Superoxide Dismutase (SOD) Activity Assay Kit

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

Operation Equipment: Spectrophotometer

Cat No: BC0170

Size: 50T/24S

Product Composition: Before use, please carefully check whether the volume of the reagent is consistent with the volume in the bottle. If you have any questions, please contact Solarbio staff in time.

Reagent name	Size	Preservation Condition
Extraction reagent	Liquid 60 mL×1	2-8℃
Reagent I	Liquid 15 mL×1	2-8℃
Reagent II	Liquid 160 μL×1	2-8℃
Reagent III	Liquid 11 mL×1	2-8℃
Reagent IV	Liquid 0.5 mL×1	2-8℃

Solution Preparation:

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

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_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. It is recommended to use BC5160 Superoxide Dismutase (SOD) Activity Assay Kit with WST-1 for **serum (plasma) samples or low activity samples**.

Reagents and Equipment Required but Not Provided:

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

Operation steps:

I. Sample preparation:

1. 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

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(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 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 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℃ 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.

Note: the supernatant extracted by this kit could be used for other kits determination of BC0090/BC0095, BC0020/BC0025, BC0200/BC0205, BC0680/BC0685.

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℃.
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℃ 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）

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 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

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

B. Tissue, bacteria or cultured cells

a) Protein concentration:

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

b) Sample weight

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

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:

1. The Sample and Reagent II working solution should be placed on ice when using.
2. 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.
3. After the reaction completed, there may be precipitation formed, which can be determined after mixing.

Experimental Examples:

1. Take 0.1 g of *Epipremnum aureum* leaves and add 1 mL of Extraction reagent for extraction and homogenization. After the supernatant is taken, the operation is carried out according to the determination steps. The results showed that $\Delta A_T = A_T - A_C = 0.532-0.085 = 0.447$, $\Delta A_B = A_{B1} – A_{B2} = 0.853-0.002 = 0.851$. Inhibition percentage = $(\Delta A_B - \Delta A_T) \div \Delta A_B \times 100\% = 47.5\%$, and the enzyme activity is calculated according to the sample mass.
   SOD activity (U/g weight) = 11.11×P÷(1-P)÷W×F = 100.5U/g weight.
2. Take 0.1 g of rat kidney and add 1 mL of Extraction reagent for extraction and homogenization. After the supernatant is taken, the operation is carried out according to the determination steps.

The results showed that $\Delta A_T = A_T - A_C = 0.608-0.109 = 0.499$, $\Delta A_B = A_{B1} - A_{B2} = 0.853-0.002 = 0.851$, Inhibition percentage = $(\Delta A_B - \Delta A_T) \div \Delta A_B \times 100\% = 41.4\%$, and the enzyme activity is calculated according to the sample mass.

SOD activity (U/g weight) = $11.11 \times P \div (1-P) \div W \times F = 78.5$ U/g weight.

3. Take 10 million cells and add 1 mL of Extraction reagent for extraction and homogenization. After the supernatant is taken, the operation is carried out according to the determination steps. The results

are as follows: $\Delta A_T = A_T - A_C = 0.614-0.015 = 0.599$, $\Delta A_B = A_{B1} - A_{B2} = 0.853-0.002 = 0.851$, Inhibition percentage = $(\Delta A_B - \Delta A_T) \times \Delta A_B \times 100\% = 29.6\%$, and the enzyme activity is calculated according to the bacteria or cell amount.

SOD activity (U/10^4 cell) = $11.11 \times P \div (1-P) \div N \times F = 0.0046$ U/10^4 cell.

Recent Protect Citations:

[1] Liu C, Zou Q, Tang H, Liu J, Zhang S, Fan C, Zhang J, Liu R, Liu Y, Liu R, Zhao Y, Wu Q, Qi Z, Shen Y. Melanin nanoparticles alleviate sepsis-induced myocardial injury by suppressing ferroptosis and inflammation. Bioact Mater. 2022 Dec 27; 24:313-321. doi: 10.1016/j.bioactmat.2022.12.026. PMID: 36632502; PMCID: PMC9813528.

[2] Nie C, A R, Wang J, Pan S, Zou R, Wang B, Xi S, Hong X, Zhou M, Wang H, Yu M, Wu L, Sun X, Yang W. Controlled Release of Hydrogen-Carrying Perfluorocarbons for Ischemia Myocardium-Targeting 19 F MRI-Guided Reperfusion Injury Therapy. Adv Sci (Weinh). 2023 Oct;10(29): e2304178. doi: 10.1002/advs.202304178. Epub 2023 Aug 18. PMID: 37596718; PMCID: PMC10582447.

[3] Zhang D, Liu J, Zhang Y, Wang H, Wei S, Zhang X, Zhang D, Ma H, Ding Q, Ma L. Morphophysiological, proteomic and metabolomic analyses reveal cadmium tolerance mechanism in common wheat (Triticum aestivum L.). J Hazard Mater. 2023 Mar 5; 445:130499. doi: 10.1016/j.jhazmat.2022.130499. Epub 2022 Nov 25. PMID: 36455318.

[4] Liu YS, Tao Y, Yang XZ, Liu YN, Shen RF, Zhu XF. Gibberellic acid alleviates cadmium toxicity in rice by regulating NO accumulation and cell wall fixation capacity of cadmium. J Hazard Mater. 2022 Oct 5; 439:129597. doi: 10.1016/j.jhazmat.2022.129597. Epub 2022 Jul 16. PMID: 35868086.

[5] Tian D, Yu Y, Yu Y, Lu L, Tong D, Zhang W, Zhang X, Shi W, Liu G. Tris(2-chloroethyl) Phosphate Exerts Hepatotoxic Impacts on Zebrafish by Disrupting Hypothalamic-Pituitary-Thyroid and Gut-Liver Axes. Environ Sci Technol. 2023 Jun 20;57(24):9043-9054. doi: 10.1021/acs.est.3c01631. Epub 2023 Jun 5. PMID: 37276532.

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.

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