Product Manual

OxiSelect™ Superoxide Dismutase Activity Assay, Trial Size

Catalog Number

STA- 340- T **20 assays**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

CELL BIOLABS, INC.
Creating Solutions for Life Science Research

Introduction

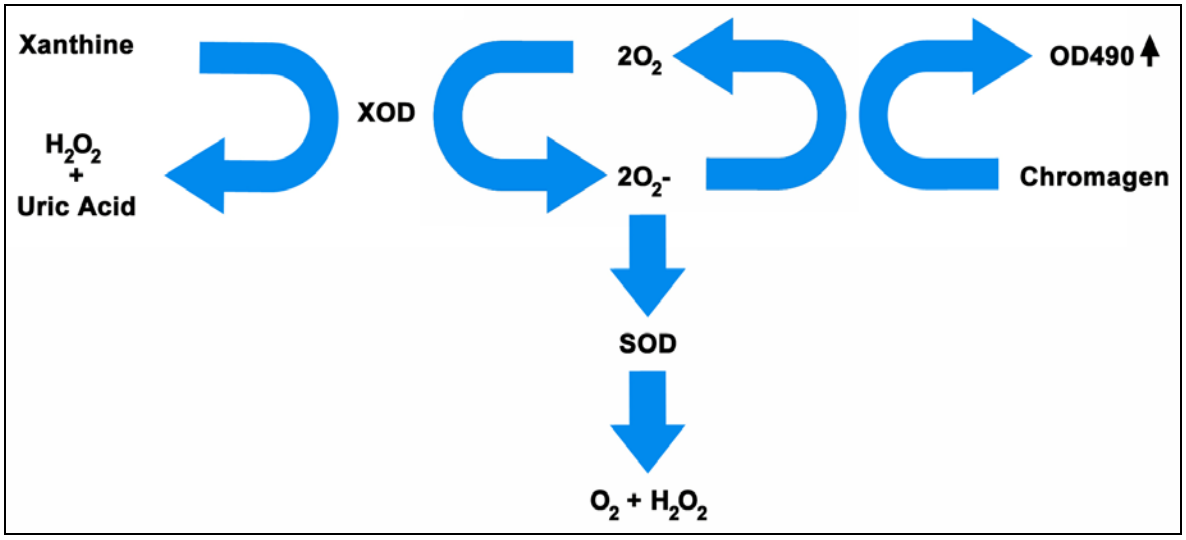
Reactive oxygen species (ROS), such as superoxide (O_2^-) and hydrogen peroxide (H_2O_2), are constantly produced during metabolic processes in all living species. Under normal physiological conditions, cellular ROS generation is counterbalanced by the action of antioxidant enzymes and other redox molecules. 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 Trial Size Superoxide Dismutase Activity Assay Kit provides sufficient reagents to perform up to 20 assays, including blanks, SOD standards and unknown protein samples.

Assay 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.



Related Products

1. STA-305: OxiSelect™ Nitrotyrosine ELISA Kit
2. STA-310: OxiSelect™ Protein Carbonyl ELISA Kit
3. STA-312: OxiSelect™ Total Glutathione (GSSG/GSH) Assay Kit
4. STA-330: OxiSelect™ TBARS Assay Kit (MDA Quantitation)
5. STA-341: OxiSelect™ Catalase Activity Assay Kit
6. STA-342: OxiSelect™ Intracellular ROS Assay Kit (Green Fluorescence)
7. STA-347: OxiSelect™ *In Vitro* ROS/RNS Assay Kit (Green Fluorescence)
8. STA-345: OxiSelect™ ORAC Activity Assay
9. STA-346: OxiSelect™ HORAC Activity Assay Kit
10. STA-832: OxiSelect™ MDA Adduct Competitive ELISA Kit
11. STA-838: OxiSelect™ HNE Adduct Competitive ELISA Kit

Kit Components

1. SOD Standard (Part No. 234001-T): One 20 µL tube provided at 5 Units/µL. 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.
2. Xanthine Solution (Part No. 234002-T): One 125 µL tube.
3. Xanthine Oxidase Solution, 150X (Part No. 234003-T): One 10 µL tube.
4. Chromagen Solution (Part No. 234004-T): One 125 µL amber tube.
5. SOD Assay Buffer, 10X (Part No. 234005): One 1.5 mL tube.

Materials Not Supplied

1. 96-well microtiter plate
2. 37ºC 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 -20ºC. Avoid multiple freeze/thaws by aliquoting. The Chromagen Solution is light sensitive and should be maintained in amber tubes.

Preparation of Reagents

- 1X SOD Assay Buffer: Dilute one vial of 10X SOD Assay Buffer to 1X with deionized water. Mix to homogeneity. Keep the second vial of 10X SOD Assay Buffer undiluted.
- 1X Xanthine Oxidase Solution: Just prior to use, dilute the 150X 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.

Preparation of Samples

- 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). 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 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 an anticoagulant such as heparin, citrate or EDTA and mix by inversion. Centrifuge the blood at 1000 x g at 4°C for 10 minutes. Collect plasma supernatant without disturbing the white buffy layer. 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. Remove the yellow serum supernatant without disturbing the white buffy layer. Samples should be tested immediately or frozen at -80°C for storage.

Assay Protocol

1. 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**

2. 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 37ºC.
3. Read absorbance at 490 nm on a microplate reader.

Preparation of SOD Standards (Optional)

1. Thaw SOD Standard at 4ºC.
2. Freshly prepare a dilution series (1:4 is suggested) of SOD Standard in the concentration range of 5 Units/µL – 1.2 mU/µL by diluting the SOD Standard in 1X Assay Buffer (see Preparation of Reagents).

Standard Tubes	SOD Standard (µL)	1X Assay Buffer (µL)	SOD (U/ µL)
1	35	0	5
2	10 of Tube #1	30	1.25
3	10 of Tube #2	30	0.312
4	10 of Tube #3	30	0.078
5	10 of Tube #4	30	0.0195
6	10 of Tube #5	30	0.0048
7	10 of Tube #6	30	0.0012
8	0	30	0

Table 1. Suggested preparation of SOD standards.

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 37ºC.
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 %) = $(OD_{blank}-OD_{sample})/(OD_{blank}) \times 100$

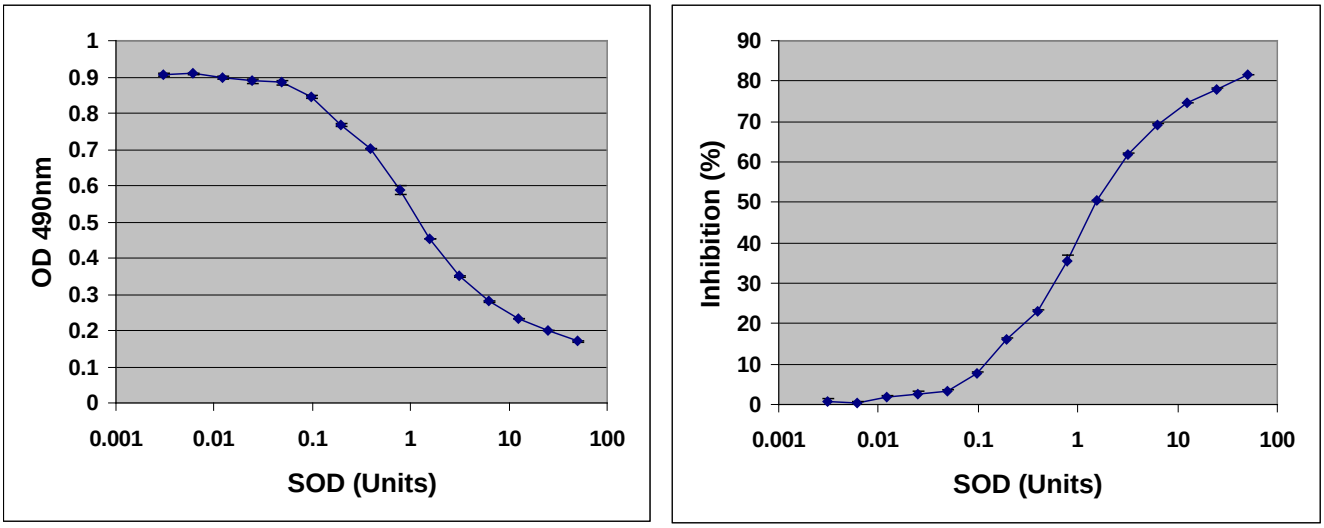


Figure 1: SOD Activity Assay Standard Curve. Left: SOD activity as a function of optical density (OD). **Right**: SOD activity as a function of inhibition percentage.

References

1. Lepock, J. R., Arnold, L. D., Torrie, B. H., Andrews, B., and Kruuv, J. (1985) *Arch. Biochem. Biophys.* **241,** 243–251.
2. Lepock, J. R., Frey, H. E., and Hallewell, R. A. (1990) *J. Biol. Chem.* **265,** 21612–21618.
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.

Recent Product Citations

1. Chan, S.H. et al. (2017). SIRT1 inhibition causes oxidative stress and inflammation in patients with coronary artery disease. *Redox Biol.* **13**:301-309.
2. Mahmoud, S.S. et al. (2017). Protective effects of ivabradine (alone and combined with atenolol or enalapril) on experimentally-induced acute myocardial infarction in albino rats. *Z.U.M.J.* **23**:2 March.
3. Choi, Y. et al. (2017). Comparative toxicity of silver nanoparticles and silver ions to *Escherichia coli. J. Env. Sci.* doi:10.1016/j.jes.2017.04.28.
4. Lei Shen et al. (2017). C−X−C motif chemokine ligand 8 promotes endothelial cell homing via the Akt−signal transducer and activator of transcription pathway to accelerate healing of ischemic and hypoxic skin ulcers. *Experimental and Therapeutic Medicine*. **13** (6): 3021-303.
5. Kim, M.H. et al. (2016). Antioxidant and hepatoprotective effects of fermented red ginseng against high fat diet-induced hyperlipidemia in rats. *Lab Anim Res*. **32**(4):217-223. doi: 10.5625/lar.2016.32.4.217.
6. Noratto, G. at al. (2016). Red raspberry decreases heart biomarkers of cardiac remodeling associated with oxidative and inflammatory stress in obese diabetic db/db mice. *Food Funct*. **227**:305-314. doi: 10.1039/C6FO01330A
7. Feng, J. et al. (2016). Inhibitor of nicotinamide phosphoribosyltransferase sensitizes glioblastoma cells to temozolomide via activating ROS/JNK signaling pathway. *BioMed Res. Int.* doi:10.1155/2016-1450843.
8. Bozkurt, M. et al. (2016). Comparative evaluation of dietary supplementation with mannan oligosaccharide and oregano essential oil in forced molted and fully fed laying hens between 82 and 106 weeks of age. *Poult Sci*. doi:10.3382/ps/pew140.
9. Kurade, M.B. et al. (2016). Insights into microalgae mediated biodegradation of diazinon by Chlorella vulgaris: Microalgal tolerance to xenobiotic pollutants and metabolism. *Algal Res.* 20:126-134.
10. Xiong, J. Q. et al. (2016). Ciprofloxacin toxicity and its co-metabolic removal by a freshwater microalga Chlamydomonas mexicana. *J Hazard Mater*. doi:10.1016/j.jhazmat.2016.04.073.
11. Bozkurt, M. et al. (2016). Effect of anticoccidial monensin with oregano essential oil on broilers experimentally challenged with mixed Eimeria spp. *Poult Sci*. doi:10.3382/ps/pew077.
12. Kara, Ö. et al. (2016). Effects of selenium on ischaemia–reperfusion injury in a rat testis model. *Andrologia.* doi:10.1111/and.12571.
13. Kara, M. et al. (2016). Evaluation of the protective effects of hesperetin against cisplatin-induced ototoxicity in a rat animal model. *Int J Pediatr Otorhinolaryngol.* **85**:12-18.
14. Erbaş, O. et al. (2016). Levetiracetam attenuates rotenone-induced toxicity: A rat model of Parkinson's disease. *Environ Toxicol Pharmacol.* **42**:226-230.
15. Liu, L. et al. (2016). Noise induced hearing loss impairs spatial learning/memory and hippocampal neurogenesis in mice. *Sci Rep*. doi:10.1038/srep20374.

16. Xiong, J. Q. et al. (2016). Biodegradation of carbamazepine using freshwater microalgae Chlamydomonas mexicana and Scenedesmus obliquus and the determination of its metabolic fate. *Bioresource Technol.* doi:10.1016/j.biortech.2016.01.038.
17. Murat, N. et al. (2016). Resveratrol protects and restores endothelium-dependent relaxation in hypercholesterolemic rabbit corpus cavernosum. *J Sex Med.* **13**:12-21.
18. Song, J. W. et al. (2015). Protective effects of manassantin A against ethanol-induced gastric injury in rats. *Biol Pharm Bull.* doi:10.1248/bpb.b15-00642.
19. Arumugam, A. et al. (2015). Desacetyl nimbinene inhibits breast cancer growth and metastasis through reactive oxygen species mediated mechanisms. *Tumor Biol.* doi:10.1007/s13277-015-4468-x.
20. Pandupuspitasari, N. S. et al. (2015). Effects of diludine on the production, oxidative status, and biochemical parameters in transition cows. *J Environ Agric Sci.* **6**:3-9.
21. Choi, M. H. et al. (2015). Phenolic acids and quercetin from Korean black raspberry seed protected against acetaminophen-induced oxidative stress in mice. *J Funct Foods.* doi:10.1016/j.jff.2015.09.052.
22. Pérez, E. et al. (2015). Biocompatibility evaluation of pH and glutathione-responsive nanohydrogels after intravenous administration. *Colloids Surf B Biointerfaces.* **136**:222-231.
23. Hou, Z. et al. (2015). Nutrigenomic effects of edible bird's nest on insulin signaling in ovariectomized rats. *Drug Des Devel Ther.* **9**:4115-25.
24. Zaghlool, et al. (2015). Assessment of protective effects of extracts of Zingiber officinale and Althaea officinalis on pyloric ligation-induced gastric ulcer in experimental animals. *UK J Pharm Biosci*. **3**:48-57.
25. Hunter, J. P. et al. (2015). Ischaemic conditioning reduces kidney injury in an experimental large-animal model of warm renal ischaemia. *Br J Surg*. doi: 10.1002/bjs.9909.

Please see the complete list of product citations: http://www.cellbiolabs.com/superoxide-dismutase-sod-assay.

Warranty

These products are warranted to perform as described in their labeling and in Cell Biolabs literature when used in accordance with their instructions. THERE ARE NO WARRANTIES THAT EXTEND BEYOND THIS EXPRESSED WARRANTY AND CELL BIOLABS DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR WARRANTY OF FITNESS FOR PARTICULAR PURPOSE. CELL BIOLABS's sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of CELL BIOLABS, to repair or replace the products. In no event shall CELL BIOLABS be liable for any proximate, incidental or consequential damages in connection with the products.

Contact Information

Cell Biolabs, Inc.
7758 Arjons Drive
San Diego, CA 92126
Worldwide: +1 858-271-6500
USA Toll-Free: 1-888-CBL-0505
E-mail: tech@cellbiolabs.com
www.cellbiolabs.com