Superoxide Dismutase (SOD) Activity Assay Kit

Note: Take two or three different samples for prediction before test.

Operation Equipment: Spectrophotometer/Microplate reader

Catalog Number: NA0615

Size: 100T/48S

Components:

Extraction reagent: 100 mL×1. Storage at 4℃.

Reagent I: 5 mL×1. Storage at 4℃.

Reagent II: 100 μL×1. Storage at 4℃. Mix by pipetting after centrifugation.

Reagent III: 4 mL×1. Storage at 4℃.

Reagent IV: Powder×1. Storage at 4℃.

Reagent V: 2 mL×1. Storage at 4℃. Add Reagent IV to Reagent V before use and dissolved by shaking with an oscillator. It can be stored for 3 months at 4℃

Reagents and Equipments Required but Not Provided:

Spectrophotometer/microplate reader, table centrifuge, transferpettor, micro glass cuvette/96 well flat-bottom plate, mortar/ homogenizer, ice and distilled water.

Product Description:

Superoxide dismutase (SOD, EC 1.15.1.1) 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 darker the blue color of the reaction solution, the lower the SOD activity. The lighter the blue color of the reaction solution, the higher the activity of SOD.

Operation steps:

I. Sample preparation:

1. Bacteria or cells: collecting bacteria or cells into the centrifuge tube, discard supernatant after centrifugation. It is suggested that 5 million of bacteria or cell amount with 1 mL of Extraction reagent. Splitting the bacteria or cell with ultrasonication (placed on ice, ultrasonic power 200W or 20%, 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: 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℃ to remove insoluble materials, and take the supernatant on ice before testing.

3. Serum (plasma) sample: detect sample directly.

II. Determination procedure:

1. Preheat the spectrophotometer/microplate reader for 30 minutes, adjust wavelength to 560 nm, set zero with distilled water.

2. Keep Reagent I, Reagent II, Reagent V in water bath for 5 minutes at 37℃(mammal) or 25℃ (other species).

3. Add reagents with the following list:

Reagent (μL)	Test tube (T)	Control tube (C)	Blank tube (B1)	Blank tube (B2)
Sample	18	18	-	-
Reagent I	45	45	45	45
Reagent II	2	-	2	-
Reagent III	35	35	35	35
Distilled water	90	92	108	110
Reagent V	10	10	10	10

Mix thoroughly and the mixture is incubated at 37℃ for 30 minutes. Add the mixture into ultra-micro cuvette/96 well flat-bottom plate, 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.

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 V must be added finally.

3. Please set one control for each sample. Blank tube 1 (B1) and blank tube 2 (B2) only need to do one or two tubes respectively.

4. After the reaction completed, there may be precipitation formed, which can be determined after mixing.

III. Calculation:

1. Inhibition percentage:

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.

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. Caculation

A. Serum (plasma) sample

SOD (U/mL)=$[P\div(1-P)\times Vrv]\div Vs\times F=11.11\times P\div(1-P)\times F$

B. Tissue, bacteria or cultured cells

a) Protein concentration:

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

b) Sample weight

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

c) Bacteria or cell amount

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

Vrv: Total reaction volume, 0.2 mL;

Vs: Sample volume, 0.018 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.

Experimental Examples:

1. 0.1 g of Rat kidney is added into 1 mL of Extraction reagent for homogenization. After the supernatant is taken, the operation is carried out according to the determination steps. The results with 96-well plates showed that $\Delta A_T = A_T - A_C = 0.54-0.112=0.428$, $\Delta A_B = A_{B1} - A_{B2} = 0.905-0.041 = 0.864$. Inhibition percentage = $(\Delta A_B - \Delta A_T) \div \Delta A_B \times 100\% = 750.463\%$, and the enzyme activity is calculated according to the sample mass.

SOD activity (U/g mass) = 11.4 × Inhibition percentage (1-Inhibition percentage) × W = 113.2 U/g mass.

2. Take 18μL rabbit serum directly follow the determination procedure. The results with 96-well plates showed that $\Delta A_T = A_T - A_C = 0.788-0.071 = 0.717$ $\Delta A_B = A_{B1} - A_{B2} = 1.152-0.043 = 1.109$, inhibition percentage = $(\Delta A_B - \Delta A_T) \div \Delta A_B \times 100\% = 35.347\%$

SOD activity (U/g mass) = 11.4 × Inhibition percentage (1-Inhibition percentage) ×W = 6.074U/g mass.

3. 10 million cells is extracted and centrifuged by adding 1 mL of Extraction reagent, and then the operation is performed according to the determination steps. The results with 96-well plates are as follows: $\Delta A_T = A_T - A_C = 0.52-0.058=0.462$, $\Delta A_B = A_{B1} - A_{B2} = 0.936-0.045=0.891$, inhibition percentage = $(\Delta A_B - \Delta A_T) \times \Delta A_B \times 100\% = 48.148\%$

SOD activity (U/10^4 cell) = Inhibition percentage ÷ (1-Inhibition percentage)$\times V_{TS}] \div (1000 \times V_S \div V_{TS})$ = 0.0102 U/10^4 cell.

Recent Protect Citations:

[1] Beibei Li,Yang Ding,Xiuli Tang,et al. Effect of L-Arginine on Maintaining Storage Quality of the White Button Mushroom (Agaricus bisporus). Food and Bioprocess Technology. April 2019; 12: 563-574. (IF3.032)

[2] Wang Li, Shi Qinghai, Li Kai, et al. Oral administration of Ginkgolide B alleviates hypoxia-induced neuronal damage in rat hippocampus by inhibiting oxidative stress and apoptosis. Iranian Journal of Basic Medical Sciences. February 2019; 22(2): 140-145. (IF1.854)

[3] Yanan Wang, Chengzhen Liang, Zhigang Meng, et al. Leveraging Atriplex hortensis choline monooxygenase to improve chilling tolerance in cotton. Environmental and Experimental Botany. June 2019; 162: 364-373. (IF3.712)

[4] Fangzhou Chen, Ying Zhao, Huizhao Chen. MicroRNA-98 reduces amyloid β-protein production and improves oxidative stress and mitochondrial dysfunction through the Notch signaling pathway via HEY3 in Alzheimer's disease mice. International Journal of Molecular Medicine. October 2018; 91-102. (IF2.784)

[5] Yang Yang, Li Jing, Wei Cong, et al. Amelioration of nonalcoholic fatty liver disease by swertiamarin in fructose-fed mice. Phytomedicine. June 2019;59. (IF4.18)

References:

[1] Spitz D R, Oberley L W. An assay for superoxide dismutase activity in mammalian tissue homogenates[J]. Analytical Biochemistry, 1989, 179(1):8-18.

[2] Masayasu M, Hiroshi Y. A simplified assay method of superoxide dismutase activity for clinical use[J]. Clinica Chimica Acta, 1979, 92(3):337-342.

Related Products:

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