# Superoxide Dismutase (SOD) Activity Assay Kit

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

**Operation Equipment:** Spectrophotometer/Microplate reader

**Catalog Number:** BC0175

**Size:** 100T/48S

**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 110 mL×1 | 2-8℃ |
| Reagent I | Liquid 5 mL×1 | 2-8℃ |
| Reagent II | Liquid 100 μL×1 | 2-8℃ |
| Reagent III | Liquid 4 mL×1 | 2-8℃ |
| Reagent IV | Liquid 0.25 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 15S) according to sample number before use.
3. Reagent IV working solution: Mix Reagent IV: distilled water=60μL: 240μL (300μL, about 30T) 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 SOD activity. 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 Equipments Required but Not Provided:**

Spectrophotometer/microplate reader, table centrifuge, transferpettor, micro glass cuvette/96 well flat-bottom plate, mortar/ homogenizer/cell ultrasonic crusher, 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. It is suggested that 5 million of bacteria /cell amount with 1 mL of Extraction



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reagent.

Splitting the bacteria or cell with ultrasonication (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/microplate reader for 30 minutes, adjust wavelength to 560 nm, set spectrophotometer counter to zero with distilled water.
2. Keep Reagent I, Reagent III, Reagent IV working solution in water bath for 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 | 20 | 20 | - | - |
| Reagent I | 45 | 45 | 45 | 45 |
| Reagent II working solution | 20 | - | 20 | - |
| Reagent III | 35 | 35 | 35 | 35 |
| Distilled water | 70 | 90 | 90 | 110 |
| Reagent IV working solution | 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 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=10×P÷(1-P)×F

B. Tissue, bacteria or cultured cells

a) Protein concentration:

SOD activity (U/mg prot)=[P÷(1-P)×Vrv] ÷1mL÷(Vs×Cpr)×F=10×P÷(1-P)÷Cpr×F

b) Sample weight

SOD activity (U/g weight)=[P÷(1-P)×Vrv] ÷1mL÷(W×Vs÷Vsv)×F=10×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=10×P÷(1-P) ÷N×F

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.

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 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 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 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 with 96-well plates showed that $\Delta A_T = A_T - A_C = 0.566-0.138=0.428$, $\Delta A_B = A_{B1} – A_{B2} =0.802-0.041 =0.761$. Inhibition percentage = $(\Delta A_B- \Delta A_T)\div \Delta A_B \times 100\% = 43.8\%$, and the enzyme is calculated according to the sample mass.

   SOD activity (U/g weight) = 10×P÷(1-P)÷W×F = 77.94U/g weight.

2. Take 0.1 g of *Epipremnum aureum* leaves and add 1 mL of Extraction reagent for extraction and homogenization. After the supernatant is taken and diluted 4 times in distilled water, the operation is carried out according to the determination steps. The results with 96-well plates

showed that $\Delta A_T = A_T - A_C = 0.498-0.078 = 0.42$, $\Delta A_B = A_{B1} - A_{B2} = 0.771-0.042 = 0.729$. Inhibition percentage = $(\Delta A_B - \Delta A_T) \div \Delta A_B \times 100\% = 42.4\%$, and the enzyme activity is calculated according to the sample mass.

SOD activity (U/g weight) = $10 \times P \div (1-P) \div W \times F$ = 294.4U/g weight.

3. Take 20μL sheep serum and diluted 50 times in distilled water, the operation is carried out according to the determination steps. The results with 96-well plates showed that $\Delta A_T = A_T - A_C = 0.467-0.049 = 0.418$, $\Delta A_B = A_{B1} - A_{B2} = 0.771-0.042 = 0.729$, inhibition percentage = $(\Delta A_B - \Delta A_T) \div \Delta A_B \times 100\% = 42.7\%$, and the enzyme activity is calculated according to the serum (plasma) sample volume .

   SOD activity (U/mL ) = $10 \times P \div (1-P) \times F$ = 372.6U/mL.

4. Take 10 million cells and add 1 mL of Extraction reagent for extraction and homogenization. After the supernatant is taken, the operation is performed according to the determination steps. The results with 96-well plates showed that $\Delta A_T = A_T - A_C = 0.52-0.058 = 0.462$, $\Delta A_B = A_{B1} - A_{B2} = 0.802-0.04 = 0.762$, Inhibition percentage = $(\Delta A_B - \Delta A_T) \div \Delta A_B \times 100\% = 39.4\%$, and the enzyme activity is calculated according to the cell amount.

   SOD activity (U/$10^4$ cell) = $10 \times P \div (1-P) \div N \times F$ = 0.0065 U/$10^4$ cell.

**Recent Protect Citations:**

[1] Xu J, Xu J, Shi T, Zhang Y, Chen F, Yang C, Guo X, Liu G, Shao D, Leong KW, Nie G. Probiotic-Inspired Nanomedicine Restores Intestinal Homeostasis in Colitis by Regulating Redox Balance, Immune Responses, and the Gut Microbiome. Adv Mater. 2023 Jan;35(3): e2207890. doi: 10.1002/adma.202207890. Epub 2022 Dec 15. PMID: 36341495.

[2] Xu J, Chu T, Yu T, Li N, Wang C, Li C, Zhang Y, Meng H, Nie G. Design of Diselenide-Bridged Hyaluronic Acid Nano-antioxidant for Efficient ROS Scavenging to Relieve Colitis. ACS Nano. 2022 Aug 23;16(8):13037-13048. doi: 10.1021/acsnano.2c05558. Epub 2022 Jul 21. PMID: 35861614.

[3] Zheng Q, Liu H, Zhang H, Han Y, Yuan J, Wang T, Gao Y, Li Z. Ameliorating Mitochondrial Dysfunction of Neurons by Biomimetic Targeting Nanoparticles Mediated Mitochondrial Biogenesis to Boost the Therapy of Parkinson's Disease. Adv Sci (Weinh). 2023 Aug;10(22): e2300758. doi: 10.1002/advs.202300758. Epub 2023 May 18. PMID: 37202595; PMCID: PMC10401119.

[4] Yan H, Meng Y, Li X, Xiang R, Hou S, Wang J, Wang L, Yu X, Xu M, Chi Y, Yang J. FAM3A maintains metabolic homeostasis by interacting with F1-ATP synthase to regulate the activity and assembly of ATP synthase. Metabolism. 2023 Feb; 139:155372. doi: 10.1016/j.metabol.2022.155372. Epub 2022 Dec 5. PMID: 36470472.

[5] Zhang S, Wang H, Liu M, Yu H, Peng J, Cao X, Wang C, Liu R, Kamali M, Qu J. Press perturbations of microplastics and antibiotics on freshwater micro-ecosystem: Case study for the ecological restoration of submerged plants. Water Res. 2022 Nov 1; 226:119248. doi: 10.1016/j.watres.2022.119248. Epub 2022 Oct 14. PMID: 36323200.

**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:**

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



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