

Superoxide Dismutase (SOD) Typed Activity Assay Kit with WST-1 (Total SOD, Cu/Zn SOD, Mn SOD Activity)

Note: The reagents have been changed, please be aware of and follow this instruction strictly.

Operation Equipment: Spectrophotometer/Microplate reader

Catalog Number: BC5255

Size: 100T/48S

Components:

Extraction Reagent I: Liquid 110 mL×1. Store at 2-8°C.

Extraction Reagent II: Liquid 18 mL×1. Store at 2-8°C.

Reagent I: Liquid 10 mL×1. Store at 2-8°C.

Reagent II: Liquid 50 μL×1. Store at 2-8°C. Centrifuge to the bottom of the tube using a palm centrifuge before using and then blowing to mix;

Reagent II working solution: according to the number of samples according to the ratio of reagent 2: distilled water = 5 μL: 245 μL (a total of 250 μL, can do about 12 Cu-Zn-SOD samples or 6 Mn-SOD samples) to prepare reagent II working solution, ready to use;

Reagent III: Liquid 8 mL×1. Store at 2-8°C.

Reagent IV: Liquid 0.5 mL×1. Store at 2-8°C.

Reagent IV working solution: Prepare the working solution according to the ratio of Reagent IV: distilled water = 20μL: 180μL (200μL in total, about 20T) according to the sample volume, and ready to use.

Product Description:

Superoxide dismutase (SOD, EC 1.15.1.1) is widely found in animals, plants, microorganisms and cultured cells. SOD represents a group of enzymes that use as cofactor copper and zinc, or manganese, or iron ions. Cu/Zn SOD is located in the cytoplasm, and Mn SOD in the mitochondria. It catalyzes the superoxide anion to form H₂O₂ and O₂. SOD is not only the superoxide anion scavenging enzyme, but also the main H₂O₂ producing enzyme, which plays an important role in the biological antioxidant system.

Superoxide anion (O₂⁻) is produced by xanthine and xanthine oxidase reaction system. O₂⁻ can reduce water-soluble tetrazolium-1(WST-1) to form a yellow formazan dye, which has absorbance in 450 nm. SOD can remove O₂⁻ 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. Cu/Zn SOD activity unchanged and Mn SOD activity inactivated after treated. Mn SOD activity could be calculated by determination of total SOD activity and Mn SOD activity.

Reagents and Equipments Required but Not Provided:

Spectrophotometer/microplate reader, centrifuge, constant temperature foster box/water-bath, vortex mixer/oscillator, transferpettor, micro glass cuvette/96 well flat-bottom plate, mortar/homogenizer/cell ultrasonic crusher, ice and sterilized water.

Operation steps:

I. Sample preparation:

1. Total SOD activity

- 1) **Bacteria or cells:** collect bacteria or cells into the centrifuge tube, discard supernatant after centrifugation. According to the proportion of bacteria or cells number (10^4): Extraction reagent I volume (mL) of 500-1000-1 to extract. It is suggested that 5 million of bacteria or cell amount with 1mL of Extraction reagent I. Split the bacteria or cell 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 reagent I volume (mL) of 1:5-10 to extract. It is suggested that 0.1 g of tissue with 1 mL of Extraction reagent I 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.

2. Cu/Zn SOD activity

- 1) Take the supernatant from the previous step. According to the proportion of supernatant volume (mL): Extraction reagent II volume (mL) of 2:3 to mix. It is suggested that 0.2 mL of supernatant with 0.3 mL of Extraction reagent II and fully mixed for 1 minute. Centrifuge at 4000 g for 10 minutes at 4°C to inactivate Mn SOD, and take the uppermost layer of the solution (Treated supernatant) to determinate Cu/Zn SOD activity.

Note: there are three layers in the solution after centrifuge and tests only need the uppermost layer.

- 2) According to the proportion of water volume (mL): Extraction reagent II volume (mL) of 2:3 to mix. It is suggested that 0.2 mL of water with 0.3 mL of Extraction reagent II and fully mixed for 1 minute. Centrifuge at 4000 g for 10 minutes at 4°C, and take the uppermost layer of the solution (Treated water) to be the blank tube of Cu/Zn SOD activity detection.

II. Determination procedure:

1. Preheat the spectrophotometer/microplate reader for 30 minutes, adjust wavelength to 450 nm, set spectrophotometer counter to zero with distilled water.
2. Preheat Reagent I, Reagent III, Reagent IV working solution for 5 minutes at 37°C.
3. Add reagents with the following list:

Reagent (μL)	Total SOD Activity				Cu/Zn SOD activity			
	Test tube 1 (T1)	Control tube 1 (C1)	Blank tube 1 (B1)	Blank tube 2 (B2)	Test tube 2 (T2)	Control tube 2 (C2)	Blank tube 3 (B3)	Blank tube 4 (B4)
Supernatant	20	20	-	-	-	-	-	-
Sample processing supernatants	-	-	-	-	20	20	-	-

Distilled water treatment supernatant	-	-	-	-	-	-	20	20
Reagent I	45	45	45	45	45	45	45	45
Reagent II	20	-	20	-	20	-	20	-
Reagent III	35	35	35	35	35	35	35	35
Sterilized water	70	90	90	110	70	90	70	90
Reagent IV	10	10	10	10	10	10	10	10

Mix thoroughly and the mixture is incubated at 37°C for 30 minutes. Add the mixture into micro glass cuvette/96 well flat-bottom plate, and detect the absorbance value of each tube at 450 nm. Blank tubes need to test once or twice and every test tube need a contrast tube.

Tubes for **Total SOD activity** is A_{T1} , A_{C1} , A_{B1} , A_{B2} , and $\Delta A_{T1}=A_{T1}-A_{C1}$, $\Delta A_{B1}=A_{B1}-A_{B2}$.

Tubes for **Cu/Zn SOD activity** is A_{T2} , A_{C2} , A_{B3} , A_{B4} , and $\Delta A_{T2}=A_{T2}-A_{C2}$, $\Delta A_{B3}=A_{B3}-A_{B4}$.

III. Calculation:

1. Inhibition percentage:

Total SOD activity Inhibition percentage= $(\Delta A_{B1}-\Delta A_{T1})\div\Delta A_{B1}\times 100\%$

Cu/Zn SOD activity Inhibition percentage= $(\Delta A_{B3}-\Delta A_{T2})\div\Delta A_{B3}\times 100\%$

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 redetermine. 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 modify the calculation formula.**

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

a) Protein concentration:

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

b) Sample weight

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

c) Bacteria or cell amount

$$\text{SOD Activity(U}/10^4 \text{ cell)}=[P\div(1-P)\times V_{rv}]\div(500\times V_s\div V_{sv})\times F=0.022\times P\div(1-P)\times F$$

C. Mn SOD Activity=Total SOD Activity-Cu/Zn SOD Activity

D.

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, %;
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 and III) can be configured according to the table. Reagent IV must be added finally.
3. These Reagents could be used to detect Mn SOD activity of 48 samples or Cn/Zn SOD activity of 96 samples.

Experimental Examples:

1. 0.106 g of *Buxus sinica* leaves is added into 1 mL of Extraction reagent I for homogenization. After the supernatant is taken and diluted 10 times, the operation is carried out according to the determination steps. The results with 96-well plates showed that $\Delta A_{T1} = A_{T1} - A_{C1} = 0.399 - 0.088 = 0.311$, $\Delta A_{B1} = A_{B1} - A_{B2} = 0.801 - 0.088 = 0.713$. Total SOD Inhibition percentage = $(\Delta A_{B1} - \Delta A_{T1}) \div \Delta A_{B1} \times 100\% = 56.381\%$; Add 0.3mL of Extraction reagent II to 0.2 mL of diluted supernatant, the operation is carried out according to the determination steps. The results with 96-well plates showed that $\Delta A_{T2} = A_{T2} - A_{C2} = 0.827 - 0.086 = 0.741$, $\Delta A_{B3} = A_{B3} - A_{B4} = 1.011 - 0.081 = 0.930$. Cn/Zn SOD Inhibition percentage = $(\Delta A_{B3} - \Delta A_{T2}) \div \Delta A_{B3} \times 100\% = 20.323\%$, and the enzyme activity is calculated according to the sample weight.

Total SOD Activity(U/g weight) = $10 \times P \div (1 - P) \div W \times F = 1219.438$ U/g weight.

Cn/Zn SOD Activity(U/g weight) = $10 \times P \div (1 - P) \div W \times F = 240.623$ U/g weight.

Mn SOD Activity(U/g weight) = $1219.438 - 240.623 = 978.815$ U/g weight.

2. 0.1104 g of rabbit spleen is added into 1 mL of Extraction reagent I for homogenization. After the supernatant is taken and diluted 10 times, the operation is carried out according to the determination steps. The results with 96-well plates showed that $\Delta A_{T1} = A_{T1} - A_{C1} = 0.348 - 0.088 = 0.260$, $\Delta A_{B1} = A_{B1} - A_{B2} = 0.801 - 0.088 = 0.713$. Total SOD Inhibition percentage = $(\Delta A_{B1} - \Delta A_{T1}) \div \Delta A_{B1} \times 100\% = 63.534\%$; Add 0.3mL of Extraction reagent II to 0.2 mL of diluted supernatant, the operation is carried out according to the determination steps. The results with 96-well plates showed that $\Delta A_{T2} = A_{T2} - A_{C2} = 0.637 - 0.084 = 0.553$, $\Delta A_{B3} = A_{B3} - A_{B4} = 1.011 - 0.081 = 0.930$. Cn/Zn SOD Inhibition percentage = $(\Delta A_{B3} - \Delta A_{T2}) \div \Delta A_{B3} \times 100\% = 40.538\%$, and the enzyme activity is calculated according to the sample weight.

Total SOD Activity(U/g weight) = $10 \times P \div (1-P) \div W \times F = 1578.177$ U/g weight.

Cn/Zn SOD Activity(U/g weight) = $10 \times P \div (1-P) \div W \times F = 617.514$ U/g weight.

Mn SOD Activity(U/g weight) = $1578.177 - 617.514 = 960.663$ U/g weight.

3. 10 million of cell amount is added into 1 mL of Extraction reagent I for homogenization. After the supernatant is taken and sample volume is doubled, the operation is carried out according to the determination steps. The results with 96-well plates showed that $\Delta A_{T1} = A_{T1} - A_{C1} = 0.336 - 0.124 = 0.212$, $\Delta A_{B1} = A_{B1} - A_{B2} = 0.714 - 0.086 = 0.628$. Total SOD Inhibition percentage = $(\Delta A_{B1} - \Delta A_{T1}) \div \Delta A_{B1} \times 100\% = 66.242\%$; Add 0.3mL of Extraction reagent II to 0.2 mL of diluted supernatant, the operation is carried out according to the determination steps. The results with 96-well plates showed that $\Delta A_{T2} = A_{T2} - A_{C2} = 0.525 - 0.085 = 0.440$, $\Delta A_{B3} = A_{B3} - A_{B4} = 0.982 - 0.081 = 0.901$. Cn/Zn SOD Inhibition percentage = $(\Delta A_{B3} - \Delta A_{T2}) \div \Delta A_{B3} \times 100\% = 51.165\%$, modify the calculation formula and the enzyme activity is calculated according to the cell amount.

Total SOD Activity(U/10⁴ cell) = $0.005 \times P \div (1-P) \times F = 0.010$ U/10⁴ cell.

Cn/Zn SOD Activity(U/10⁴ cell) = $0.005 \times P \div (1-P) \times F = 0.005$ U/10⁴ cell.

Mn SOD Activity(U/10⁴ cell) = $0.010 - 0.005 = 0.005$ U/10⁴ cell.

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):157-166.
- [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.
- [3] Cristiana, F. , Elena, A. and Nina, Z. Superoxide Dismutase: Therapeutic Targets in SOD Related Pathology[J]. Health, 2014, 06(10):975-988.

Related Products:

BC0170/BC0175	Superoxide Dismutase (SOD) Activity Assay Kit
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
BC5160/BC5165	Superoxide Dismutase (SOD) Activity Assay Kit with WST-1