SOD Activity Assay Kit

SOD Activity Assay Kit is a detection kit for the quantification of Superoxide Dismutase in serum, plasma and cell / tissue lysates.

Catalog number: ARG81353

Package: 100 assays

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MANUFACTURED BY:

Arigo Biolaboratories Corporation

Address: No. 22, Ln. 227, Gongyuan Rd., Hsinchu City 300, Taiwan

Phone: +886 (3) 562 1738

Fax: +886 (3) 561 3008

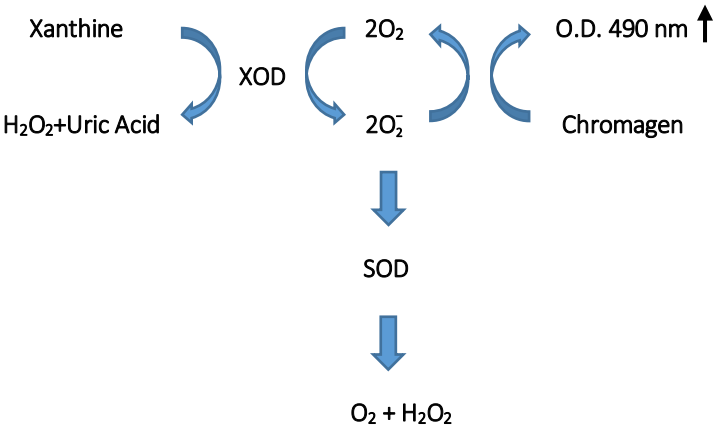
Email: info@arigobio.com

INTRODUCTION

Superoxide dismutase (SOD, EC 1.15.1.1) is an enzyme that alternately catalyzes the dismutation (or partitioning) of the superoxide (O_2^-) radical into ordinary molecular oxygen (O_2) and hydrogen peroxide (H_2O_2). Superoxide is produced as a by-product of oxygen metabolism and, if not regulated, causes many types of cell damage. Hydrogen peroxide is also damaging and is degraded by other enzymes such as catalase. Thus, SOD is an important antioxidant defense in nearly all living cells exposed to oxygen. One exception is *Lactobacillus plantarum* and related lactobacilli, which use a different mechanism to prevent damage from reactive O_2^-. [Provide by Wikipedia: Superoxide dismutase]

PRINCIPLE OF THE ASSAY

This SOD Activity Assay Kit employs a convenient colorimetric method for the detection of SOD activity from cell lysate, plasma, serum, and tissue homogenates. Superoxide anions (O_2^-) are generated by a Xanthine / Xanthine Oxidase (XOD) system, and then detected with a Chromagen Solution. However, in the presence of SOD, these superoxide anion concentrations are reduced, yielding less colorimetric signal. The intensity of the color is measured at a wavelength of 490 nm. The IU of activity SOD in the samples is then determined by comparing the O.D of samples to the standard curve.



MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at -20°C. Avoid multiple freeze/thaw cycles. Use the kit before expiration date.

Component	Quantity	Storage information
SOD Standards (5 Units/µL)	20 µL	-20°C
Xanthine Solution	125 µL (ready to use)	-20°C
150X Xanthine Oxidase Solution	10 µL	-20°C
Chromagen Solution	125 µL (ready to use)	-20°C (protect from light)
10X SOD Assay Buffer	2 X 1.5 mL	-20°C

Unit Definition: One unit will inhibit the rate of reduction of cytochrome c by 50% in a coupled system, using xanthine and xanthine oxidase, at pH 7.8 at 25°C in a 3.0 ml reaction volume.

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at 490 nm
- 37°C incubator or water bath
- Sonicator or homogenizer
- Deionized or Distilled water
- 96 well microtiter plate
- 1X PBS
- 1X Lysis Buffer (10 mM Tris, pH 7.5, 150 mM NaCl, 0.1 mM EDTA)
- Pipettes and pipette tips
- Multichannel micropipette reservoir

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at -20°C at all times.
- Avoid the use of reducing agents, such as DTT, in the assay due to interference with the Chromagen Solution.
- The concentrations can vary over a wide range depending on the different samples. For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- It is highly recommended assaying the Standards and samples in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Suspension Cells: Centrifuge 3-6 x 10^6 cells at 1200 x g for 2 minutes and discard supernatant. Wash cell pellet once with ice-cold PBS, centrifuge, and discard the supernatant. Resuspend cell pellet in 0.5 mL of cold 1X Lysis Buffer (10 mM Tris, pH 7.5, 150 mM NaCl, 0.1 mM EDTA). Lyse cells with sonication or homogenation. Centrifuge at 12000 x g for 10 minutes and collect the cell lysate supernatant.

Adherent Cells: Wash 1-5 x 10^6 cells once with 10 mL ice-cold PBS per 100 mm dish. Harvest cells with a cell scraper in 1 mL of cold 1X Lysis Buffer (10 mM Tris, pH 7.5, 150 mM NaCl, 0.1 mM EDTA). Lyse cells with sonication or homogenation. Centrifuge at 12000 x g for 10 minutes and collect the cell lysate supernatant.

Tissue Lysates: Homogenize tissue sample in 5-10 mL of cold 1X Lysis Buffer (10 mM Tris, pH 7.5, 150 mM NaCl, 0.1 mM EDTA) per gram tissue. Lyse cells with sonication or homogenation. Centrifuge at 12000 x g for 10 minutes and collect the tissue lysate supernatant.

Plasma: Collect blood with EDTA, heparin or citrate and centrifuge at 2000 x g and 4°C for 10 minutes. Sample should be tested immediately or frozen at -80°C for storage.

Serum: Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 30 minutes. Centrifuge at 2500 x g for 20 minutes. Sample should be tested immediately or frozen at -80°C for storage.

REAGENT PREPARATION

- **1X SOD Assay Buffer:** Dilute 10X SOD Assay Buffer into deionized water to yield 1X SOD Assay Buffer. (E.g., add 1.5 mL of 10X SOD Assay Buffer into 13.5 mL of deionized water to a final volume of 15 mL) Keep the second vial of 10X SOD Assay Buffer undiluted.
- **1X Xanthine Oxidase Solution:** Dilute 150X Xanthine Oxidase Solution into 1X SOD Assay Buffer to yield 1X Xanthine Oxidase Solution. (E.g., add 2 µL of 150X Xanthine Oxidase Solution into 298 µL of 1X SOD Assay Buffer to a final volume of 300 µL)
- **SOD Standards:** Thaw SOD Standard at 4°C. Fresh prepare a dilution series (1:4 is suggested) of SOD Standard in the concentration range of 5 Units/µL – 1.2 mU/µL with 1X SOD Assay Buffer.

Dilute SOD Standards as according to the table below:

Standard tube	Final SOD conc. (U/µL)	Volume of 1X SOD Assay Buffer (µL)	Volume of standard (µL)
S1	5	0	35 of SOD standards
S2	1.25	30	10 of S1
S3	0.312	30	10 of S2
S4	0.078	30	10 of S3
S5	0.0195	30	10 of S4
S6	0.0048	30	10 of S5
S7	0.0012	30	10 of S6
S0	0	30	0

ASSAY PROCEDURE

Standards and samples should be assayed in duplicates or triplicate.

1. Add **10 µL** of **sample**, **Blank (deionized water only)**, and serial **diluted SOD Standards** into 96-well microplate.
2. Then add **5 µL** of **Xanthine Solution, 5 µL** of **Chromagen Solution** and **10 µL** of **10X SDS Assay Buffer** into the appropriate wells of 96-Well Microplate.
3. Add **deionized water** to **70 µL** each well. Allow pre-incubation time if inhibitor is used.

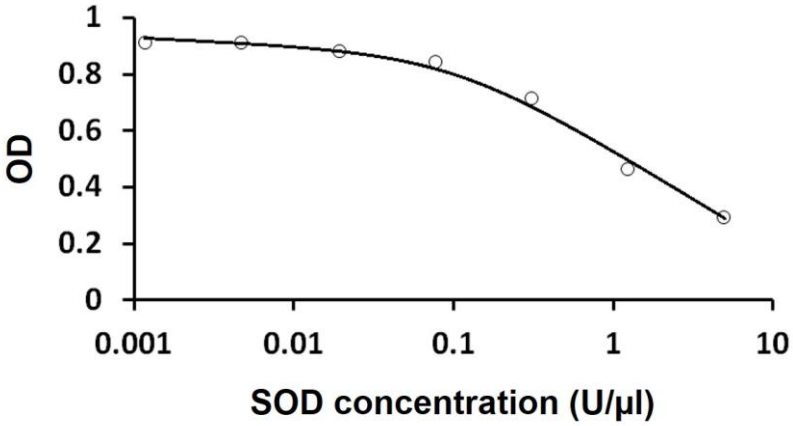
   Note: refer to the supplier's datasheet for the amount of inhibitor.
4. Finally, add **10 µL** of **pre-diluted 1X Xanthine Oxidase Solution** to each well. Mix well and incubate for **1 hour** at **37°C**.
5. Read the OD with a microplate reader at **490 nm** immediately.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards and samples.
2. Using log-log, semi-log or linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Use the mean absorbance value for each sample determine the corresponding concentration from the standard curve.
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit or linear curve fit.
5. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for the detail. (https://www.arigobio.com/elisa-analysis)

EXAMPLE OF TYPICAL STANDARD CURVE

The following figures demonstrate typical results with the SOD Activity Assay Kit. One should use the data below for reference only. This data should not be used to interpret actual results.



QUALITY ASSURANCE

Intra-assay and Inter-assay precision

The CV value of intra-assay and inter-assay was ≤ 10 %