

Technical Bulletin

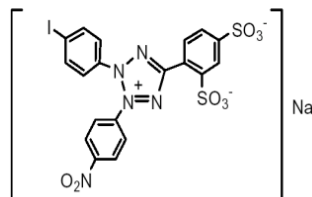
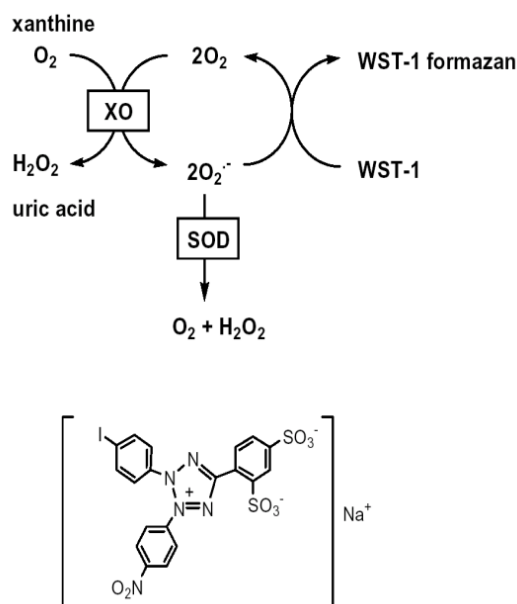
## SOD Assay Kit

Catalogue Number 19160

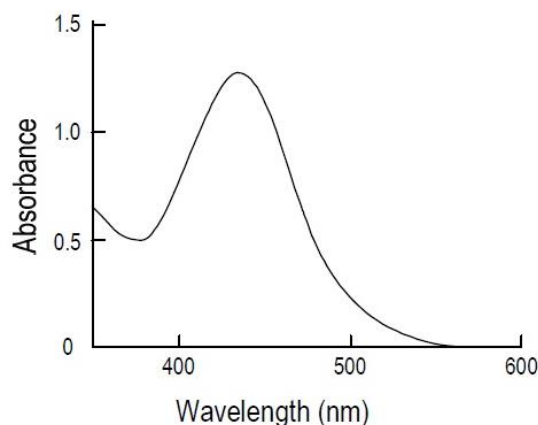
### Product Description

Superoxide dismutase (SOD), which catalyzes the dismutation of the superoxide anion ( $O_2^-$ ) 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.

The SOD Assay Kit allows very convenient SOD assaying by utilizing the 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  $O_2^-$  is 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. 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 1.**  
SOD Inhibition Assay Mechanism



**Figure 2.**  
Absorption spectrum of WST-1 formazan



## Components

The kit is sufficient for 500 colorimetric assays in 96-well plates.

- Reagent Working Solution (WST Solution) 5 mL  
Catalogue Number 53456-5ML-F
- Buffer Solution 100 mL  
Catalogue Number 43525-100ML-F
- Enzyme Working Solution 100 µL  
Catalogue Number 74043-100UL-F
- Dilution Buffer 50 mL  
Catalogue Number 07437-50ML-F

## Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (e.g., multichannel pipettor)
- Plate reader capable of 450 nm.
- Clear plates for colorimetric assays (Catalogue number M2936 or equivalent)  
Cell culture or tissue culture treated plates are not recommended.
- Incubator
- Superoxide dismutase (SOD), if necessary for the preparation of an inhibition curve.  
(Catalogue number S7571 or equivalent)

## Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

## Storage/Stability

Store all components at 2-8 °C.

## Preparation Instructions

### WST Working Solution

Dilute 1 ml of Reagent Working Solution (WST Solution) with 19 ml of Buffer Solution. The WST Working Solution is stable for 2 months at 2-8 °C.

### Enzyme Working Solution

Centrifuge the Enzyme Working Solution tube for 5 seconds. Mix by pipetting, dilute 15 µl of Enzyme Solution with 2.5 ml of Dilution Buffer. The Enzyme Working Solution is stable for 3 weeks at 2-8 °C.

### Sample Preparation

**Cells (Adherent cells:  $9 \times 10^6$  cells, Leukocytes:  $1.2 \times 10^7$  cells)**

1. Harvest cells with a scraper, centrifuge at 2,000 g for 10 min at 4 °C and discard the supernatant.
2. Wash the cells with 1 ml PBS and centrifuge at 2,000 g for 10 min at 4 °C. Discard the supernatant. Repeat this step.
3. Break cells using the freeze-thaw method (-20 °C for 20 min, then 37 °C bath 10 min, repeat twice).
4. Add 1 mL PBS. If necessary, sonicate the cell lysate on an ice bath (60 W with 0.5 sec interval for 15 min).
5. Centrifuge at 10,000 g for 15 min at 4°C.

### Plant or Vegetable (200 mg)

1. Add 1 mL distilled water and homogenize the sample using a homogenizer with beads.
2. Filter the homogenate with paper filter and lyophilize the filtrate.
3. Measure the weight of the lyophilized sample and dissolve with 0.1 M phosphate buffer (pH 7.4) to prepare sample solution.

### Tissue (100 mg)

1. Wash the tissue with saline to remove as much blood as possible. Blot the tissue with paper towels and measure its weight.
2. Add 400-900 µL sucrose buffer (0.25 M sucrose, 10 mM Tris, 1 mM EDTA, pH 7.4) and homogenize the sample using Teflon homogenizer. If necessary, sonicate the homogenized sample on an ice bath (60W with 0.5 second intervals for 15 min).
3. Centrifuge the homogenized sample at 10,000 g for 60 min at 4°C and transfer the supernatant to a new tube.

### Tea (antioxidant activity detection)

1. Add 60 mL boiled water to 10 g of tea, and leave it for 2.5 min.
2. Filter the extract with paper filter and then filter again with a 0.45 µm membrane filter.
3. Dilute the filtrate with distilled water to prepare sample solution.

### Erythrocytes or Plasma

1. Centrifuge 2-3 mL of anticoagulant treated blood (such as heparin 10 U/mL final concentration) at 600 g for 10 min at 4°C.
2. Collect the supernatant and dilute it with saline to use as a plasma sample.
3. Add the same volume of saline as the original sample (in step 1) to the pellet and suspend. Transfer the 0.4 mL of suspension to the 10 mL glass centrifuge tube and add 9.6 mL of saline.
4. Centrifuge the suspension at 600 g for 10 min at 4°C.
5. Discard the supernatant, then add the 10 mL of saline to the pellet and suspend. Centrifuge the suspension at 600 g for 10 min at 4°C.
6. Repeat step 5 twice.
7. Discard the supernatant, then add 4 mL of distilled water to the pellet and suspend (Erythrocytes are hemolyzed and SOD are released.). Add 1 mL of ethanol and 0.6 mL of chloroform to the hemolysate.
8. Shake vigorously the stopped test tube with a shaker for 15 min at 4°C.
9. Centrifuge 600 g for 10 min at 4°C, then transfer the upper water-ethanol phase to a new tube.
10. Take 0.1 mL of water-ethanol phase and mix with 0.7 mL of distilled water to prepare the erythrocytes sample.  
\*If the erythrocytes sample needs to be diluted, use 0.25% ethanol to dilute.

### Extracellular SOD (EC-SOD)

1. Prepare a 0.5 mL volume of Con A-sepharose column equilibrated with PBS.
2. Apply supernatant of a tissue homogenate on the column and leave

the column for 5 min at room temperature.

3. Add total 10 mL PBS to wash the column.
4. Add 1 mL of 0.5 M α-methylmannoside/PBS, and collect the eluate. Repeat 5 times.
5. Use the eluate for the SOD assay without dilution. If the SOD activity is high enough, dilute the eluate with PBS.

### Wine (antioxidant activity detection)

1. Filter wine with a 0.45 µm membrane filter.
2. Dilute the filtrate with distilled water to prepare sample solution.

### Sample Dilution

Dilute sample solution with Dilution buffer or saline to prepare sample solution. Using 6 tubes, serial dilute the sample 1:5 by adding 50 µl of sample into 200 µl Dilution Buffer.

## Procedure

### General Protocol

1. Add 20 µl of sample solution to each sample and blank 2 well and add 20 µl of ddH<sub>2</sub>O (double distilled water) to each blank 1 and blank 3 well. See Table 1.
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 mix thoroughly.

**Note:** 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.

**Table 1.**

Sample and Blank preparation

	Sample	Blank1	Blank2	Blank3
<b>Sample Solution</b>	20 µL	-	20 µL	-
<b>ddH<sub>2</sub>O</b>	-	20 µL	-	20 µL
<b>WST Working Solution</b>	200 µL	200 µL	200 µL	200 µL
<b>Dilution Buffer</b>	-	-	20 µL	20 µL
<b>Enzyme Working Solution</b>	20 µL	20 µL	-	-

Blank1: Coloring without inhibitor

Blank2: Sample blank

Blank3: Reagent blank

**Note:** If the color of the sample solution is strong, measure blank2 at each dilution of the sample.

5. Incubate the plate at 37°C for 20 min.
6. Read the absorbance at 450 nm using a microplate reader.

Calculate the SOD activity (inhibition rate %) using the following equation.

$$\frac{(A_{\text{blank1}} - A_{\text{blank3}}) - (A_{\text{sample}} - A_{\text{blank2}})}{(A_{\text{blank1}} - A_{\text{blank3}})} \times 100$$

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:

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

Where:

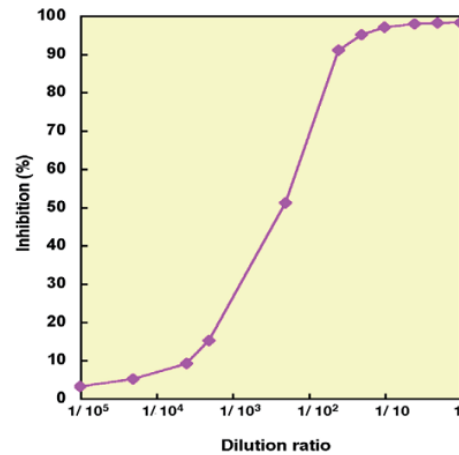
S1: slope of blank 1

S2: slope of blank 2

S3: slope of blank 3

SS: slope of sample

Unit Definition: One unit of SOD is defined as the amount of the enzyme in 20 µl of sample solution that inhibits the reduction reaction of WST-1 with superoxide anion by 50%.

**Figure 3.**

Inhibition Curve of Cu, Zn-SOD

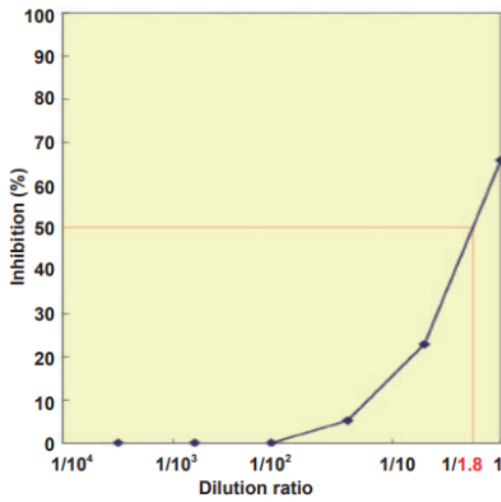
### Determination of SOD Activity

1. Read the dilution ratio at 50% inhibition (IC<sub>50</sub>) from inhibition curve.
2. Multiply the dilution ratio at IC<sub>50</sub> and at the sample preparation to obtain the SOD activity.

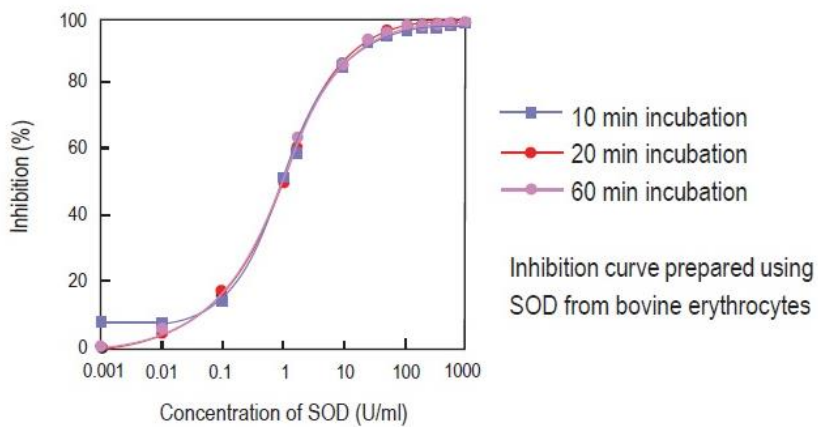
### Example of SOD Activity in Erythrocytes

1. Read the dilution ratio at 50% inhibition (IC<sub>50</sub>) from the inhibition curve (Fig.3). The dilution ratio at IC<sub>50</sub> is 1/1.8.
2. The SOD activity before dilution is "1.8 U/20 µL " from the definition above.
3. The SOD activity in 1 mL of sample is 1.8 / 0.02 = 90.0 U/mL.
4. Multiply the SOD activity calculated above by the necessary dilution ratio for sample preparation. In the case blood is diluted to 1/108 during the sample preparation, the SOD activity in blood is calculated as below.  
108 x 90.0 = 9,720 U/mL of blood

**Note:** Mn-SOD activity can be measured by adding potassium cyanide (final concentration: 1 mmol/l) or diethyldithiocarbamate (final concentration: 1 mmol/l) to the sample solution. These reagents inactivate Cu, Zn-SOD and extracellular-SOD activities.



**Figure 4.**  
Inhibition curve of erythrocytes



**Figure 5.**  
Inhibition curve by WST method



## Interference

Table 2 shows compatible concentration of possible interfering materials. If a sample contains these materials, please dilute the sample to be below their compatible concentration.

Since 2-mercaptoethanol and dithiothreitol cause a significant increase of the O.D. value, please remove them when a sample contains these materials.

**Table 2.**

Compatible concentration of possible interfering materials.

<b>Detergents</b>		<b>Solvents</b>		<b>Reducing Agents</b>		<b>Others</b>	
SDS	0.05%	Ethanol	25%	Glutathione reduced form	1.25 mmol/l	EDTA	2 mmol/l
Tween 20	0.5%	DMSO	5%	Ascorbic Acid	0.1 mmol/l	BSA	1% w/v
NP-40	0.5%						

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