KAMIYA BIOMEDICAL COMPANY

SOD Assay

For the measurement of Superoxide Dismutase (SOD) Inhibition Activity

Cat. No. KT-019 (500 tests)

For Research Use Only. Not for Use in Diagnostic Procedures.

PRODUCT INFORMATION

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PRODUCT
The **K-ASSAY®** SOD Assay is for the measurement of Superoxide Dismutase (SOD) inhibition activity.

PRINCIPLE
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 **K-ASSAY®** SOD Assay allows very convenient SOD assaying by utilizing **KAMIYA BIOMEDICAL COMPANY**'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^- 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.

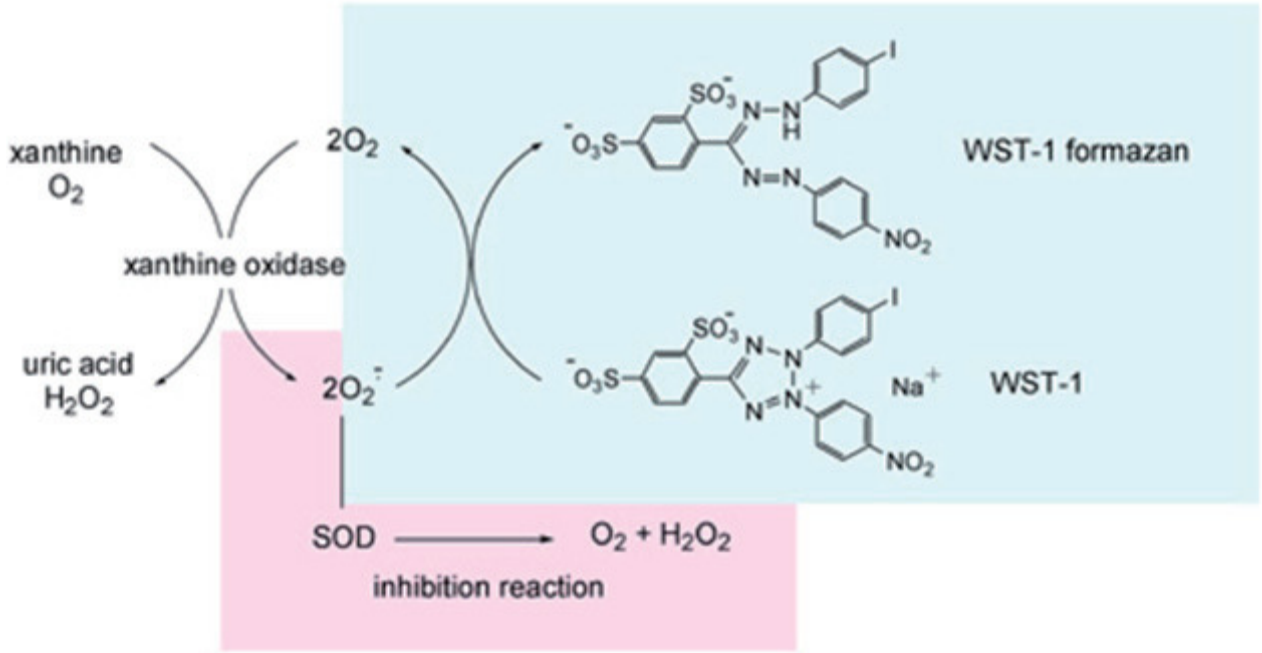

Fig. 1 Principle of the determination of SOD activity using SOD Assay Kit.

COMPONENTS

KT-019 (500 Tests)

- 1 x 5 mL WST Solution
- 1 x 100 µL Enzyme Solution
- 1 x 100 mL Buffer Solution
- 1 x 50 mL Dilution Buffer

MATERIALS OR EQUIPMENT REQUIRED BUT NOT PROVIDED

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

PROTOCOLS

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 4℃.
- Enzyme Working Solution
  Centrifuge the Enzyme Solution tube for 5 sec. Mix by pipeting, and dilute 15 µL of Enzyme Solution with 2.5 mL of Dilution Buffer. Enzyme working solution is stable at 4℃ for 3 weeks.
- Sample Solution
  Dilute sample solution with Dilution buffer or saline to prepare sample solution as follows.
  Dilution ratio: 1, 1/5, $1/5^2$, $1/5^3$, $1/5^4$, $1/5^5$, $1/5^6$

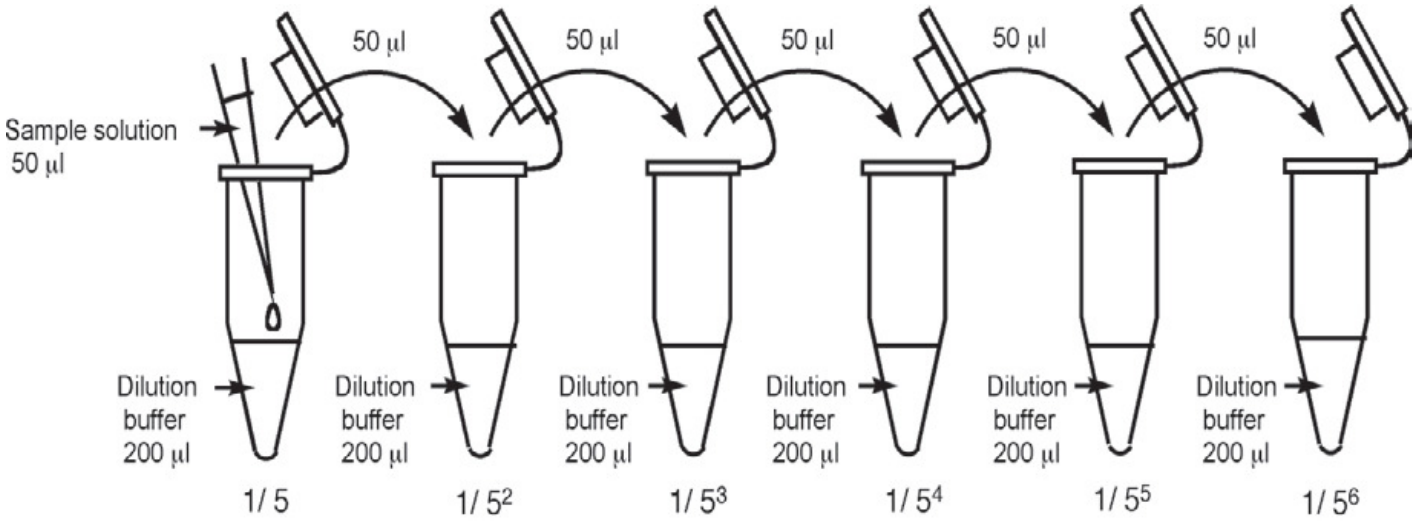

Fig. 2 Preparation of sample solution

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

	sample	blank 1	blank 2*	blank 3
Sample Solution	20 µL	-	20 µL	-
ddH_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

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

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 %) = $\{[(S1 - S3) - (SS - S2)] / (S1 - S3)\} \times 100$
S1: slope of blank1, S2: slope of blank2, S3: slope of blank3, SS: slope of sample

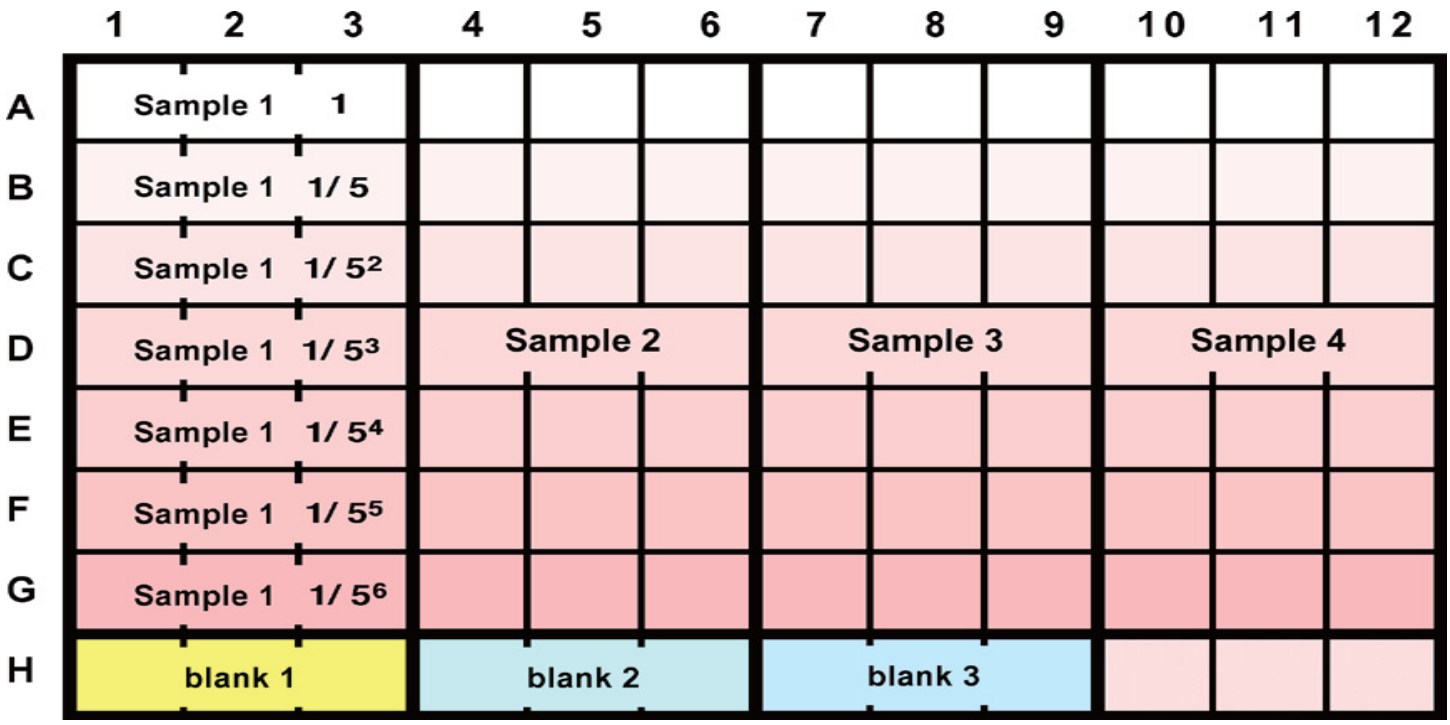

Fig 3. Example of sample and blank arrangement on a 96-well plate

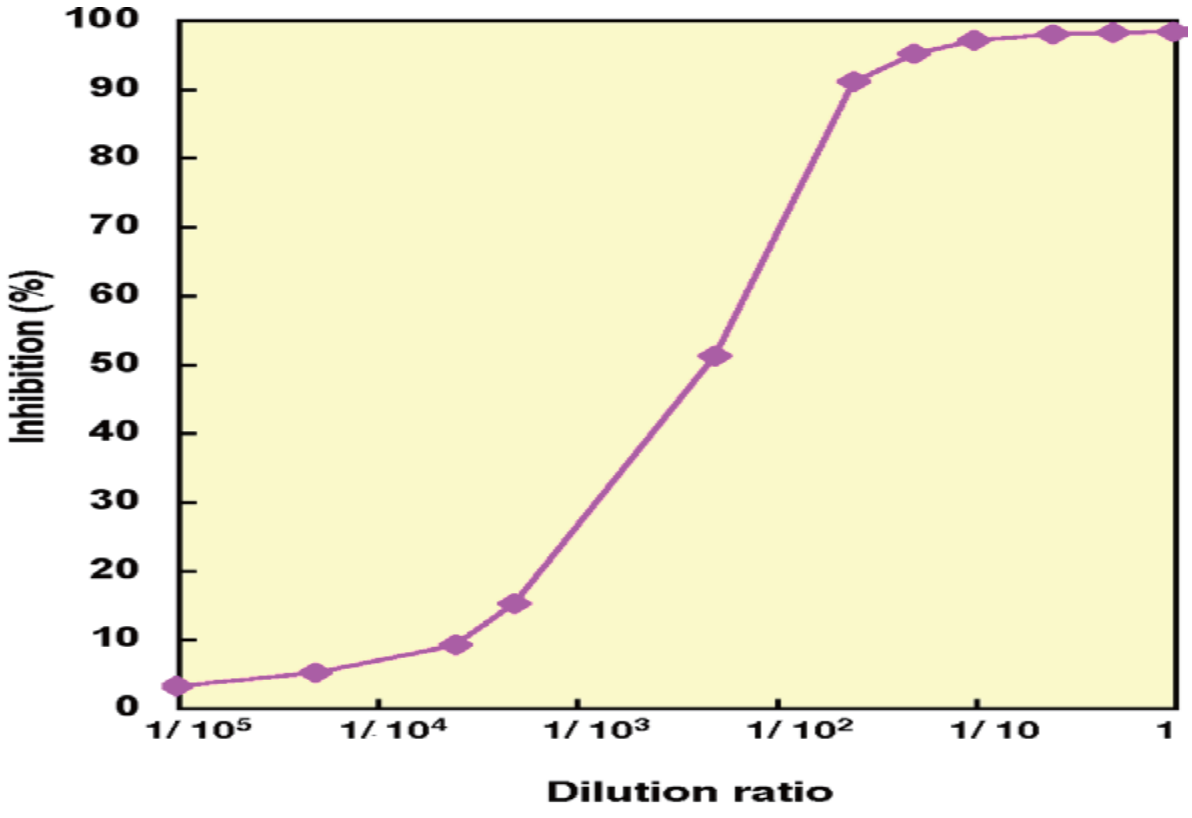

Fig 4. Inhibition curve of Cu,Zn-SOD (SIGMA Cat#:S2515)

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 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 in calculated as below.
   108 x 90.0 = 9,720 U/mL of blood.

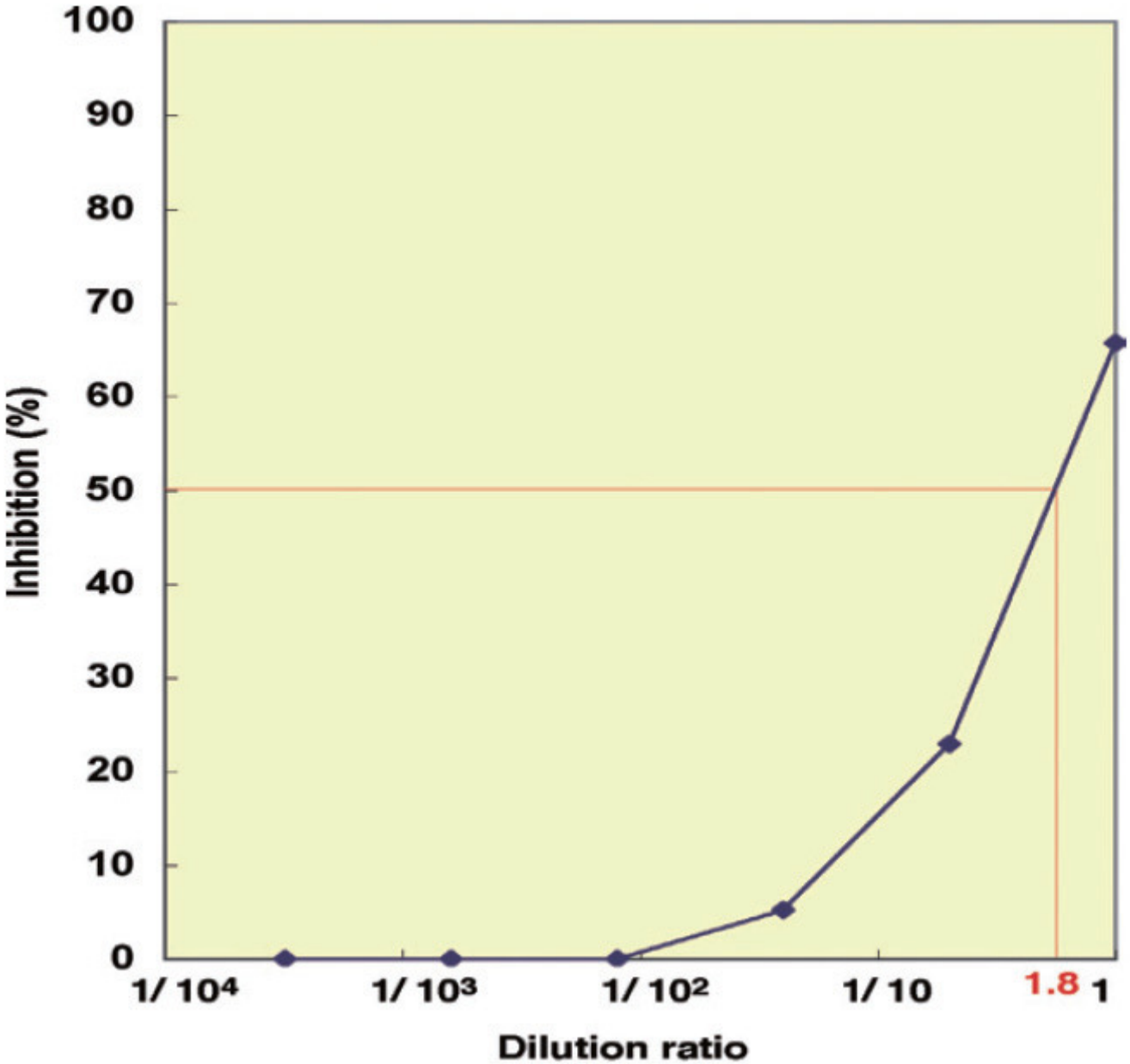

Fig.5 Inhibition curve of erythrocyte

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

Below shows compatible concentration 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.

Detergents: SDS (0.05%), Tween 20 (0.5%), NP-40 (0.5%)
Solvents: Ethanol (25%), DMSO (5%)
Reducing agents: Glutathione reduced form (1.25 mmol/L), Ascorbic acid (0.1 mmol/L)
Others: EDTA (2 mM), BSA (1%w/v)

PRECAUTION

1. For dilution of sample, use Dilution buffer or saline.
2. The Enzyme solution is separated into two layers. Therefore, omitting the pipetting process will result in inaccurate experimental results.
3. For an accurate measurement, the use of multiple wells per sample is recommended.
4. 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.

STORAGE

Please store at 4°C. Protect the WST Solution and WST Working Solution from light.

FOR RESEARCH USE ONLY
NOT FOR USE IN DIAGNOSTIC PROCEDURES

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