SOD Assay Kit - WST 500 tests

Technical Manual

Technical Manual (Japanese version) is available at http://www.dojindo.co.jp/manual/s311.pdf

General Information

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 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^- 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).

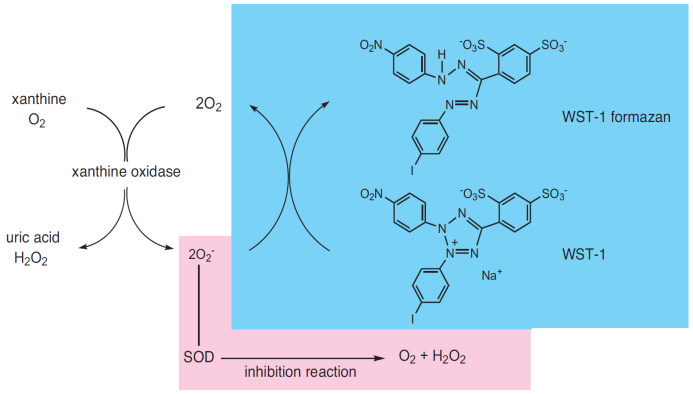

Fig.1 Principle of the determination of SOD activity using SOD Assay Kit - WST

Kit Contents

- WST solution	5 ml x 1	- Enzyme solution	100 µl x 1
- Buffer solution	100 ml x 1	- Dilution buffer	50 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, $1/5^2$, $1/5^3$, $1/5^4$, $1/5^5$, $1/5^6$

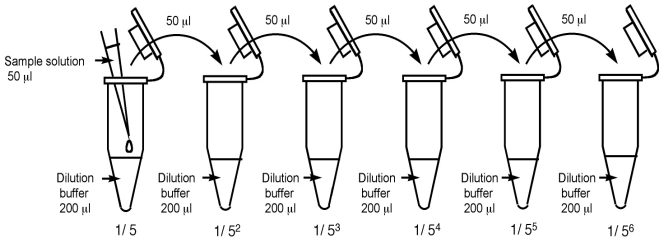

Fig. 2 Preparation of sample solution

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

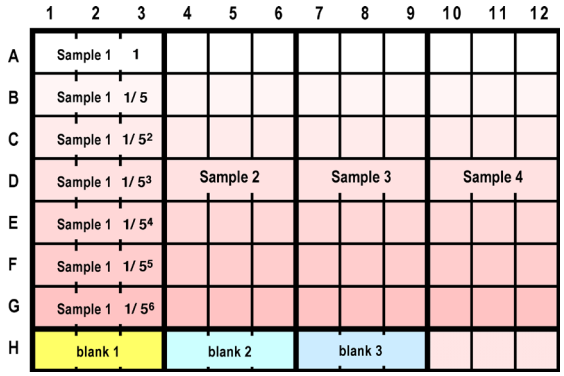

Fig.3 Example of sample and blank arrangement on a 96-well plate

Inhibition Curve

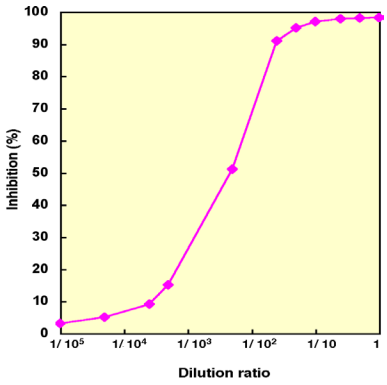

Fig.4 Inhibition curve of Cu, Zn-SOD

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

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

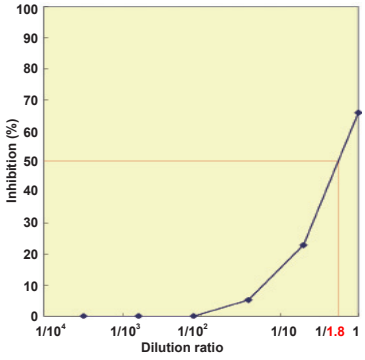

Fig.5 Inhibition curve of erythrocyte

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 our technical service.

www.interchim.com
interchim

211 bis Avenue Kennedy - BP 1140
03103 Montluçon - France
33 (0) 4 70 03 88 55
Fax 33 (0) 4 70 03 82 60
e-mail interchim@interchim.com

Agence Paris - Normandie
33 (0) 1 41 32 34 40
Fax 33 (0) 1 47 91 23 90
e-mail interchim.paris@interchim.com