19160 SOD Determination Kit

Application

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 Determination 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 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.
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.

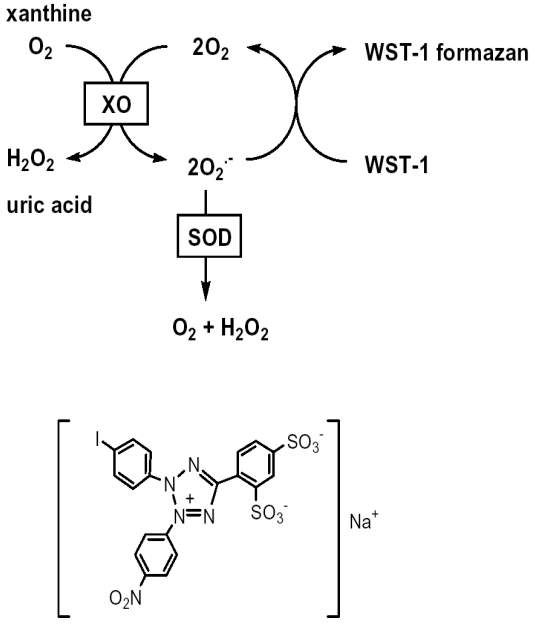

Figure 1: Principle of the SOD Assay Kit

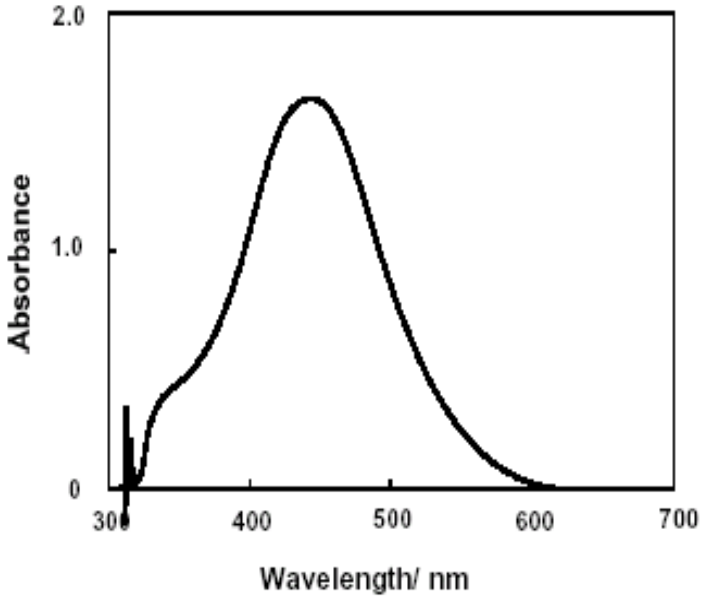

Figure 2. Absorption spectrum of WST-1 formazan.

Components

- WST Solution 5 ml
- Enzyme Solution 100 µl
- Buffer Solution 100 ml
- Dilution Buffer 50 ml

Reagents and Equipment Required but not Provided

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

Precautions and Disclaimer:

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage

Store all components at 2-8 °C. WST Working Solution is stable for 2 months at 2-8 °C. Enzyme Working Solution is stable for 3 weeks at 2-8 °C. Protect WST Solution and WST Working Solution from light.

Preparation of Working Solutions

- 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 seconds. Mix by pipeting, and dilute 15 µl of Enzyme Solution with 2.5 ml of Dilution Buffer.
- 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

Procedure

Concentrated protein assay

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.

Note: If Sample Solution has visible color, set up a separate "Blank 2" lane.

1) Add 20 µl of sample solution to each Sample and Blank 2 well and add 20 µl of ultrapure H_2O 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*.

	Sample	Blank 1	Blank2*	Blank 3
Sample solution	20 µl	---	20 µl	---
Ultrapure H_2O	---	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

Table 1: Amount of each Solution for Sample and, Blanks 1, 2 and 3
*If Sample Solution has visible color, set up separate "blank 2" lane.

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:

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

Notes

1) For an accurate measurement, the use of multiple wells per sample is recommended (see Figure 3).
2) 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.
3) Inhibition activity can also be determined by a kinetic method. 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

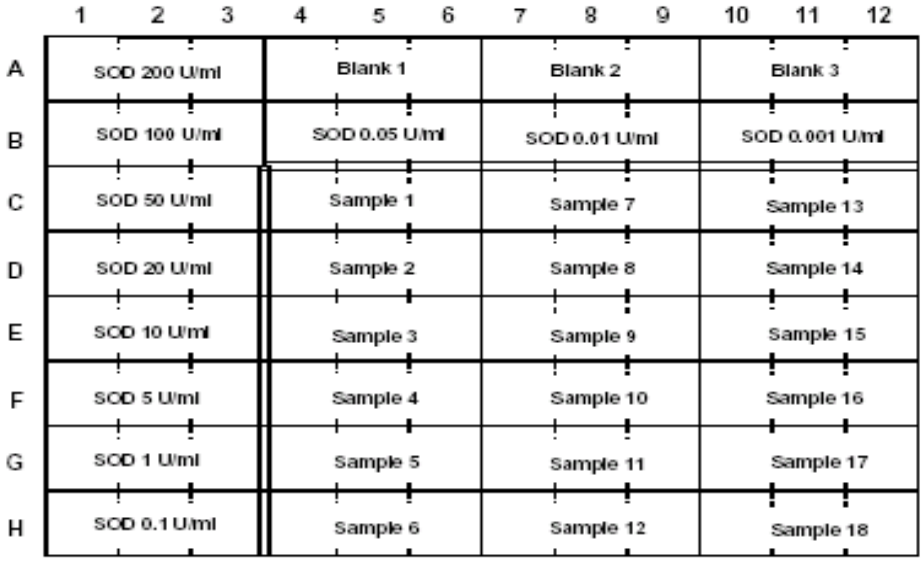

Figure 3: Sample and blank arrangement on a 96-well plate including SOD standard solutions.

Interfering agents

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
Ascorbic acid	0.1 mM
Glutathione, reduced form	5 mM

Inhibition curve

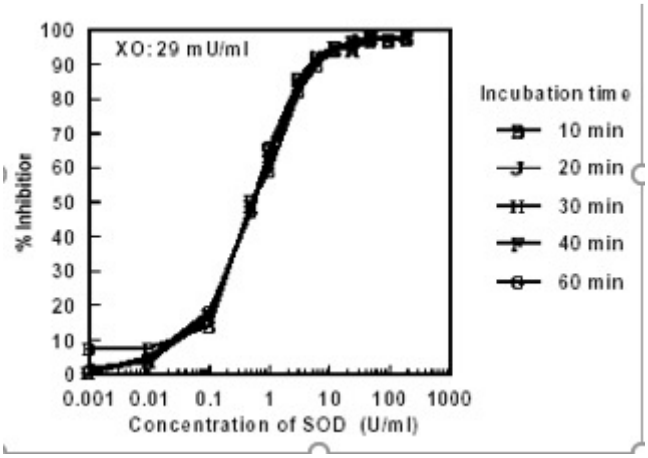

Figure 4: Example of inhibition curves prepared by WST-1 assay using superoxide dismutase from bovine erythrocytes and with different incubation times. Measurements taken at 450 nm.