

# EZ-SOD Assay Kit

Superoxide Dismutase Assay Kit

Oxidative Stress Assay Kit

Cat. No. DG-SOD400

FOR RESEARCH USE ONLY

NOT FOR USE IN DIAGNOSTIC PROCEDURES



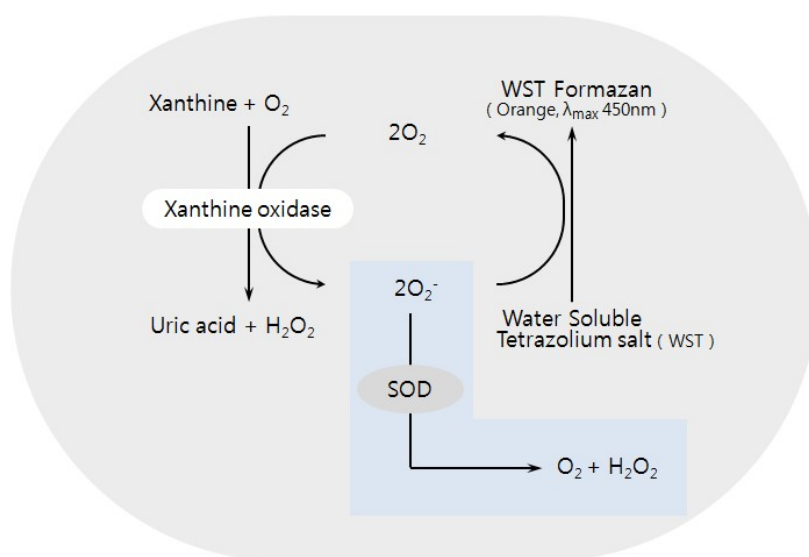
## ▪ Product Description

SOD is an important antioxidant enzyme that catalyzes the dismutation reaction of superoxide anion (or radical), one of the reactive oxygen species constantly generated during metabolic processes in various cells, into oxygen and hydrogen peroxide.

SOD, which exists widely not only in animals but also in plants, is a metalloenzyme and its types vary depending on the type of metal bound to it. Representative types include Cu/Zn SOD, Mn SOD, and Fe SOD. Cu/Zn SOD is mainly present in the cytoplasm of eukaryotic cells, and Mn SOD is present in human mitochondria and some *E. coli*.

SOD is a representative antioxidant enzyme and has been measured as an indicator of antioxidant ability. NBT has been widely used as a representative method, but accurate measurement has been difficult due to problems such as the formation of formazan, which is poorly soluble in water, and direct reaction with xanthine oxidase.

DoGENBio's EZ-SOD Assay kit is a product that can measure SOD activity more stably and accurately by using Water Soluble Tetrazolium salt (WST) to solve these problems.



Detection mechanism with EZ-SOD assay kit

Xanthine oxidase oxidizes xanthine to produce uric acid and  $H_2O_2$  while also producing  $O_2^-$ . The  $O_2^-$  generated in this way reduces WST to produce colored WST-formazan, which absorbs light at 450 nm. It will be formed. However, when SOD is active,  $O_2^-$  generated by xanthine oxidase is inhibited by SOD, which converts it into  $O_2$  and  $H_2O_2$ . It inhibits the formation of WST-formazan by converting it into  $O_2$  and  $H_2O_2$ .

The degree of inhibition is related to SOD enzyme activity, and the greater the SOD enzyme activity, the lower the absorbance at 450 nm.



## ▪ Kit Contents and Storage Conditions

Component	400 assay
WST Solution	4mℓ
Buffer Solution	50 mℓ x 2
Xanthine Oxidase	70 μℓ
Dilution Buffer	50 mℓ

\* Before opening, the kit is stable for 6 months at 4°C.

## ▪ Preparation of Working Solution

Solution	Preparation	Storage and Stability
WST Working Solution (Protect from light)	Mix 1 ml WST Solution and 19 ml Buffer Solution.	The mixed solution is stable for 3 weeks at 4°C, but <u>it is recommended to mix it immediately before use.</u>
Enzyme Working Solution (Xanthine Oxidase)	15μℓ Xanthine Oxidase and 2.5mℓ Dilution Buffer Mix. * Xanthine Oxidase is separated into two layers, and must be mixed evenly by pipetting after spinning down before use.	

\* Buffer solution is used after sufficiently warming up to room temperature before experiment.

\* When using a cold buffer, enzyme activity may be inhibited, affecting measurement results.

## ▪ Interference

1. If the sample contains 2-mercaptoethanol or itiothreitol (DTT), the absorbance will be affected. Please remove all of it through the preprocessing process before performing the experiment.
2. If the sample contains the following substances, please dilute the sample to a lower concentration than the given concentration.

Detergent		Solvents	
SDS	0.05%	Ethanol	25%
Tween20	0.5%	DMSO	5%
NP-40	0.5%	Methanol	25%
Reducing agents		Others	
Glutathione reduced form	1.25 mmol/l	EDTA	2 mmol/l
Ascorbic acid	0.1 mmol/l	BSA	1%(W/V)



## ▪ Preparation of Sample

**Cells** (0.5~1.5x10<sup>7</sup> cells, Determine the appropriate number of cells depending on the type of cell.)

- ① Centrifuge the suspended cells at 2,000xg (10 min at 4°C) and remove the supernatant.
- ② After adding 1 ml of PBS, centrifuge at 2,000xg (10 min at 4°C) and remove the supernatant.
- ③ Cells are disrupted using the Freeze-Thaw method.  
(-20°C for 20 min, 37°C bath 10 min, Repeat 3 times)

\* option : Add 1 ml of PBS and disrupt the cells using a homogenizer or sonication.

- ④ Add 1 ml of PBS, centrifuge at 10,000xg (10 min at 4 °C), transfer the supernatant to a new tube, and use it as a sample.

**Plant** (200 mg)

- ① After adding 1 ml of distilled water, disrupt the cells with a homogenizer.
- ② Separate the filtrate using a paper filter and freeze-dry it.
- ③ After measuring the weight of the sample, dissolve it in 0.1 M phosphate buffer (pH 7.4).

**Tissue** (100 mg)

- ① Wash the sample with saline to remove as much blood as possible.
- ② Remove moisture using a paper towel and measure the weight.
- ③ Add 0.4~0.9 ml of sucrose buffer and homogenize with a homogenizer.  
\* option : Cells are disrupted by sonication in a tube on ice.
- ④ Centrifuge at 10,000xg (10 min at 4 °C), transfer the supernatant to a new tube, and use it as a sample.

**Erythrocytes or Plasma**

- ① Centrifuge 2-3 ml of anticoagulant-treated blood at 600xg (10 min at 4°C).
- ② Remove the supernatant, dilute with saline and use as plasma.
- ③ Add saline to the pellet, centrifuge at 600xg (10 min at 4 °C), and remove the supernatant..
- ④ Repeat ③ two more times.
- ⑤ After adding 4 ml of distilled water to the pellet, add 1 ml of ethanol and 0.6 ml of chloroform.
- ⑥ Shake for 15 min at 4°C.
- ⑦ Centrifuge at 600xg (10 min at 4 °C) and transfer the water-ethanol layer to a new tube.
- ⑧ D.W. added to 0.1 ml of water-ethanol layer. Add 0.7 ml to use as sample.  
(Dilute with 0.25% ethanol if necessary.)



## ▪ Preparation of Blank

The blanks commonly required during experiments are as follows.

### 1) Blank 1

Measure the maximum absorbance. Since the superoxide anion generated during the reaction in the absence of SOD is not decomposed and is all involved in the formation of WST formazan, the highest absorbance should be measured as a result of the experiment.

\* The absorbance value of the sample being tested must be lower than blank 1. If the absorbance value of the sample is similar to or higher than blank 1, please increase the dilution factor of the sample.

### 2) Blank 2

Measure the background of the test sample.

When using a diluted test sample, the blank of each diluted test sample must be measured.

### 3) Blank 3

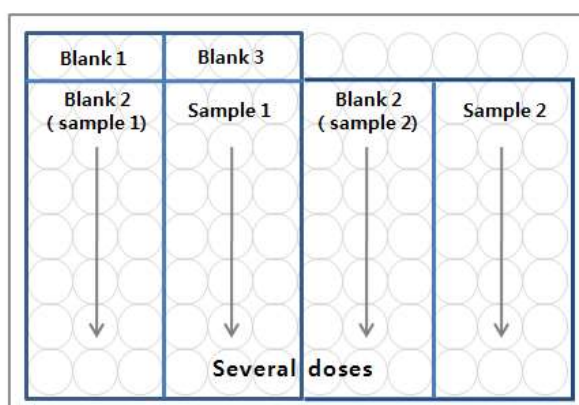
Measure the background of the solutions excluding the test sample.

In addition to the background of the solutions, you can check whether the experiment results are affected by contamination of kit components or external factors during the experiment.

## ▪ General Protocol

1. Add 20  $\mu\text{l}$  of samples diluted to various concentrations into each sample well and Blank 2 well. At this time, add 20  $\mu\text{l}$  of ddH<sub>2</sub>O to Blank 1 and Blank 3 wells.

\* When calculating the SOD activity of a sample as a unit, please use the SOD standard (sold separately, Sigma S7571) or serially dilute the sample.



Example arrangement on a 96 well plate

2. Add 200  $\mu\text{l}$  of WST working solution to each well.

3. Add 20  $\mu\text{l}$  of Dilution Buffer to each well of Blank 2 and Blank 3.



4. Using a multi-channel pipette, add 20µl of Enzyme working solution to Blank 1 and sample well and mix carefully.

\* Since superoxide is generated and color development occurs as soon as the enzyme working solution is added, it is recommended to minimize the gap between wells using a multi-channel pipette.

	Blank 1	Blank 2	Blank 3	Test sample
Sample	—	20µl	—	20µl
ddH <sub>2</sub> O	20µl	—	20µl	—
WST working solution	200µl	200µl	200µl	200µl
Dilution Buffer	—	20µl	20µl	—
Enzyme working solution	20µl	—	—	20µl
Total volume	240µl	240µl	240µl	240µl

5. Incubate the plate at 37°C for 20 minutes.

\* The optimal reaction temperature for xanthine oxidase is 37°C. If the ambient temperature is low or the solution is not sufficiently warmed up, the absorbance may be low. If the absorbance of Blank 1 is lower than 0.7, you can increase the reaction time and measure it. (  $0.7 \leq \text{blank1} \leq 1$  )

6. Measure absorbance at 450 nm using a plate reader.

## ▪ Calculation

$$1. \text{ SOD activity ( Inhibition rate \% ) } = \frac{(\text{OD}_{\text{blank1}} - \text{OD}_{\text{blank3}}) - (\text{OD}_{\text{sample}} - \text{OD}_{\text{blank2}})}{(\text{OD}_{\text{blank1}} - \text{OD}_{\text{blank3}})} \times 100$$

2. SOD activity (unit)

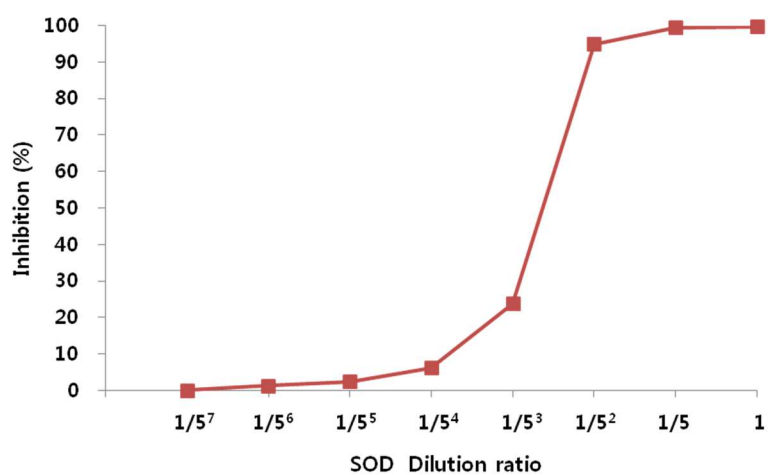
1) Dilute until the sample reaches 50% inhibition rate (IC<sub>50</sub>) and calculate the dilution ratio.

2) The concentration of SOD contained in 20µl of sample that inhibits the reduction reaction of WST-1 by 50% (IC<sub>50</sub>) is expressed as 1 unit.

Ex) When calculating total SOD unit in blood sample

- If the IC<sub>50</sub> value of the experimental results is determined at a dilution ratio of 1/25  
: 1 U x 25 (dilution ratio) = 25 U
- Since the volume of the sample used in the experiment was 20µl  
: 25 U/20 µl = 1.25 U/µl = 1,250 U/ml
- If the blood sample was prepared by diluting the high concentration stock sample 10 times  
: 1,250 U/ml x 10 = 12,500 U/ml of blood.





Inhibition curve of Cu,Zn-SOD



## ▪ Related Product

	Products	Catalog No.	Assay
<b>Oxidative Stress Assay Kit</b>	EZ-Superoxide Dismutase (SOD) Assay Kit (Colorimetric)	DG-SOD400	400 Assay
	EZ-Glutathione Assay Kit (Colorimetric)	DG-GLU200	200 Assay
	EZ-Catalase Assay Kit (Fluorometric/Colorimetric)	DG-CAT400	400 Assay
	EZ-Hydrogen peroxide/Peroxidase Assay Kit (Fluorometric/Colorimetric)	DG-PER500	500 Assay
	EZ-Lipid Peroxidation (TBARS) Assay Kit (Colorimetric)	DG-TBA200	200 Assay
	EZ-Total Antioxidant Capacity (TAC) Assay Kit (Colorimetric)	DG-TAC200	200 Assay
	EZ-DPPH Antioxidant Assay Kit (Colorimetric)	DG-DPH400	400 Assay
	EZ-ABTS Antioxidant Assay Kit (Colorimetric)	DG-ABT400	400 Assay
	EZ-Glutathione Peroxidase Assay Kit (Colorimetric)	DG-GPX100	100 Assay
	EZ-Lactate Assay Kit (Colorimetric)	DG-LAC100	100 Assay
	EZ-Acetylcholinesterase Assay Kit (Colorimetric)	DG-ACE100	100 Assay
	EZ-Ascorbic Acid Assay Kit (Colorimetric)	DG-ASC100	100 Assay
	EZ-ATP Assay Kit (Fluorometric/Colorimetric)	DG-ATP100	100 Assay
	EZ-Free Fatty Acid Assay Kit (Fluorometric/Colorimetric)	DG-FFA100	100 Assay
	EZ-Free Glycerol Assay Kit (Fluorometric/Colorimetric)	DG-FGC100	100 Assay
<b>Metabolism Assay Kit</b>	EZ-Glucose Assay Kit (Fluorometric/Colorimetric)	DG-GCS100	100 Assay
	EZ-HDL, LDL/VLDL Assay Kit (Fluorometric/Colorimetric)	DG-CHO100	100 Assay
	EZ-Total Cholesterol Assay Kit (Fluorometric/Colorimetric)	DG-TSC100	100 Assay
	EZ-Triglyceride Quantification Assay Kit (Fluorometric/Colorimetric)	DG-TGC100	100 Assay
	EZ-Nitric Oxide Assay Kit (Colorimetric)	DG-NO500	500 Assay
	EZ-Ethanol Assay Kit (Colorimetric)	DG-ETH100	100 Assay



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EZ-Total Collagen Assay Kit  
(Colorimetric)

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DG-COL100

100 Assay