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

(Total SOD, Cu/Zn SOD, Mn SOD Activity)

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

Operation Equipment: Spectrophotometer/Microplate reader

Catalog Number: NA0162

Size: 100T/48S

Components:

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

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

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

Reagent II: Liquid 24 μL×1. Store at 2-8℃. Mix by pipetting after centrifugation, and dilute 100 times with sterilized water according to the number of samples before use.

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

Reagent IV: Liquid 0.5 mL×1. Store at 2-8℃. Dilute 10 times with sterilized water according to the number of samples before 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 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 xanthine and xanthine oxidase reaction system. O_2^- can reduce water-soluble tetrazolium-1(WST-1) to form a yellow formazan dye, which has absorbance in 450 nm. SOD can remove O_2^- 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℃ 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℃ 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℃ 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℃, 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 for 5 minutes at 37℃.
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	-	-	-	-	-	-
Treated supernatant	-	-	-	-	20	20	-	-
Treated water	-	-	-	-	-	-	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℃ 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=$(\Delta A_{B1}-\Delta A_{T1})\div\Delta A_{B1}\times100\%$

Cu/Zn SOD activity Inhibition percentage=$(\Delta A_{B3}-\Delta A_{T2})\div\Delta A_{B3}\times100\%$

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

SOD Activity(U/mL)=[P÷(1-P)×Vrv]÷Vs×F=11.11×P÷(1-P)×F

B. Tissue, bacteria or cultured cells

a) Protein concentration:

SOD Activity(U/mg prot)=[P÷(1-P)×Vrv]÷(Vs×Cpr)×F=11.11×P÷(1-P)÷Cpr×F

b) Sample weight

SOD Activity(U/g weight)=[P÷(1-P)×Vrv]÷(W×Vs÷Vsv)×F=11.11×P÷(1-P)÷W×F

c) Bacteria or cell amount

SOD Activity(U/10^4 cell)=[P÷(1-P)×Vrv]÷(500×Vs÷Vsv)×F=0.022×P÷(1-P)×F

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

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 = $(\Delta A_{B1}-\Delta A_{T1})\div\Delta A_{B1}\times100\%$ = 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= $(\Delta A_{B3}-\Delta A_{T2})\div\Delta A_{B3}\times100\%$ = 20.323%, and the enzyme activity is calculated according to the sample weight.
   Total SOD Activity(U/g weight) = 10×P÷(1-P)÷W×F = 1219.438 U/g weight.
   Cn/Zn SOD Activity(U/g weight) = 10×P÷(1-P)÷W×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 = $(\Delta A_{B1}-\Delta A_{T1})\div\Delta A_{B1}\times100\%$ = 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= $(\Delta A_{B3}-\Delta A_{T2})\div\Delta A_{B3}\times100\%$ = 40.538%, and the enzyme activity is calculated according to the sample weight.
   Total SOD Activity(U/g weight) = 10×P÷(1-P)÷W×F = 1578.177 U/g weight.
   Cn/Zn SOD Activity(U/g weight) = 10×P÷(1-P)÷W×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 = $(\Delta A_{B1}-\Delta A_{T1})\div\Delta A_{B1}\times100\%$ = 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=

$(\Delta A_{B3}-\Delta A_{T2})\div\Delta A_{B3}\times100\%$ = 51.165%, modify the calculation formula and the enzyme activity is calculated according to the cell amount.

Total SOD Activity(U/10^4 cell) = 0.005×P÷(1-P)×F = 0.010 U/10^4 cell.

Cn/Zn SOD Activity(U/10^4 cell) = 0.005×P÷(1-P)×F = 0.005 U/10^4 cell.

Mn SOD Activity(U/10^4 cell) = 0.010-0.005=0.005 U/10^4 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:

NA0857/NA0615	Superoxide Dismutase (SOD) Activity Assay Kit
NA0855/NA0613	Polyphenol Oxidase (PPO) Activity Assay Kit
NA0853/NA0611	Phenylalnine Ammonialyase (PAL) Activity Assay Kit
NA0854/NA0386	Catalase (CAT) Activity Assay Kit
NA0864/NA0621	Peroxidase (POD) Activity Assay Kit
NA0181/NA0180	Superoxide Dismutase (SOD) Activity Assay Kit with WST-1