Superoxide Dismutase (SOD)

Colorimetric Assay kit

(192 Tests)

Zellbio GmbH (Germany)

CAT No. ZX-44108-192

www.zellx.de

Sample types validated for:

Serum, plasma, Cells, Tissues and Erythrocyte Lysates

!!! Caution: This product is for Research Use Only. Not for *in-vitro* Diagnostics !!!

Table of Contents

Please read this insert completely prior to using the product.

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Introduction

Background

Superoxide Dismutases (SODs, EC1.15.1.1) are enzymes which detoxify superoxide anions (O_2^-) by converting (dismutating) them into two less damaging species: oxygen (O_2) and hydrogen peroxide (H_2O_2). The dismutation reaction occurs in nearly all cells as an important antioxidant mechanism in response to O_2^- exposure to protect against its toxicity.

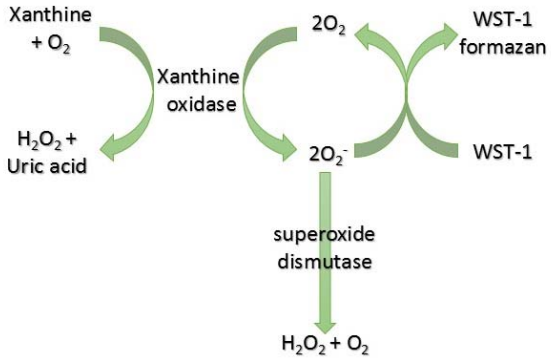
There are three major families of superoxide dismutases, which are classified based on their metal cofactor: Cu/Zn, Fe/Mn, and Ni. The major intracellular SOD is a 32-kD copper and zinc containing homodimer (Cu/Zn SOD).

Abnormal activities of SODs are associated with different health disorders such as familial Amyotrophic Lateral Sclerosis (ALS), perinatal lethality, neural disorders, downs syndrome, thyroid dysfunction and cancer.

Assay principle

The ZellX® Superoxide Dismutase (SOD) Activity Kit is designed to quantitatively measure SOD activity in a variety of samples. The kit can be used to evaluate the activity of all SOD types, including Cu/Zn, Mn, and Fe SOD types. A bovine erythrocyte SOD standard is provided to generate a standard curve for the assay and all samples should be read off of the standard curve.

Samples are diluted in Assay Buffer and added to the wells. The Substrate is added followed by Xanthine Oxidase Reagent and incubated at room temperature for 20 minutes. The Xanthine Oxidase generates superoxide in the presence of oxygen, which converts a colorless substrate in the Detection Reagent into a yellow colored product. The colored product is read at 450 nm. Increasing levels of SOD in the samples causes a decrease in superoxide concentration and a reduction in yellow product. The results are expressed in terms of units of SOD activity per mL (U/mL).



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General information

Materials supplied in the Kit

Component	*Quantity*
Superoxide Dismutase Standard (4 U/mL)	1 Vial
Xanthine Oxidase Concentrate (25 X)	225 µL
Xanthine Oxidase Buffer	6 mL
Assay Buffer	50 mL
Substrate Concentrate (10 X)	1.1 mL
Substrate Diluent	12 mL
Clear Half Area 96 Well Plate	2 plate

All reagents should be stored at 4° C until the expiration date of the kit. Upon reconstitution, the Superoxide Dismutase Standard should be aliquoted and stored at -20°C.

Materials required but not supplied

2 mM Potassium Cyanide solution for inhibition of Cu/Zn and extracellular SOD if desired

Deionized water (diH_2O)

Phosphate Buffer Saline (PBS)

Microplate/ELISA reader capable of reading optical absorption at 450 nm

Centrifuge, Vortex mixer

Precision pipettes and multichannel pipette and disposable pipette tips

Disposable 1.5-2 mL microtubes for sample preparation

Precautions

This kit should only be used by qualified personnel who have had laboratory safety instruction. The complete insert should be read and understood before attempting to use the product.

General remarks

- Equilibrate all kit components to room temperature (RT) 30 minutes before use.

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- The instruction must be strictly followed.
- The reading of Microplate/ELISA reader must be set at appropriate wavelength.
- Pipette tips should not be used more than once to prevent cross contamination.
- Reagents of different batches should not be mixed or used after their expiration dates.

Assay protocol

Reagent preparation

i. **Xanthine Oxidase Solution:** Vortex the solution prior to use (**Pipet from the base of the tube**); prepare a 1:25 dilution of Xanthine Oxidase Concentrate with Xanthine Oxidase Buffer (mix 1 part Xanthine Oxidase Concentrate with 24 parts Xanthine Oxidase Buffer).

ii. **Substrate Working Solution:** Vortex the solution prior to use; Prepare a 1:10 dilution of Substrate Concentrate with Substrate Diluent (mix 1 mL of Substrate Concentrate with 9 mL of Substrate Diluent). **Substrate Diluent and Substrate Working Solution must be kept tightly capped.**

Sample preparation

After collecting the sample, extraction should be immediately carried out in accordance with the related instruction. After extraction, experiment should be conducted immediately, otherwise, keep the sample at -70 or -80˚C. Avoid repeated freeze-thaw cycles.

Samples should be kept on ice to maintain enzyme activity.

I. **Serum, and Plasma:**

- Collect plasma in heparin tubes.
- Centrifuge at 700 g for 15 min at 4˚C and Aspirate off the pale yellow supernatant.
- Collect blood in serum tubes.
- Centrifuge at 700 g for 15 min at 4˚C and Aspirate off the serum supernatant.
- Plasma and serum should be diluted ≥ 1:5 by taking one part of sample and adding 4 or more parts of Assay Buffer prior to conducting the assay.

❖ Some serum and plasma samples may contain significant hemoglobin concentrations which may result in a high background signal. Therefore the optical density at 450 nm should be determined prior to running the assay. After addition of the Substrate Working Solution to all the used wells, the optical density at 450 nm should be read and subtracted from the optical density recorded at the end of the 20-minute incubation.

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II. **Erythrocytes, Red Blood Cells:**

- Erythrocytes can be lysed by taking the pelleted RBCs from the Plasma step above and adding 4 volumes of ice-cold diH_2O.
- Centrifuge at 10000 g for 15 min at 4˚C.
- RBCs should be diluted ≥ 1:100 by taking one part of lysed RBCs and adding 99 or more parts of Assay Buffer prior to conducting the assay.

❖ Lysed RBCs will exhibit high background color. After adding the Detection Substrate Working Solution read the blank OD at 450 nm prior to addition of Xanthine Oxidase Solution.

III. **Cell lysate:**

- Collect 1× 10^6 cells and wash with 1 mL cold PBS. (For adherent cells trypsinize them gently and then collect the cells).
- Centrifuge at 250 g for 10 min at 4˚C and discard the supernatant.
- Wash the cell pellet with ice-cold PBS.
- Centrifuge at 250 g for 10 min at 4˚C and discard the supernatant.
- Homogenize or sonicate the cell pellet in 0.5-1 mL of PBS per 100 mg of cells.
- Centrifuge at 1500 g for 10 min at 4°C and collect the supernatant.
- Cell samples should be diluted ≥ 1:4 by taking one part of sample (supernatant) and adding 3 or more parts of Assay Buffer prior to conducting the assay.

❖ To measure cytosolic (SOD1, Cu/Zn) and/or mitochondrial SOD (SOD2, Mn), the sample supernatants should be centrifuged at 10000 g for 15 min at 4°C. The supernatants will contain the cytosolic SOD and the cell pellets will contain mitochondrial SOD.

❖ To determine Mn SOD (SOD2) activity, treat the sample with 2 mM potassium cyanide. Addition of cyanide will inactivate other SOD enzymes.

IV. **Tissue sample:**

- Wash fresh tissue with cold PBS to remove blood, and blot it on a filter paper.
- Incise sample and weigh up.
- Homogenize or sonicate the tissue in 0.5-1 mL of PBS per 100 mg of tissue.
- Centrifuge at 1500 g for 10 min at 4°C and collect the supernatant.
- Tissue samples should be diluted ≥ 1:4 by taking one part of sample (supernatant) and adding 3 or more parts of Assay Buffer prior to conducting the assay.
- To measure cytosolic (SOD1, Cu/Zn) and/or mitochondrial SOD (SOD2, Mn), the sample supernatants should be centrifuged at 10000 g for 15 min at 4°C. The supernatants will contain the cytosolic SOD and the cell pellets will contain mitochondrial SOD.
- To determine Mn SOD (SOD2) activity, treat the sample with 2 mM potassium cyanide. Addition of cyanide will inactivate other SOD enzymes

Experiment should be conducted immediately. Otherwise, aliquots of the sample should be kept at -70˚C or lower temperature, preferably after being frozen in liquid nitrogen.

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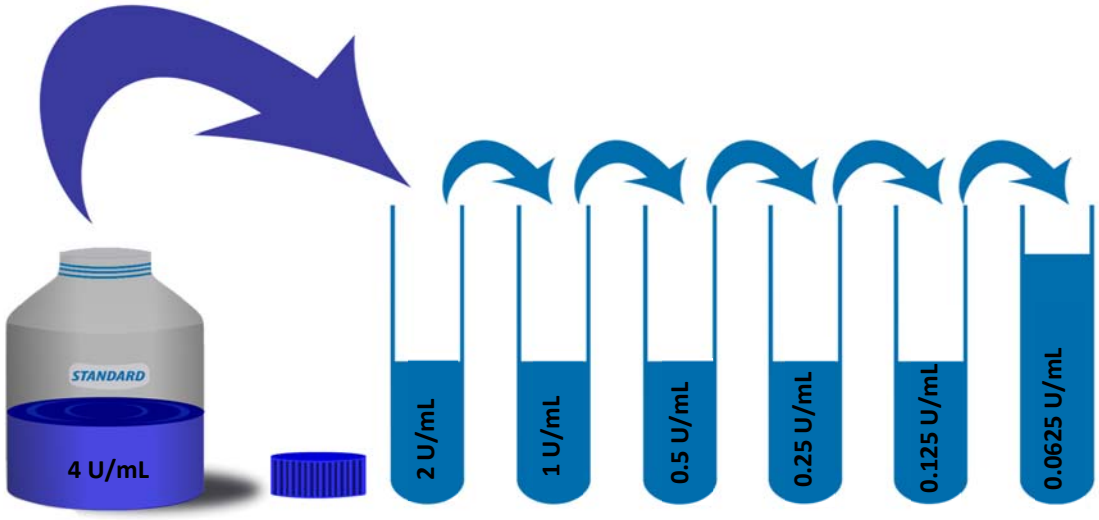
Standard preparation

- Add 250 µL of Assay Buffer to the lyophilized vial of SOD Standard, vortex well and incubate for 5 minutes at RT to make the Standard stock or Standard No.7 (4 U/mL).
- Prepare a 1:2 dilution of the Standard No.7 with Assay Buffer (mix 75 µL of prepared standard with 75 µL of Assay buffer), and label as the Standard No.6 (2 U/mL).
- Make series of lower dilutions as described in the table.
- The Assay Buffer is used as the 0 U/mL standard.

No.	*Concentration SOD*	*Material needed*
Standard No.6	2 U/mL	75 µL SOD Standard No.7 + 75 µL Assay Buffer
Standard No.5	1 U/mL	75 µL Standard No.6 + 75 µL Assay Buffer
Standard No.4	0.5 U/mL	75 µL Standard No.5 + 75 µL Assay Buffer
Standard No.3	0.25 U/mL	75 µL Standard No.4 + 75 µL Assay Buffer
Standard No.2	0.125 U/mL	75 µL Standard No.3 + 75 µL Assay Buffer
Standard No.1	0.0625 U/mL	75 µL Standard No.2 + 75 µL Assay Buffer
Standard No.0	0 U/mL	75 µL Assay Buffer



All standard must be used within 2 hours of preparation. Aliquot the reconstituted SOD vial and keep at -20°C.

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Assay Procedure

The Assay Buffer contains detergents. When pipetting samples or standards into the wells, **carefully** add the sample slowly down the side of the well. **Avoid bubbles!**

1. Pipette 10 µL samples or standards into duplicate wells in the plate.
2. Pipette 10 µL Assay Buffer into duplicate wells as the Zero standard.
3. Add 50 µL of the Substrate Working Solution to each well using a multichannel pipette. **NOTE: If your samples have significant yellow coloration then pre-read the optical density at 450 nm**
4. Add 25 µL of the Xanthine Oxidase Solution to each of the wells using a multichannel pipette.
5. Incubate at room temperature for 20 minutes.
6. Read the optical density at 450 nm.

Calculation

- Average the duplicate optical density (OD) readings for each standard and sample.
- Create a standard curve by reducing the data using the four parameter logistic curve (4PLC) fitting routine on the plate reader using the adjusted OD values
- The concentrations obtained should be multiplied by the dilution factor to obtain sample values.

$$SOD\ inhibition = \frac{(mean\ OD\ of\ Standard\ or\ Sample)}{(mean\ OD\ of\ Zero\ Standard)} \times 100$$

SOD Unit Definition:

One unit of SOD is defined as the amount of enzyme causing half the maximum inhibition of the reduction of 1.5 mM Nitro blue tetrazolium in the presence of riboflavin at 25°C and pH 7.8.

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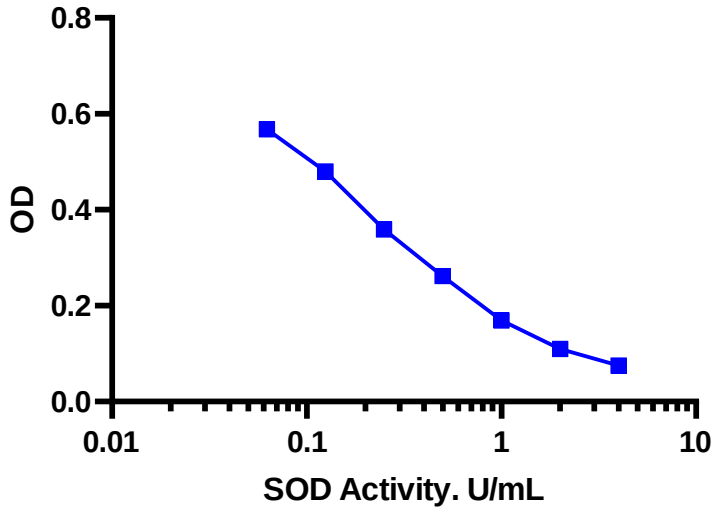
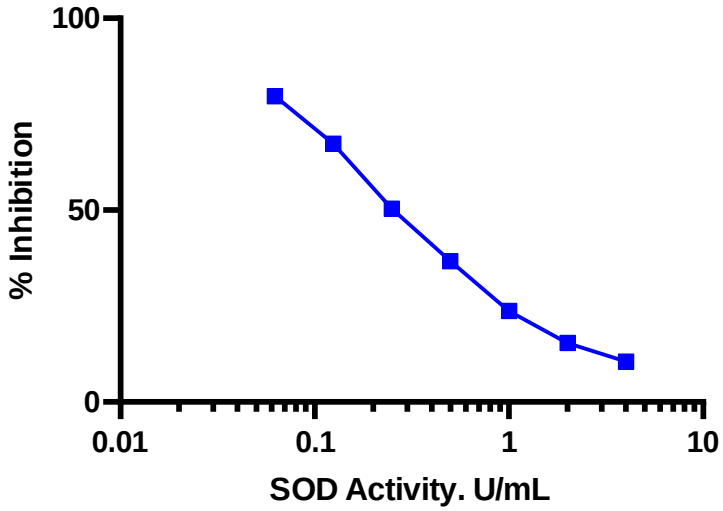


A typical standard curve of ZellX® SOD Assay kit based on OD values

Run your own standard curves for calculation of results



A typical standard curve of ZellX® SOD Assay kit based on the % inhibition

Run your own standard curves for calculation of results

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Sensitivity

The sensitivity of the ZellX® SOD assay was determined as 0.044 U/mL.

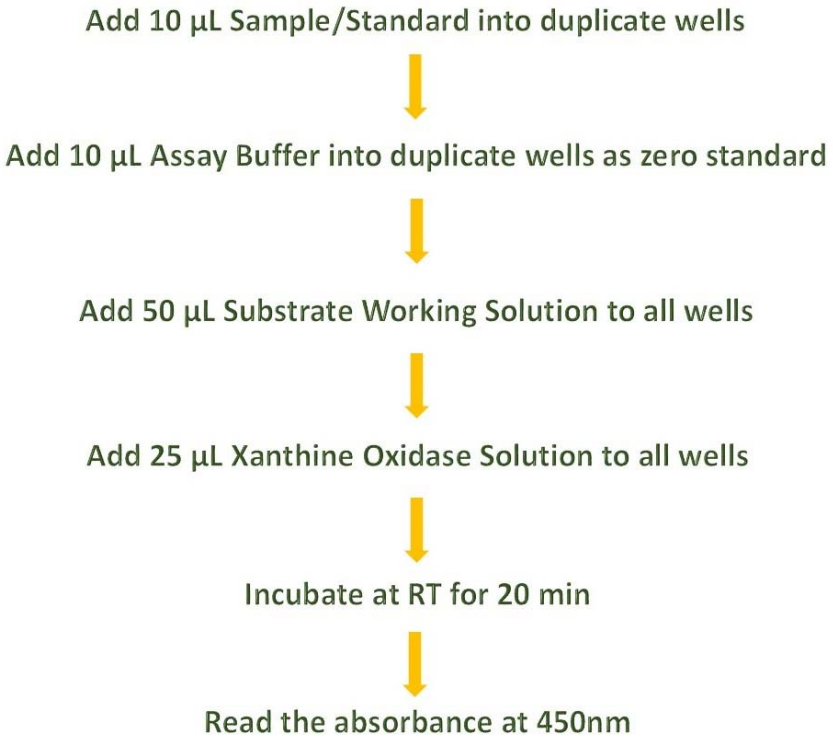
Precision

Intra-Assay Precision (Precision within an assay): 3 samples were tested 20 times in an assay.

Inter-Assay Precision (Precision between assays): 3 samples were tested in duplicate on 16 different assays over multiple days.

Item	*%CV*
Intra assay	7.3, 4.6, 16.8
Inter assay	10.5, 6.1, 13.8

Protocol summary



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References

1. Torsdottir, G. et al. “Case-control studies on ceruloplasmin and superoxide dismutase (SOD1) in neurodegenerative diseases: a short review”. J.Neurol.Sci. 2010. 299(1-2):51-54.
2. Giacco, F & Brownlee, M. “Oxidative stress and diabetic complications”. Circ.Res. 2010. 107(9):1058-1070.
3. Bae, S-C, et al. “Inadequate antioxidant nutrient intake and altered plasma antioxidant status of rheumatoid arthritis patients”, J.Amer.Coll.Nutr. 2003. 22(4):311-315.
4. Akbostanci, MC, et al. “Erythrocyte superoxide dismutase activity differs in clinical subgroups of Parkinson’s disease patients”. Acta Neurol.Belg. 2001. 101:180-183.
5. Shainkin-Kestenbaum, R, et al. “Reduced superoxide dismutase activity in erythrocytes of dialysis patients: a possible factor in the etiology of uremic anemia”. Nephron. 1990. 55(3):251-253.
6. Saito, T. “Superoxide dismutase level in human erythrocytes and its clinical application to patients with cancers and thyroidal dysfunctions”. Hokkaido Igaku Zasshi. 1987. 62(2):257-268.
7. Liocher, SI and Fridovich, I., “The Effects of Superoxide Dismutase on H2O2 Formation”., Free Rad. Biol. Med., 2007, 42:1465-1469

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