A_B \times 100\% = 26.77\%$ , and the enzyme activity is calculated according to the sample weight.

$$\text{SOD activity (U/g weight)} = 11.11 \times \text{Inhibition percentage} \div (1 - \text{Inhibition percentage}) \div W \times F = 2030.6 \text{ U/g}$$

2. 1 mL of Extraction reagent is added to 0.1 g of mice kidney for homogenization. After the supernatant is taken and diluted 50 times, the operation is carried out according to the determination steps. The results showed that  $\Delta A_T = A_T - A_C = 0.3921 - 0.0591 = 0.3330$ ,  $\Delta A_B = A_{B1} - A_{B2} = 0.8505 - 0.0542 = 0.7963$ , inhibition percentage =  $(\Delta A_B - \Delta A_T) \div \Delta A_B \times 100\% = 58.18\%$

$$\text{SOD activity (U/g weight)} = 11.11 \times \text{Inhibition percentage} \div (1 - \text{Inhibition percentage}) \div W \times F = 7728.1 \text{ U/g}$$

**References:**

- [1] Peskin A V, Winterbourn C C. A microtiter plate assay for superoxide dismutase using a water-soluble tetrazolium salt (WST-1) [J]. *Clinica chimica acta*, 2000, 293(1-2):157-166ates.
- [2] Hou Z, Zhao L, Wang Y, et al. Purification and characterization of superoxide dismutases from sea

buckthorn and chestnut rose[J]. Journal of food science, 2019, 84(4): 746-753.

**Related Products:**

- BC0190/BC0195 Polyphenol Oxidase (PPO) Activity Assay Kit
- BC0210/BC0215 Phenylalanine Ammonialyase (PAL) Activity Assay Kit
- BC0200/BC0205 Catalase (CAT) Activity Assay Kit
- BC0090/BC0095 Peroxidase (POD) Activity Assay Kit