BT LAB
Bioassay Technology Laboratory

Optimize Your Research

Superoxide Dismutase (SOD) Assay Kit

Catalog No: SH0039
Method: WST-1 Method
Specification: 96T

Application

The kit is used for the determination of SOD in serum, plasma, cerebrospinal fluid, pleural effusion, ascites, renal dialysis fluid, urine, semen, red blood cells, white blood cells, 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.

Detection significance

Superoxide dismutase is an enzyme that alternately catalyzes the dismutation (or partitioning) of the superoxide (O2-) radical into either ordinary molecular oxygen (O2) or hydrogen peroxide (H2O2). Superoxide is produced as a by-product of oxygen metabolism and, if not regulated, causes many types of cell damage. Hydrogen peroxide is also damaging and is degraded by other enzymes such as catalase. Thus, SOD is an important antioxidant defense in nearly all living cells exposed to oxygen. One exception is Lactobacillus plantarum and related lactobacilli, which use a different mechanism to prevent damage from reactive (O2-).

Detection principle

The activity of SOD was measured by WST-1 method in this kit and the principles of the WST-1 method refer to Fig.1. Xanthine Oxidase (XO) can catalyze WST-1 react with O2 to generate a water-soluble formazan dye. SOD can catalyze the disproportionation of superoxide anions, so the reaction can be inhibited by SOD, and the activity of SOD is negatively correlated with the amount of formazan dye. Therefore, the activity of SOD can be determined by the colorimetric analysis of WST-1 products.

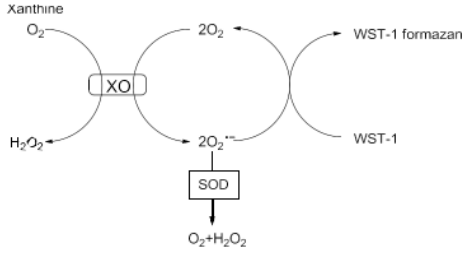

Fig. 1

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Kit components

	Components	Specifications	Storage
Reagent 1	Buffer Solution	15 mL × 1 vial	4 ℃, 3 months
Reagent 2	Substrate Solution	0.075 mL × 1 vial	4 ℃, 3 months, shading light
Preparation of substrate applied solution: Mix the Reagent 1 and Reagent 2 at the ratio of 200:1 thoroughly. Prepare it when it will be used, the remaining substrate applied solution can be stored for 7 days at 2~8 ℃.			
Reagent 3	Enzyme Solution	0.15 mL ×1 vial	-20 ℃, 3 months
Reagent 4	Enzyme Diluent	2 mL × 1 vial	4 ℃, 3 months
Preparation of enzyme working solution: Mix the Reagent 3 and Reagent 4 at the ratio of 1:10 thoroughly. Prepare it when it will be used, the remaining enzyme working solution can be stored for 3 days at 4 ℃.			

Note: Reagent 2 should melt slowly on ice. It is recommended to aliquot the Reagent 2 into smaller quantities for optimal storage. Avoid repeated freeze-thaw cycles. Bring all reagents to room temperature before use.

Experimental instrument

96-well microplate, Micropipette, Multichannel pipettor, Vortex mixer, Centrifuge, Microplate reader (450 nm).

Preparation of sample

1. Serum/plasma:

(1) Observe the serum/plasma samples, centrifuge for 10 min at 3500 r/m if it's muddy. Collect the supernatant and carry out the assay immediately.

(2) The supernatant is diluted into difference concentration with normal saline, then take the pre- experiment.

2. Tissue:

10 % tissue homogenate: It is recommended to get detailed references from other literatures before assay aiming at different tissue types. Mince the tissues to small pieces, then be weighed and homogenized in normal saline on ice, the volume of normal saline (mL): the weight of the tissue (g)
=9:1. The tissue homogenate is centrifuged for 10 min at 2500~3000 r/m, Collect the

supernatant and carry out the assay immediately. The supernatant is diluted into difference concentration with PBS, then take the pre-experiment. Meanwhile, determine the concentration of supernatant (E-BC- K318, E-BC-K168, E-BC-K165).
Note: The supernatant must be clarification, the rotational speed and time can be increased appropriately.

3. Cells:
(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 r/m, discard supernatant.
(2) Resuspend adherent cells in 1 mL cold PBS, centrifuge for 10 min at 1000 r/m, discard supernatant.
(3) Resuspend cells in PBS (1 ×) 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

1. **Control well**: add 20 μL of double distilled water and 20 μL of enzyme working solution.
Blank$_{control}$ well: add 20 μL of double distilled water and 20 μL of enzyme diluent.
Sample well: add 20 μL of Sample and 20 μL of enzyme working solution.
Blank$_{Sample}$ well: add 20 μL of Sample and 20 μL of enzyme diluent.
2. Add 200 μL of substrate applied solution with a multi-channel pipettor into each well and mix fully.
3. Incubate at 37 ℃ for 20 min. Measure the OD values of each well with Microplate Reader.
Note: It can be refer to the following operating table

	Control well	Blank$_{Control}$ well	Sample well	Blank$_{Sample}$ well
Sample (μL)			20	20
ddH_2O (μL)	20	20		
Enzyme working solution (μL)	20		20	
Enzyme diluent (μL)		20		20
Substrate applied solution (μL)	200	200	200	200
Mix fully and incubate at 37 ℃ for 20 min. Measure the OD values of each well with Microplate Reader.				

[Notices]: Control, BlankControl, Blanksample experiment only need 1-2 wells for each experiment.

Note

1.In order to reduce errors in different well, please use multiple-channel pipettes to add substrate applied solution.

2.It is recommended to take a 96 wells microplate and a multi-channel pipettor for operation to reduce errors between wells, mix fully to ensure the samples fully contact with reagents.

3.The SOD inhibition ratio can arrive 100%.

4.Before the formal experiment, it needs to choose one or two samples for diluting a series of diluent and determine the dilution factor when the SOD inhibition ratio is 40%~60%.

Calculation of results

1. Definition:

When SOD inhibition ratio in this reaction system reach 50%, the corresponding enzyme level is 1 SOD activity unit (U).

2. Calculation formula:

For serum/plasma sample:

Inhibition ratio of SOD (%)

$$= \frac{(A_{Control}-A_{Blank\ control})-(A_{Sample}-A_{Blank\ sample})}{A_{Control}-A_{Blank\ control}} \times 100\%$$

SOD activity (U/mL)

$$= \text{Inhibition ratio of SOD (\%)} \div 50\% \times \left(\frac{0.24mL}{0.02mL}\right) \times \text{Dilution factor of sample beforetested}$$

For Tissue and Cells:

Inhibition ratio of SOD (%)

$$= \frac{(A_{Control}-A_{Blank\ control})-(A_{Sample}-A_{Blank\ sample})}{A_{Control}-A_{Blank\ control}} \times 100\%$$

SOD activity (U/mg prot)

$$= \text{Inhibition ratio of SOD(\%)} \div 50\% \times \left(\frac{0.24mL}{0.02mL}\right) \div \text{Concentration of the protein tested (mgprot/mL)}$$

Technical parameters

1. The sensitivity of the kit is 0.2 U/mL.
2. The intra-assay CV is 2.9 % and the inter-assay CV is 3.7%.

3. The recovery of the kit is 96.6 %.

Notes

1. This kit is for research use only.
2. Please progress strictly with operation procedures.
3. Do not use components from different batches of kit.

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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