# SOD Assay Kit - WST 100 tests — Technical Manual

**Technical Manual ( Japanese version) is available at http://www.dojindo.co.jp/manual/s311_100tests.pdf**

## 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 SOD activity, several direct and indirect methods have been developed. Among these methods, the indirect method using nitroblue tetrazolium (NBT) is commonly used due to the convenience and ease to use. However, there are several disadvantages in 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 assay by utilizing Dojindo's highly water-soluble tetrazolium salt, WST-1 (2-(4-Iodophenyl)- 3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2*H*-tetrazolium, monosodium salt) that produces a water-soluble formazan dye upon reduction with the superoxide anion. The rate of the reduction of WST-1 with $O_2^{\cdot-}$ are linearly related to the xanthine oxidase (XO) activity, and this reduction is inhibited by SOD, as shown in Fig. 1. Therefore, the $IC_{50}$ (50% inhibition activity of SOD or SOD-like materials) can be determined by the colorimetric method (Patent filing).

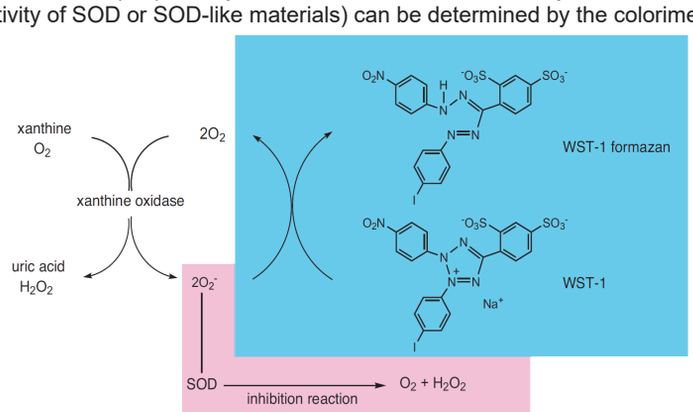


Fig.1 Principle of the determination of SOD activity using SOD Assay Kit - WST

## Kit Contents

| | | | |
|---|---|---|---|
| - WST solution | 1 ml x 1 | - Enzyme solution | 20 µl x 1 |
| - Buffer solution | 11 ml x 2 | - Dilution buffer | 10 ml x 1 |

## Storage Condition

Store at 0-5°C. Protect the WST solution and WST working solution from light.

## Required Equipment and Materials

- Plate reader (450 nm filter)
- 2-20 µl and 20-200 µl pipettes, a multi channel pipette
- 96-well microplate
- Incubator

## Precaution

-For dilution of sample, use Dilution buffer or saline.
-The Enzyme solution is separate into two layers. Therefore, omitting the pipetting process will result in inaccurate experimental results.
-For an accurate measurement, the use of multiple wells per sample is recommended.
-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.

## Sample preparation

General preparation methods are indicated in our home page. Access www.dojindo.com and go to SOD Assay Kit product information.

## Preparation of Solutions

**For one 96-well plate**

### WST working solution

Dilute 1 ml of WST solution with 19 ml of Buffer solution.
*WST working solution is stable for 2 months at 0-5 °C.

### Enzyme working solution

Centrifuge the Enzyme solution tube for 5 sec. Mix by pipetting, dilute 15 µl of Enzyme solution with 2.5 ml of Dilution buffer.
*Enzyme working solution is stable at 0-5 °C for 3 weeks.

### Sample solution

Dilute sample solution with Dilution buffer or saline to prepare sample solution as follows.
Dilution ratio : 1, 1/5, $1/5^2$, $1/5^3$, $1/5^4$, $1/5^5$, $1/5^6$

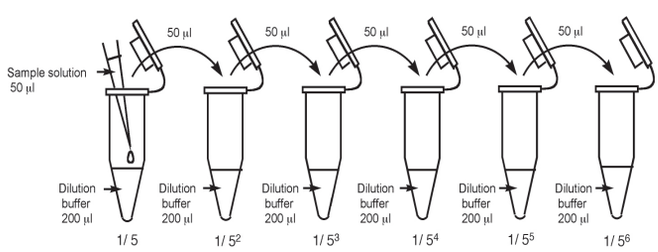


Fig. 2 Preparation of sample solution

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

| | sample | blank1 | blank2 | blank3 |
|---|---|---|---|---|
| Sample solution | 20 µl | - | 20 µl | - |
| $ddH_2O$ | - | 20 µl | - | 20 µl |
| WST working solution | 200 µl | 200 µl | 200 µl | 200 µl |
| Dilution buffer | - | - | 20 µl | 20 µl |
| Enzyme working solution | 20 µl | 20 µl | - | - |

**blank 1** : coloring without inhibitor
**blank 2** : sample blank
**blank 3** : reagent blank
∗ If the color of the sample solution is strong, measure blank 2 at each dilution of the sample.

## General Protocol

See Table 1, Fig.3 and 4.

1) Add 20 µl of sample solution to each sample and blank 2 well, and add 20 µl of $ddH_2O$ (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.

**SOD activity (inhibition rate %) = $[(A_{blank\ 1} - A_{blank\ 3}) - (A_{sample} - A_{blank\ 2})]/ (A_{blank\ 1} - A_{blank\ 3}) \times 100$**

** 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.*

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%) = [(S1 - S3) - (SS - S2)] / (S1 - S3) x 100**

S1: slope of blank 1, S2: slope of blank 2, S3: slope of blank 3, SS: slope of sample

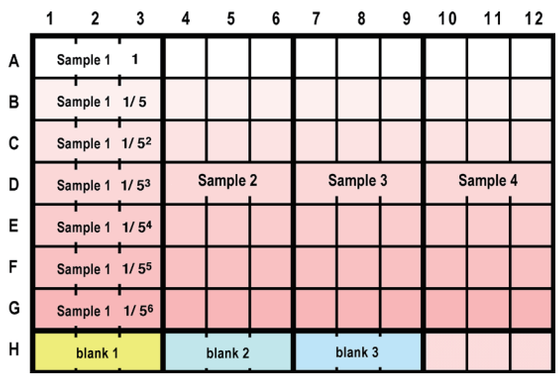


Fig.3 Example of sample and blank arrangement on a 96-well plate

## Inhibition Curve

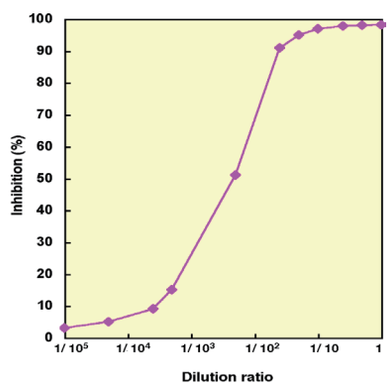


Fig.4 Inhibition curve of Cu, Zn-SOD

## Definition of SOD Unit

**『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%.』**

## Determination of SOD activity

1) Read the dilution ratio at 50% inhibition ($IC_{50}$) from inhibition curve.
2) Multiply the dilution ratio at $IC_{50}$ and at the sample preparation to obtain the SOD activity.

**Example : Determination of SOD activity in erythrocytes**

(The 1/108 diluted blood has been used as a sample solution.)

1) Read the dilution ratio at 50% inhibition ($IC_{50}$) from the inhibition curve (Fig.5). The dilution ratio at $IC_{50}$ 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 1ml 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

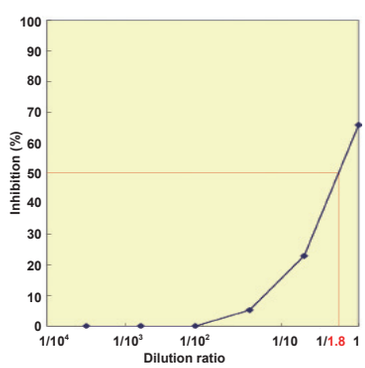


Fig.5 Inhibition curve of erythrocyte

## Determination of Mn-SOD activity

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.

## Interference

Table 2 shows compatible concetration of possible interfering materials. If 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 sample contains these materials.

Table 2. Compatible concentration of possible interfering materials for SOD assay

| Detergents | | Solvents | | Reducing agents | | others | |
|---|---|---|---|---|---|---|---|
| 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% | | | | | | |

*If you need more information, please contact Dojindo technical service.*


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