BT LAB
Bioassay Technology Laboratory

Optimize Your Research

Total-Superoxide Dismutase (SOD) Assay Kit

(Hydroxylamine method)

Catalog No: SH0120
Method: Colorimetric method
Specification: 100 tubes/96 samples

Application

This kit adopts the xanthine oxidase (hydroxylamine method) to measure T-SOD activity. The activity of SOD in serum, plasma, cerebrospinal fluid, pleural effusion ascites, renal dialysis fluid, urine, erythrocyte, leukocyte, platelets, myocardial cells, tumor cells and a variety of plant and animal tissues and cells, subcellular level (mitochondria and microsome) can be tested by this kit. And the activity of SOD in microorganisms, medicine, food, beverage, cosmetics and other samples can be tested by this kit too.

Kit components

Reagent 1 working solution: 10 mL ×1 vial. Store at 4-10℃ for 1 year
Note: It will appear crystal in bottle when the temperature is low, please dissolve it completely with hot water bath.

Reagent 2: 10 mL ×1 vial. Store at 4-10℃ for 1 year

Reagent 3: 10 mL ×1 vial. Store at 4-10℃ for 1 year

Reagent 4 working solution: 350μL×2 vials. Store at -20℃

Diluent for Reagent 4 : 10 mL×1 vial. 4℃ for 6 months
Note: Dilute Reagent 4 at a ratio of 1:14. Prepare the fresh solution before use. Unused reagent can be stored at 4℃, avoid frozen
All nozzles are disposable nozzles

Reagent 5: Powder, 1 vial

Dissolve a vial of powder with 70-80℃ double distilled water to a final volume of 75 mL. It can be store at 4℃ with shading light for 1 year.

Reagent 6: Powder, 1 vial.

Dissolve a vial of powder with double distilled water to a final volume of 75 mL. It can be store at 4℃ for 6 months

Preparation of chromogenic agent: Prepare chromogenic agent at ratio of Reagent 5 : Reagent 6: Glacial acetic acid =3:3:2. Prepare the fresh solution before use and store prepared chromogenic agent at 4 ℃ in the dark for 3 months.

Note: Glacial acetic acid (analytical grade, acetic acid concentration ≥99.5%)

Reagent 5, Reagent 6 must be configured separately.

Do not mix reagent 5 and reagent 6 before disposing, otherwise it will not develop color.

Experimental instrument

Test tube, Vortex mixer, Micropipettor, 37 ℃ water bath, Spectrophotometer (550 nm)

Detection principle

The superoxide anion free radical (O_2^-) can be produced by xanthine and xanthine oxidase reaction system, O_2^- oxidize hydroxylamine to form nitrite, it turn to purple under the reaction of developer. When the measured samples containing SOD, the SOD can specifically inhibit superoxide anion free radical (O_2^-). The inhibitory effect of SOD can reduce the formation of nitrite, the absorbance value of sample tube is lower than control tube. Calculate the SOD of sample according to the computational formula.

Sample pretreatment

1. **Serum (Plasma) :** Centrifuge the serum (plasma) at 3500 rpm for 10 min if it's turbid, then take the supernatant to measure. The clarified serum (plasma) was diluted into different concentrations with normal saline to do a pre-experiment.
2. **10% Tissue homogenate:** Weigh the tissue accurately. Adding 9 times of the volume of PBS (0.1 M, pH 7-7.4) according to the proportion of Weight (g): Volume (mL) =1:9. Homogenized mechanically with a homogenizer in ice-bath, then centrifuge at 1500 g for 10 min. Take the supernatant and preserve it on ice for detection. The supernatant was diluted into different concentrations with phosphate buffer to do a pre-experiment. Meanwhile, determine the concentration of supernatant.
3. **Tissue (plant) sample:**
    1) Adherent cells should be detached with trypsin or a cell scraper and then collected sedimentary cells by centrifugation. (Suspension cells can be collected sediment by centrifugation directly). Centrifuge for 10 min at 1000 g, discard supernatant.
    2) Resuspend adherent cells in 1 mL cold PBS, centrifuge for 10 min at 1000 g, discard supernatant.
    3) Resuspend cells in PBS (0.1 M, pH 7-7.4) or normal saline. Sonicate or grind with hand-operated in ice water bath to break the cells. (or Freeze cells at ≤ -20℃. Thaw cells with gentle mixing. Repeat the freeze/thaw cycle for 3 times.)

Operation steps

Reagent	Sample tube	Control tube
Reagent 1 working solution (mL)	1.0	1.0
Sample (mL)	a*	
Double distilled water(mL)		a*
Reagent 2 (mL)	0.1	0.1
Reagent 3 (mL)	0.1	0.1
Reagent 4 working solution (mL)	0.1	0.1
Mix fully with a vortex instrument, incubate for 40 min at 37 ℃.		
Chromogenic agent	2	2
Mix fully and stand for 10 min at room temperature. Set to zero with double-distilled water and measure the OD value of each tube at 550 nm with 1 cm diameter cuvette.		

Note: a* is the sampling volume of sample and double distilled water.

Calculation of results

1. **For serum (plasma), culture cell and other liquid samples:**

Definition: The amount of SOD when the inhibition ratio reaches 50% in 1 mL reaction solution is defined as 1 SOD activity unit (U).

SOD activity (*U/mL*)

$$= \frac{OD_{Control} - OD_{Sample}}{OD\ Control} \div 50\% \times \text{Dilution multiple of reaction system}$$

× Dilution multiple of sample before tested

2. **For animal tissue sample:**

Definition: The amount of SOD when the inhibition ratio reaches 50% of 1 mg tissue protein in 1 mL reaction solution is defined as 1 SOD activity unit (U).

SOD activity (U/*mgprot*)=

$$= \frac{OD_{Control} - OD_{Sample}}{OD\ Control} \div 50\% \times \frac{\text{Total volume of reaction liquid } (mL)}{\text{Volume of sample}(mL)}$$

÷Protein concentration of sample(mgprot/ml)

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3. **For plant tissue:**

Definition: The amount of SOD when the inhibition ratio reaches 50% of 1 g tissue in 1 mL reaction solution is defined as 1 SOD activity unit (U).

SOD activity (*U/g* tissue) =

$$\frac{OD_{Control}-OD_{Sample}}{OD\ Control} \div 50\% \times \frac{Total\ volume\ of\ reaction\ liquid\ (mL)}{Volume\ of\ sample(mL)} \div Homogen\ concentration\ (g/ml)$$

Note: Homogen concentration= Tissue wet weight(g)÷Homogenate medium volume(ml)

Notes

1. Determine optimal sampling volume of each sample before formal experiment. Calculate the inhibition ratio of serial sampling volume, and choose the optimal sampling volume when inhibition ratio in the range of 45%~55%.

$$\text{Inhibition ratio}=\frac{OD_{control}-OD_{sample}}{OD_{control}} \times 100\%$$

2. The optimal sampling volume are different for different species, the SOD also are different for different samples. So it is best to do a pre-test to determining optimal sampling volume for a new sample.
3. Please follow the operation table to add reagents orderly, reagent 1 can be mixed with reagent 2 or reagent 3, the mixed reagent can be taken 1 mL, it will not affect the result. Note: It can't mix reagent 1, reagent 2, reagent 3 and reagent 4 simultaneously, this can affect the result.
4. It is best to reserve 3 paralleled tubes with different sampling volumes in pre-test for determining the optimal sampling volume. The sampling volume in examples (page 5) as median, increase by 10 μL and decrease by 10 μL. Take the pre-test with 3 paralleled tubes and 1 control tube to determining the optimal sampling volume.
5. Adjust sampling volume: If inhibition ratio > 60%, need to dilute the sample or decrease the sampling volume than take the test. If inhibition ratio < 20%, need to increase the sampling volume.
6. All the reagents should be prepared at the day before the experiment, in order to let the reagents dissolve fully. The prepared reagents can be stored at 4℃ for 3~6 months(except reagent 4). Please bring all the reagents and samples to room temperature for 30 min before the assay.
7. The incubation time is 40 min, the incubation time can be extended to 45 min when the room temperature is lower than 20℃. Ensure the incubation temperature is 37℃.
8. In the formal experiment, need to test 2 control tubes interlaced between sample tubes, take the average value when calculation. Or test 1 control tube for each 9 sample tubes.
9. EDTA should not be as anticoagulation, suggest to use heparin plasma.

Reference values for samples

1. Mouse

- T-SOD activity in serum(plasma): 110.446 ± 21.325 U/mL (The recommended sampling volume is 20 μL);
- T-SOD activity in liver tissue: 269.274±23.448 U/mgprot (0.25% tissue homogenate, the recommended sampling volume is 50 μL);
- T-SOD activity in brain tissue: 108.790 ± 13.494 U/mgprot (1% tissue homogenate, the recommended sampling volume is 50 μL);
- T-SOD activity in kidney tissue: 154.277±15.646 U/mgprot (0.5% tissue homogenate, the recommended sampling volume is 50 μL);
- T-SOD activity in skin tissue: 69.01 ± 19.95 U/mgprot (1% tissue homogenate, the recommended sampling volume is 50 μL);
- T-SOD activity in skeletal muscle tissue: 101.717±12.190 U/mgprot (1% tissue homogenate, the recommended sampling volume is 50 μL).

2. Rat

- T-SOD activity in serum(plasma): 262.786 ± 23.240 U/mL (The recommended sampling volume is 5 μL);

- T-SOD activity in whole blood: 21.554±2.116 U/mgHb
- T-SOD activity in liver tissue: 214.689±38.803 U/mgprot (0.25% tissue homogenate, the recommended sampling volume is 50 μL);
- T-SOD activity in brain tissue: 140.177 ±26.878 U/mgprot (1% tissue homogenate, the recommended sampling volume is 50 μL);
- T-SOD activity in kidney tissue: 136.825±24.763 U/mgprot (0.5% tissue homogenate, the recommended sampling volume is 50 μL);
- T-SOD activity in intestine tissue: 74.738±11.351 U/mgprot (1% tissue homogenate, the recommended sampling volume is 50 μL);
- T-SOD activity in lung tissue: 35.542 ± 15.465 U/mgprot (2% tissue homogenate, the recommended sampling volume is 50 μL);
- T-SOD activity in cerebral cortex tissue: 79.037±3.996 U/mgprot (1% tissue homogenate, the recommended sampling volume is 50 μL);
- T-SOD activity in sea horse tissue: 136.863±36.472 U/mgprot (1% tissue homogenate, the recommended sampling volume is 50 μL);
- T-SOD activity in cardiac muscle tissue: 128.292±9.129 U/mgprot (0.5% tissue homogenate, the recommended sampling volume is 50 μL).

3. **Rabbit**
   - T-SOD activity in serum(plasma): 429.04±31.60 U/mL (The recommended sampling volume is 10 μL).
4. **Human**
   - T-SOD activity in serum(plasma): 104.2±18.8 U/mL (The recommended sampling volume is 30 μL);
   - T-SOD activity in red blood cell: 19246±132 U/gHb (The recommended sampling volume is 10 μL);
   - T-SOD activity in whole blood: 21.554±2.117 U/mgHb

Note: Suggest every lab establish the own reference values range for samples, the reference value we provided just for reference.

This manual must be read attentively and completely before using this product.
May you have any problems, please contact our Technical Service Center for help.
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