

SOD Assay Kit-WST

Technical Manual

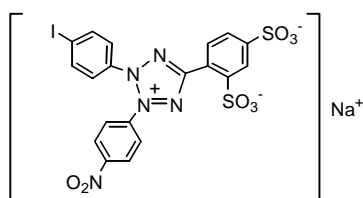
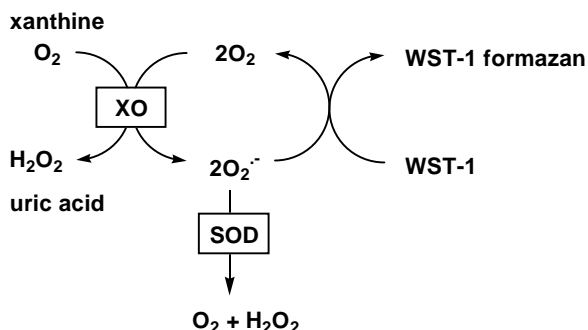
Product Code: S311-10 (500 tests)

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GENERAL INFORMATION

Superoxide dismutase (SOD), which catalyzes the dismutation of the superoxide anion ($O_2^{\cdot-}$) into hydrogen peroxide and molecular oxygen, is one of the most important antioxidative enzymes. In order to determine the SOD activity, several direct and indirect methods have been developed. Among these methods, an indirect method using nitroblue tetrazolium (NBT) is commonly used due to its convenience and ease of use. However, there are several disadvantages to the NBT method, such as poor water solubility of the formazan dye and the interaction with the reduced form of xanthine oxidase. **SOD Assay Kit-WST** allows very convenient SOD assaying by utilizing Dojindo's highly water-soluble tetrazolium salt, WST-1 (2-(4-iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt) that produces a water-soluble formazan dye upon reduction with a superoxide anion. The rate of the reduction with $O_2^{\cdot-}$ are linearly related to the xanthine oxidase (XO) activity, and is inhibited by SOD, as shown in Figure 1. Therefore, the IC_{50} (50% inhibition activity of SOD or SOD-like materials) can be determined by a colorimetric method (Patent filing).



WST-1

Figure 1. Principle of the SOD Assay Kit

Figure 2 shows the absorption spectrum of WST-1 formazan. Since the absorbance at 440 nm is proportional to the amount of superoxide anion, the SOD activity as an inhibition activity can be quantified by measuring the decrease in the color development at 440 nm.

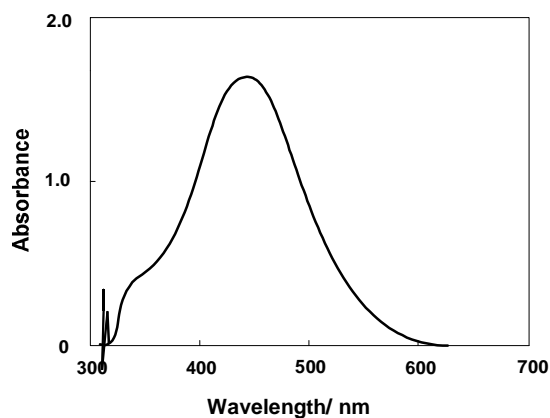


Figure 2. Absorption spectrum of WST-1 formazan.

CONTENTS OF THE KIT

- WST Solution 5 ml x 1
- Enzyme Solution 100 μ l x 1
- Buffer Solution 100 ml x 1
- Dilution Buffer 50 ml x 1
- Manual 1 booklet

STORAGE

Please store at 0-5 °C. The kit is stable for 1 year at 0-5 °C storage. WST Working Solution is stable for 2 months at 4 °C, and Enzyme Working Solution is stable for 3 weeks at 4 °C. Protect WST Solution and WST Working Solution from light.

HOW TO USE SOD ASSAY KIT

1. Required Equipments and Materials

- Plate reader (450 nm filter)
- 96-well microplate
- 10 μ l & 100-200 μ l pipettes and a multi-channel pipette
- Incubator
- Superoxide dismutase (SOD), if necessary for the preparation of an inhibition curve

2. Protocol

Preparation of Solutions (for one 96-well plate)

- WST Working Solution
Dilute 1 ml of WST Solution with 19 ml of Buffer Solution.
- Enzyme Working Solution
Centrifuge the Enzyme Solution tube for 5 sec. Mix by pipeting*, and dilute 15 μ l of Enzyme Solution with 2.5 ml of Dilution Buffer.

* The Enzyme Solution is separated into two layers. Therefore, omitting the pipeting process will result in inaccurate experiment results.

- SOD Solution (for assay monitoring, if necessary):
Dilute SOD with Dilution Buffer to prepare SOD Standard Solution as follows.

200 U/ml, 100 U/ml, 50 U/ml, 20 U/ml, 10 U/ml, 5 U/ml,
1 U/ml, 0.1 U/ml, 0.05 U/ml, 0.01 U/ml, 0.001 U/ml

General Protocol (illustrated on page 4)

* Refer to Table 1 for the amount of solutions in each well. If you are using a SOD standard, set up wells for it in the same manner as the sample.

- 1) Add 20 µl of sample solution to each sample and blank 2 well, and add 20 µl of ddH₂O (double distilled water) to each blank 1 and blank 3 well.
- 2) Add 200 µl of WST Working Solution to each well, and mix.
- 3) Add 20 µl of Dilution Buffer to each blank 2 and blank 3 well.
- 4) Add 20 µl of Enzyme Working Solution to each sample and blank 1 well, and then mix thoroughly*.
- 5) Incubate the plate at 37 °C for 20 min.
- 6) Read the absorbance at 450 nm using a microplate reader.
- 7) Calculate the SOD activity (inhibition rate %) using the following equation.

$$\text{SOD activity (inhibition rate \%)} = \frac{\{(A_{\text{blank 1}} - A_{\text{blank 3}}) - (A_{\text{sample}} - A_{\text{blank 2}})\}}{(A_{\text{blank 1}} - A_{\text{blank 3}})} \times 100$$

* Since the superoxide will be release immediately after the addition of Enzyme Working Solution to a well, use a multi-channel pipette to avoid the reaction time lag of each well.

Table 1: Amount of each solution for sample, blank 1, 2 and 3

	sample	blank 1	blank 2*	blank 3
Sample Solution	20 µl	-	20 µl	-
ddH ₂ O	-	20 µl	-	20 µl
WST Working Solution	200 µl	200 µl	200 µl	200 µl
Enzyme Working Solution	20 µl	20 µl	-	-
Dilution Buffer	-	-	20 µl	20 µl

* If Sample Solution has visible color, set up separate "blank 2" lane.

	1	2	3	4	5	6	7	8	9	10	11	12
A	SOD 200 U/ml			Blank 1			Blank 2			Blank 3		
B	SOD 100 U/ml			SOD 0.05 U/ml			SOD 0.01 U/ml			SOD 0.001 U/ml		
C	SOD 50 U/ml			Sample 1			Sample 7			Sample 13		
D	SOD 20 U/ml			Sample 2			Sample 8			Sample 14		
E	SOD 10 U/ml			Sample 3			Sample 9			Sample 15		
F	SOD 5 U/ml			Sample 4			Sample 10			Sample 16		
G	SOD 1 U/ml			Sample 5			Sample 11			Sample 17		
H	SOD 0.1 U/ml			Sample 6			Sample 12			Sample 18		

Fig. 3. Sample and blank arrangement on a 96-well plate including SOD standard solutions.

If SOD standard solutions are not necessary, use these wells for sample solutions

3. Interference

Reducing agents, such as ascorbic acids and reduced forms of glutathiones, interfere with the SOD assay. The following are the concentrations of materials that cause 10% increase in the O.D. value. Please note that since the increase in the O.D. values can be subtracted as the O.D. of blank 2, these materials do not interfere with the actual SOD assay.

Bovine Serum Albumin: 5% w/v
(no O.D. increase is observed)

Ascorbic acid: 0.1 mM

Glutathione, reduced form: 5 mM

4. Inhibition curve

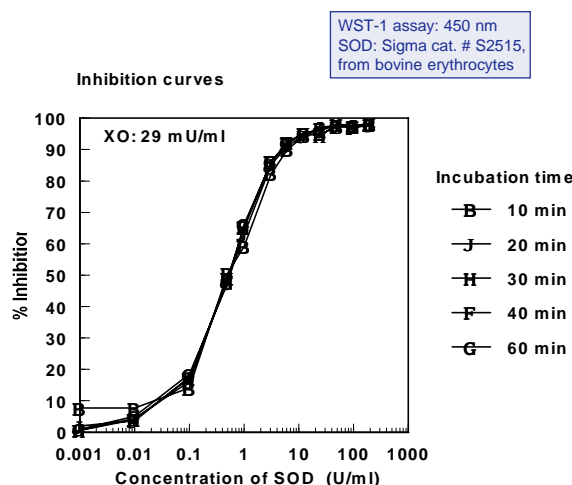


Fig. 4: Inhibition curves prepared by WST-1 assay with different incubation time

NOTES

1. Store at 0-5 °C. Do not use the kit after the expiration date.
2. For an accurate measurement, the use of multiple wells per sample is recommended (see Fig. 3).
3. Since superoxide will be released immediately after the addition of Enzyme Working Solution to a well, use a multi-channel pipette to avoid the reaction time lag of each well.
4. Inhibition activity can also be determined by a kinetic method. Please determine an incubation time range that has a linearity of the slope before the assay. A good linearity should be observed up to 20 min. For the calculation, use the following equation:

SOD activity (inhibition rate %) =

$$\frac{\{(S1 - S3) - (SS - S2)\}}{(S1 - S3)} \times 100$$

S1: slope of blank 1

S2: slope of blank 2

S3: slope of blank 3

SS: slope of sample

PRODUCT CODE AND PRICE

Product	Unit	Product code	Price (\$)
SOD Assay Kit-WST	500 tests	S311-10	210.00

RELATED PRODUCTS

Product Name	Unit	Product Code	Price (\$)
WST-1	100 mg	W201-10	90.00
WST-1	500 mg	W201-12	280.00
Total Glutathione Quantification Kit	100 tests	T419-10	175.00

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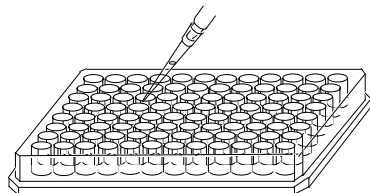
- NOTES -

General Protocol at a Glance

Read Technical Information carefully prior to using this General Protocol

Step 1)

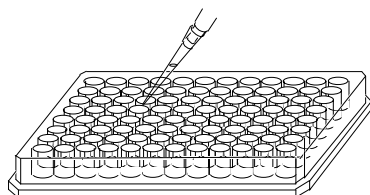
Add 20 μ l of sample solution to each sample and Blank 2 well, and add 20 μ l of ddH₂O to each Blank 1 and Blank 3 well^{a)}.



a) see Fig.3, page 2

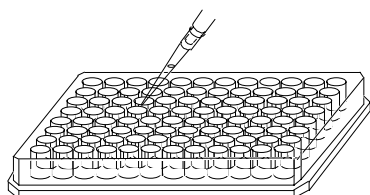
Step 2)

Add 200 μ l of WST Working Solution to each well, and mix.



Step 3)

Add 20 μ l of Dilution Buffer to each Blank 2 and Blank 3 well.^{b)}

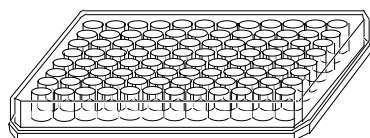


b) see Fig. 3, page 2

Step 4)

Using a multi-channel pipette, add 20 μ l of Enzyme Working Solution to each sample and Blank 1 well^{b)}, and then mix thoroughly. Incubate the plate at 37 °C for 20 min.

37 °C, 20 min incubation



Step 5)

Read the absorbance at 450 nm using a microplate reader.