(FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS!)

Total Superoxide Dismutase (T-SOD) Activity Assay Kit (WST-8 Method)

Catalog No.: BC00014

Size: 100T

Please read the instructions carefully before use. If you have any questions or need further help during experiment, please don't hesitate to contact us through the following methods:

✉ Email (Sale) order@enkilife.com

✉ Email (Techsupport) techsupport@enkilife.com

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Shelf life: Please refer to the label on the outer package.

Techsupport: In order to provide you with better service, please inform us the lot number on the label of the outer package.

Basic Information

Product Name	Total Superoxide Dismutase(T-SOD) Activity Assay Kit(WST-8 Method)
Detection Method	Colorimetric
Sample Type	Serum, Whole blood, Red blood cell extracts, Cells, Tissues
Assay Type	Enzyme activity
Detection Instrument	Microplate reader (420-480 nm, optimal detection wavelength is 450 nm)

Product Introduction

Total SOD Activity Detection Kit (WST-8 method) is a kit based on the colorimetric reaction of WST-8, which detects the activity of SOD, i.e. superoxide dismutase, in cells, tissues or other samples by colorimetry.
Superoxide dismutase (SOD) can catalyze the dismutation of superoxide anions to generate hydrogen peroxide (H_2O_2) and oxygen (O_2), and is an important antioxidant enzyme in organisms. There are currently a variety of SOD activity assays, among which the NBT (nitroblue tetrazolium) method is widely used due to its ease of use. However, the formazan dye produced by the NBT method has poor water solubility and is easily interacted with the reduced xanthine oxidase, resulting in an inhibition rate that is less than 100%, which affects the sensitivity and accuracy of the assay. The cytochrome C method is also a commonly used method for detecting SOD activity, but cytochrome C has high oxidative activity and is easily interfered by reducing agents in the sample. In addition, this method requires continuous measurement of absorbance values, and has a relatively low sensitivity for SOD detection, and is not suitable for the detection of large quantities of samples. The WST-1 method is similar to the WST-8 method, and is more advanced than the above methods. This kit adopts the WST-8 method, which has better stability and higher sensitivity among the current SOD determination methods, and can detect SOD as low as 0.5U/ml.

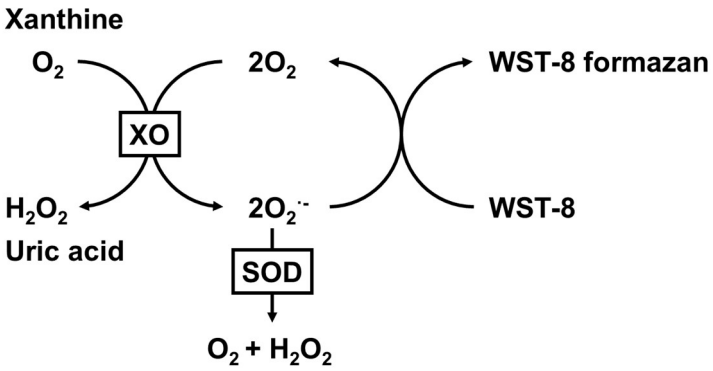
Product Features

● **Methodological advantages:**
1. The reaction product of WST-8 is a stable water-soluble product. The SOD enzyme activity can be measured by absorbance detection at a single time point, which is suitable for high-throughput screening research. When measuring SOD enzyme activity with the WST-8 method, the maximum inhibition percentage can be close to 100 %, and can not be interfered by some common interference factors, so that the detection effect is significantly improved than some other common methods.
2. The performance of this kit is better than the total SOD activity detection kit (NBT method) of similar products.

3. The purpose of this product is the same as the total SOD activity detection kit (NBT method) of similar products. The change in absorbance caused by the same amount of SOD is significantly greater than that of the NBT method, and the linear range is wider.
● **Convenience:** The SOD sample preparation solution provided in this kit can directly lyse cells without homogenization, making the operation more convenient.

Principle

WST-8 can react with superoxide anions (O2-) produced by xanthine oxidase (XO) to produce water-soluble formazan dye. Since SOD can catalyze the dismutation of superoxide anions, this reaction step can be inhibited by SOD. Therefore, the activity of SOD is negatively correlated with the amount of formazan dye generated, so the enzyme activity of SOD can be calculated by colorimetric analysis of the WST-8 product. The schematic diagram of this kit is shown as below



Components

Serial number	Components	Size(100T)	Storage
Reagent 1	SOD Sample Preparation Solution	50ml	-20 ℃
Reagent 2	SOD Assay Buffer	50ml	-20 ℃
Reagent 3	WST-8	800μl	-20 ℃ , avoid light
Reagent 4	Enzyme Solution	100μl	-20 ℃
Reagent 5	Reaction Start Solution (16X)	150μl	-20 ℃
	Microplate(96 wells)	1 plate	RT
	Plate Sealer	2 pieces	RT

Storage

The unopened kit can be stored at -20 ℃ for 12 months .

Experimental Preparation

- Sample processing

1. Cell samples

For adherent cells, aspirate the cell culture medium, wash once with PBS or saline precooled at 4°C or in an ice bath, add 100-200μl of the SOD sample preparation solution provided in this kit per 1 million cells, and blow appropriately to fully lyse the cells; for suspended cells, centrifuge at 600 x g for 5 minutes to collect the cells, wash once with PBS or saline precooled at 4°C or in an ice bath, add 100-200 μl of the SOD sample preparation solution per 1 million cells, and blow appropriately to fully lyse the cells. Centrifuge at 4°C at about 12,000 x g for 3-5 minutes, and take the supernatant as the sample to be tested.

2. Tissue samples

The animals were perfused with physiological saline (0.9% NaCl, containing 0.16mg/ml heparin sodium) to remove blood and obtain tissue samples. Take an appropriate amount of tissue sample and add 100μl of SOD sample preparation solution per 10mg of tissue for homogenization at 4°C or in an ice bath (a glass homogenizer or various common electric homogenizers can be used). Centrifuge at about 12,000 x g at 4°C for 3-5 minutes, and take the supernatant as the sample to be tested.

3. Plasma or red blood cell samples

Collect blood in an anticoagulant tube and mix thoroughly by inversion. Centrifuge at 600 x g for 10 minutes at 4℃, transfer the supernatant to another new 1ml centrifuge tube, and dilute with an appropriate amount of saline to use as a plasma sample for testing. For red blood cell samples, refer to the preparation method of suspended cell samples in step (1).

- Sample dilution

1. After the above samples are prepared, the protein concentration can be measured using BCA Protein Assay Kit (BCA Method)(BC00006) produced by EnkiLife . The average activity of SOD in a cell or tissue homogenate sample of 1 μg protein is about 1 unit (different cells and tissues will vary greatly, and this activity range is only for preliminary reference). Prepare 20-100 μg protein for each sample is usually sufficient for subsequent detection.

2. According to the protein concentration and the expected amount of protein used, dilute the sample appropriately with the SOD detection buffer provided in this kit. For example, the supernatant of 10% homogenate of mouse liver tissue (the weight ratio of tissue to homogenate is 1:10) usually needs to be diluted 10-100 times. If the prepared sample is measured on the same day, it can be stored in an ice bath; if the measurement cannot be completed on the same day, it can be frozen at -70℃, but it is recommended to complete the measurement on the same day as much as possible.

- Preparation of the assay kit

1. Preparation of WST-8/enzyme working solution
Prepare an appropriate amount of WST-8/enzyme working solution according to the volume of 160μl per reaction. Evenly mix 151μl SOD assay buffer, 8μl WST-8 and 1μl enzyme solution to prepare 160μl WST-8/enzyme working solution. Prepare an appropriate amount of WST-8/enzyme working solution according to the number of samples to be tested (including standards). For specific preparation methods, please refer to the table below. The prepared WST-8/enzyme working solution can be stored at 4℃ or in an ice bath and can be used on the same day, but it is recommended to prepare it as soon as possible. Note: Since the amount of enzyme solution is small and it is easy to precipitate, it must be carefully centrifuged before use, and then properly mixed before use.

Sample No.to be tested	**1**	**10**	**20**	**50**
SOD Assay Buffer (μl)	151	1510	3020	7550
WST-8(μl)	8	80	160	400
Enzyme solution (μl)	1	10	20	50
WST-8/enzyme working solution (μl)	160	1600	3200	8000

2. Preparation of reaction start solution
Dissolve the reaction start solution (16X) in the kit and mix well. Dilute it by adding 15μl SOD detection buffer to every 1μl reaction start solution (16X). Mix well to get the reaction start working solution. Prepare an appropriate amount of reaction start working solution according to the number of samples to be tested (including standards). The prepared reaction start working solution can be stored at 4℃ or in an ice bath and can be used on the same day, but it is recommended to prepare it as soon as possible.
3. (Optional) Preparation of SOD Standard
You need to prepare your own SOD standard. Use the SOD sample preparation solution provided by this kit (when the sample is prepared with the SOD sample preparation solution provided by the kit) or SOD detection buffer (when the sample is a sample that does not require treatment such as blood) to dilute the SOD standard by half dilution to the following series of concentrations: 100U/ml, 50U/ml, 25U/ml, 12.5U/ml, 6.25U/ml. In the subsequent tests, take 20 microliters each and test with the reference sample. The detection effect of the SOD standard is shown in Figure 2. Note: In order to avoid the decrease of SOD enzyme activity after dilution, the SOD standard should be diluted and used immediately; this kit does not require SOD as a standard for the detection of SOD, but the SOD standard can be used as a positive control or as a reference for the quantification of SOD activity.

Operation process

1.Please refer to the table below to set up sample wells and various blank control wells

using a 96-well plate. Add the sample to be tested and other solutions in sequence according to the table below. Add the reaction start working solution and mix thoroughly.

	Sample/Standard	Blank control 1 (Blank1)	Blank control 2 (Blank2)	Blank control 3 (Blank3)
Samples to be tested	20μl	--	--	20μl
SOD Assay Buffer	--	20μl	40μl	20μl
WST-8/Enzyme working solution	160μl	160μl	160μl	160μl
Reaction start solution	20μl	20μl	--	--

Notice:

- If the sample has no color and does not contain antioxidants, blank control 3 may not be performed.
- Note: The reaction will start immediately after adding the reaction start working solution. You can operate at a low temperature or use a discharge gun to reduce the error caused by the difference in the time of adding the reaction start working solution between each well.

2.Reaction: Incubate at 37°C for 30 minutes.

3.Detection: Measure absorbance at 450nm. If there is no 450nm filter, a 420-480nm filter can be used. You can choose to set 600nm (or above 600nm, such as 650nm) as the reference wavelength (also called reference wavelength), and the absorbance reading at 450nm minus the absorbance reading at the reference wavelength can be used as the actual measured reading.

Result calculation

1. Calculation of inhibition percent

If the sample is colored or contains antioxidants, refer to the following formula to calculate the inhibition percentage:

$$\text{Inhibition percentage}=\frac{(A_{\text{Blank control1}}-A_{\text{Blank control2}})-(A_{\text{Sample}}-A_{\text{Blank control3}})}{A_{\text{Blank control1}}-A_{\text{Blank control2}}}\ X100\%$$

If the sample has no color and does not contain antioxidants, calculate the inhibition percentage using the following formula:

$$\text{Inhibition percentage}=\frac{A_{\text{Blank control1}}-A_{\text{Sample}}}{A_{\text{Blank control1}}-A_{\text{Blank control2}}}\ X100\%$$

If the calculated inhibition percentage is less than 10% or greater than 90%, the sample usually needs to be re-measured. Try to keep the inhibition percentage of the sample within the range of 10-90%. If the inhibition percentage measured is too high, the sample needs to be appropriately diluted; if the inhibition percentage measured is too low, a higher concentration of the sample needs to be re-prepared.

2. Definition of SOD enzyme activity unit

When the inhibition percentage in the above xanthine oxidase coupled reaction system is 50%, the SOD enzyme activity in the reaction system is defined as one enzyme activity unit (unit or U). Note: There are many ways to define the activity unit of SOD, and different activity units need to be appropriately converted according to their different definitions.

3. Calculation of SOD enzyme activity:

The calculation formula of SOD enzyme activity is as follows:

$$\text{SOD enzyme activity units in the sample to be tested} = \left(\frac{\text{Inhibition percentage}}{\text{1-Inhibition percentage}}\right) \text{units}$$

For example, when the inhibition percentage is 50%, the SOD enzyme activity unit in the test sample = 50%/(1-50%) units = 1 unit; when the inhibition percentage is 60%, the SOD enzyme activity unit in the test sample = 60% / (1-60%) units = 1.5 units.

4. If the sample is a homogenate of cells or tissues, the SOD activity unit can be converted to U/g or U/mg protein according to the protein concentration and dilution multiple of the sample. If the sample is a red blood cell extract, it can be converted to U/g hemoglobin or U/mg hemoglobin according to the hemoglobin content.

Notes

1. The sample to be tested can be stored at -70℃ for 1 month. Please note that repeated freezing and thawing will cause partial inactivation of SOD.
2. If a kit cannot be used up within 3 times, the WST-8 in it needs to be appropriately packaged when used for the first time to avoid a decrease in detection effect due to repeated freezing and thawing.
3. The diluent used when diluting the standard should be as consistent as possible with the sample. When calculating the actual enzyme activity in the sample, pay attention to whether it has been diluted. If so, it should be multiplied by the dilution factor.
4. Antioxidants will interfere with the detection of this kit. For example, 0.1mM ascorbic acid and 5mM GSH will significantly increase the measured absorbance. Although the sample has no color at this time, if a blank control 3 is set, the interference of antioxidants in the sample can be eliminated.
5. This product is for Research Use Only and shall not be used for clinical diagnosis or treatment, food or medicine, or stored in ordinary residences.
6. This kit can obtain the test results without drawing a standard curve. If the user needs and prepares the SOD standard by himself (not provided in this kit), the standard curve method can also be used to calculate the SOD enzyme activity. The reference scheme of the standard curve method is: first use this kit to draw the inhibition percentage curve of the SOD standard (as shown in Figure 2C), and then calculate the SOD enzyme activity

unit in the sample based on the inhibition percentage detected in the sample and the inhibition percentage curve of the standard.

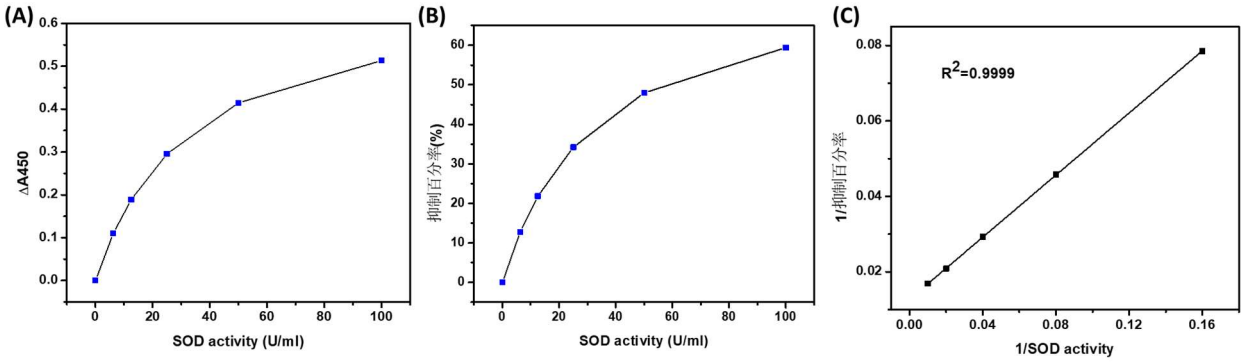


Figure 2. The detection effect of this kit on SOD standard. A) The vertical axis ΔA450 is the absorbance difference between the blank control 1 and the SOD standard well after incubation for 30 minutes. B) The SOD enzyme activity and ΔA450 (Figure A) and the inhibition percentage (Figure B) are nonlinear. C) 1/SOD enzyme activity and 1/inhibition percentage are linearly related and can be used as a standard curve. The data in the figure are for reference only. The slope and intercept of the standard curve obtained in the actual measurement may be significantly different from the above figure due to different specific reaction conditions.

7. If conditions permit, the kinetic method can also be used to detect the enzyme activity of SOD when using this kit. Usually, after step 3a, the sample can be incubated at 37°C and the absorbance can be continuously measured at 450nm for 30 minutes. The percentage of inhibition is calculated based on the slope of the absorbance change within 30 minutes:

$$\text{Inhibition percentage}=\frac{(\text{Slope}_{\text{Blank control1}}-\text{Slope}_{\text{Blank control2}})-(\text{Slope}_{\text{Sample}}-\text{Slope}_{\text{Blank control3}})}{\text{Slope}_{\text{Blank control1}}-\text{Slope}_{\text{Blank control2}}}\ X100\%$$

The remaining calculation methods are the same as the above non-kinetic calculation methods. The detection and calculation of the kinetic method are more accurate, but the detection is relatively troublesome. This kit usually uses the non-kinetic method.