Product Manual

OxiSelect™ Superoxide Dismutase Activity Assay

Catalog Number

STA-340 100 assays

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

CELL BIOLABS, INC.
Creating Solutions for Life Science Research

Introduction

Reactive oxygen species (ROS), such as superoxide (O_2^-) and hydrogen peroxide (H_2O_2), are constantly produced during metabolic processes in all living species. Under normal physiological conditions, cellular ROS generation is counterbalanced by the action of antioxidant enzymes and other redox molecules. However, excessive ROS accumulation will lead to cellular injury, such as damage to DNA, protein, and lipid membrane. Because of their potential harmful effects, excessive ROS must be promptly eliminated from the cells by a variety of antioxidant defense mechanisms. Superoxide dismutase (SOD), which catalyzes the dismutation of the superoxide anion into hydrogen peroxide and molecular oxygen, is one of the most important antioxidative enzymes. SOD enzymes are classified into three groups: cytosolic Cu/Zn-SOD, mitochondrial Mn-SOD, and extracellular Ec-SOD.

Our OxiSelect™ Superoxide Dismutase Activity Assay uses a xanthine/xanthine oxidase (XOD) system to generate superoxide anions. The included chromagen produces a water-soluble formazan dye upon reduction by superoxide anions. The activity of SOD is determined as the inhibition of chromagen reduction (See Figure 1).

The OxiSelect™ Superoxide Dismutase Activity Assay is a fast and reliable kit for the measurement of SOD activity from cell lysate, plasma, serum, tissue homogenates. Each kit provides sufficient reagents to perform up to 100 assays, including blanks, SOD standards and unknown protein samples.

Test Principle

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.

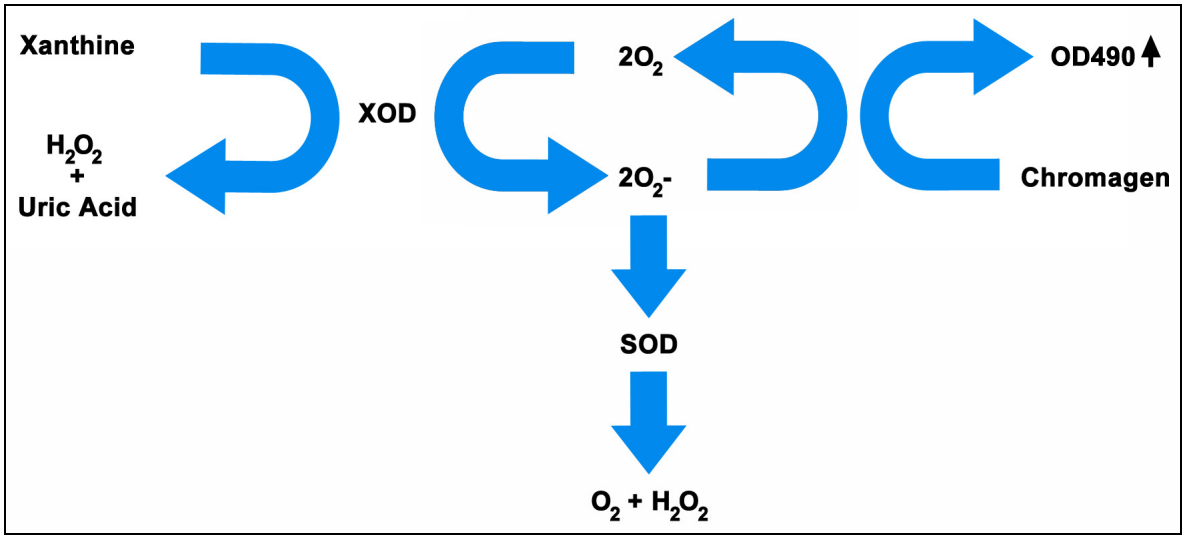

Figure 1: Assay Principle

Related Products

1. STA-305: OxiSelect™ Nitrotyrosine ELISA Kit (96 Assays)
2. STA-310: OxiSelect™ Protein Carbonyl ELISA Kit (96 Assays)
3. STA-320: OxiSelect™ Oxidative DNA Damage ELISA (8-OHdG, 96 Assays)
4. STA-330: OxiSelect™ TBARS Assay Kit (MDA Quantitation)
5. STA-332: OxiSelect™ MDA Adduct ELISA Kit (96 Assays)

Kit Components

1. SOD Standard (Part No. 234001): One 100 µL vial provided at 5 Units/µL.
2. Xanthine Solution (Part No. 234002): One 550 µL vial.
3. Xanthine Oxidase Solution, 10X (Part No. 234003): One 100 µL vial.
4. Chromagen Solution (Part No. 234004): One 550 µL amber vial.
5. SOD Assay Buffer, 10X (Part No. 234005): One 1.5 mL vial.

Materials Not Supplied

1. 96-well microtiter plate
2. 37 °C incubator or water bath
3. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips
4. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
5. Multichannel micropipette reservoir
6. Microplate reader capable of reading at 490 nm

Storage

Store kit components at -20 °C until their expiration dates. Avoid multiple freeze/thaws by aliquoting. Chromagen Solution is light sensitive and should be maintained in amber tubes.

Preparation of Reagents

- 1X SOD Assay Buffer: Dilute the 10X SOD Assay Buffer to 1X with deionized water. Mix to homogeneity.
- 1X Xanthine Oxidase Solution: Just prior to use, dilute the 10X Xanthine Oxidase Solution to 1X with 1X SOD Assay Buffer. Mix to homogeneity.

Special Precautions

Avoid the use of reducing agents, such as DTT, in the assay due to interference with the Chromagen Solution.

Assay Protocol

1. 1) Cytosolic fraction preparation:

   a) Suspension Cells- Centrifuge 3-6 x 10^6 cells at 700 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, 0.5% Triton-100) and incubate on ice for 10 minutes. Centrifuge at 12000 x g for 10 minutes and collect the cell lysate supernatant.

   b) Adherent Cells- Wash 1-5 x 10^6 cells once with 10 mL ice-cold PBS per 100 mm dish. Place dish on ice. Add 1 mL of cold 1X Lysis Buffer (10 mM Tris, pH 7.5, 150 mM NaCl, 0.1 mM EDTA, 0.5% Triton-100) and incubate on ice for 10 minutes. Collect cells/debris with a rubber policeman. Transfer the cell extract to a microfuge tube and centrifuge at 12000 x g for 10 minutes. Collect the cell lysate supernatant.

   2) Tissue lysate preparation: 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, 0.5% Triton-100) per gram tissue. Centrifuge at 12000 x g for 10 minutes and collect the tissue lysate supernatant.

2. Prepare samples including a blank in a 96-well microtiter plate according to the below table. Allow pre-incubation time if inhibitor is used.

Component	**Blank**	**Sample**
SOD Sample	0 µL	X µL
Inhibitor (optional)	0 µL	Y µL
Xanthine Solution	5 µL	5 µL
Chromagen Solution	5 µL	5 µL
10X SOD Assay Buffer	10 µL	10 µL
DI Water	70 µL	70-(X+Y) µL
Total	**90 µL**	**90 µL**

3. Finally, add 10 µL of pre-diluted 1X Xanthine Oxidase Solution (see Preparation of Reagents) to each well. Mix well and incubate for 1 hour at 37 °C.
4. Read absorbance at 490 nm on a microplate reader.

Preparation of SOD Standards (Optional)

1. Thaw SOD Standard at 4 °C.
2. Freshly prepare a dilution series (1:4 is suggested) of SOD Standard in the concentration range of 5 Units/µL – 0.61 mU/µL by diluting the SOD Standard in 1X Assay Buffer (Table 1).
3. Transfer 10 µL of each dilution to a 96-well microtiter plate, including a 1X Assay Buffer blank.

4. Prepare the following master mixture, adjusting for the required number of wells.

Component	Volume per well
Xanthine Solution	5 µL
Chromagen Solution	5 µL
10X SOD Assay Buffer	10 µL
DI Water	60 µL
Total	**80 µL**

5. Transfer 80 µL of the above master mixture to each well.
6. Finally, add 10 µL of pre-diluted 1X Xanthine Oxidase Solution (see Preparation of Reagents) to each well. Mix well and incubate for 1 hour at 37 °C.
7. Read absorbance at 490 nm on a microplate reader.

Example of Results

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

SOD Activity (inhibition %) = $(OD_{blank}-OD_{sample})/(OD_{blank}) \times 100$

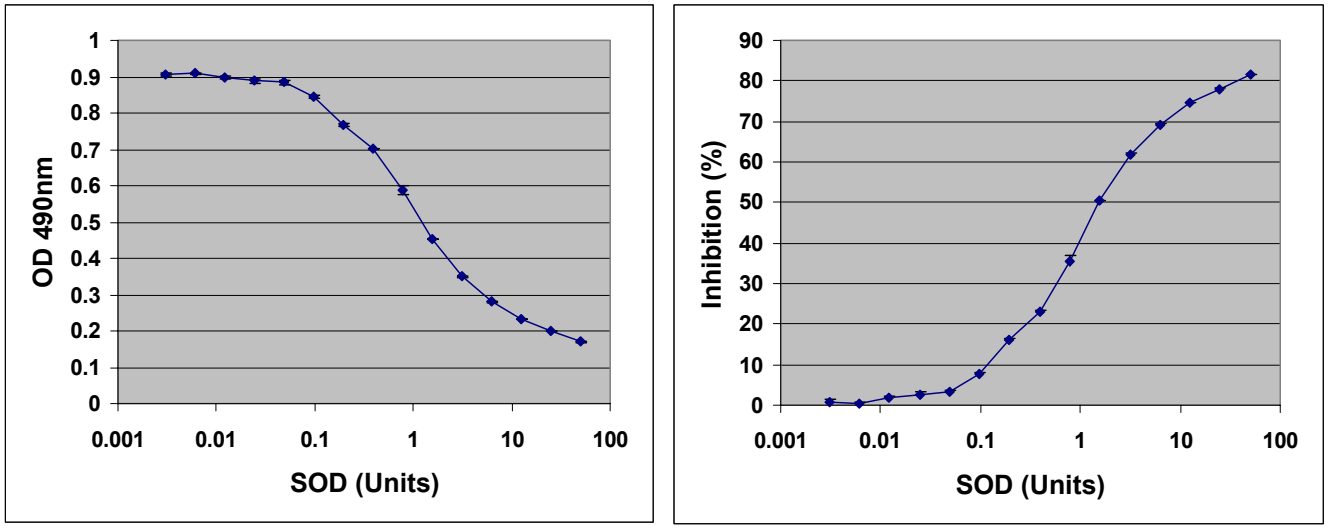

Figure 2: SOD Activity Assay Standard Curve.

References

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3. Valentine, J. S., and Hart, P. J. (2003) *Proc. Natl. Acad. Sci. U. S. A.* **100,** 3617-3622.
4. Zelko, I. N., Mariani, T. J., and Folz, R. J. (2002) *Free Radic. Biol. Med.* **33,** 337-349.

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