

ARBOR ASSAYS™
Interactive Assay Solutions™



DetectX[®]

Superoxide Dismutase (SOD) Colorimetric Activity Kit

2 Plate Kit Catalog Number K028-H1

Species Independent

Sample Types Validated:

**Serum, Plasma, Cells, Tissues
and Erythrocyte Lysates**

Please read this insert completely prior to using the product.
For research use only. Not for use in diagnostic procedures.

www.ArborAssays.com   

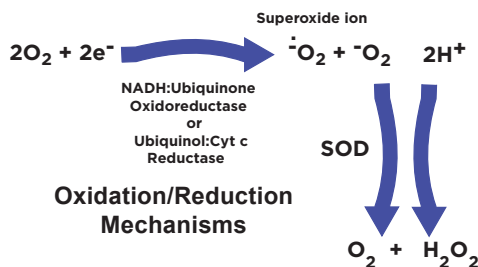
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BACKGROUND

Short-lived and highly reactive oxygen species (ROS) such as O_2^- (superoxide), $^{\bullet}OH$ (hydroxyl radical), and H_2O_2 (hydrogen peroxide) are continuously generated *in vivo*. In the resting state, the balance between antioxidants and oxidants is sufficient to prevent the disruption of normal physiologic functions; however, either increases in oxidants or decreases in antioxidants can disrupt this balance giving rise to elevated levels of reactive oxygen species (ROS)^{1,2}.



The cellular levels of ROS are controlled by antioxidant enzymes and small molecule antioxidants. The major antioxidant enzymes, superoxide dismutases (SODs), including copper-zinc superoxide dismutase (Cu/ZnSOD, SOD1), manganese superoxide dismutase (Mn-SOD, SOD2) and extracellular superoxide dismutase (EC-SOD, SOD3), all play critical roles in scavenging O_2^- . Decreased SOD activity results in elevated level of superoxide which in turn leads to decreased NO but increased peroxynitrite concentrations. The major intracellular SOD is a 32-kD copper and zinc containing homodimer (Cu/Zn SOD). The mitochondrial SOD (MnSOD) is a manganese-containing 93-kD homotetramer that is synthesized in the cytoplasm and translocated to the inner matrix of mitochondria. EC-SOD is the primary extracellular SOD enzyme and is highly expressed in many organs. Increased SOD activity levels are seen in Downs Syndrome³ while decreased activity is seen in diabetes, Alzheimer's disease, rheumatoid arthritis, Parkinson's disease, uremic anemia, atherosclerosis, some cancers, and thyroid dysfunction³⁻⁸.

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2. Imlay, JA. "Cellular Defences Against Superoxide and Hydrogen peroxide", Ann Rev. Biochem., 2008, 77:755-776.
3. 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.
4. Giacco, F & Brownlee, M. "Oxidative stress and diabetic complications". Circ.Res. 2010. 107(9):1058-1070.
5. 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.
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8. 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.
9. Okado-Matsumoto, A, and Fridovich, I., "Subcellular Distribution of Superoxide Dismutases (SOD) in Rat Liver". J. Biol. Chem. 2001, 276:38,388-38,393.

ASSAY PRINCIPLE

The DetectX® Superoxide Dismutase (SOD) Activity Kit is designed to quantitatively measure SOD activity in a variety of samples. The assay measures all types of SOD activity, including Cu/Zn, Mn, and FeSOD types. Please read the complete kit insert before performing this assay. 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 our specially colored 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 activity of the SOD in the sample is calculated after making a suitable correction for any dilution, using software available with most plate readers. The results are expressed in terms of units of SOD activity per mL.

RELATED PRODUCTS

Kits	Catalog No.
Catalase Colorimetric Detection Kit	K033-H1
Catalase Fluorescent Detection Kit	K033-F1
Glutathione Colorimetric Detection Kit	K006-H1
Glutathione Fluorescent Detection Kits	K006-F1/F5
Glutathione Reductase Fluorescent Activity Kit	K009-F1
Glutathione S-Transferase Fluorescent Activity Kit	K008-F1
Hemoglobin Dual Range Detection Kit	K013-H1
Hydrogen Peroxide Colorimetric Activity Kit	K034-H1
Hydrogen Peroxide Fluorescent Activity Kit	K034-F1
Nitric Oxide Colorimetric Detection Kit	K024-H1



SUPPLIED COMPONENTS

Clear 96 well Half Area Plates

See www.ArborAssays.com/resources/#general-info for plate dimension data.

2 Plates

Catalog Number X018-2EA

Superoxide Dismutase Standard

1 Unit/vial of bovine Erythrocyte Superoxide Dismutase lyophilized.

1 Vial

Catalog Number C098-1EA

Assay Buffer

Buffer containing detergents, stabilizers and dye.

50 mL

Catalog Number X100-50ML

Xanthine Oxidase Buffer

Buffer containing detergents and stabilizers.

6 mL

Catalog Number X102-6ML

Xanthine Oxidase Concentrate

A 25X concentrated suspension of Xanthine Oxidase.

225 µL

Catalog Number C099-225UL

Substrate Diluent

Substrate buffer. **Keep tightly capped.**

12 mL

Catalog Number X101-12ML

Substrate Concentrate

A 10X concentrate of the Detection Reagent.

1.1 mL

Catalog Number C100-1.1ML

STORAGE INSTRUCTIONS

All components of this kit should be stored at 4°C until the expiration date of the kit.

Once reconstituted, the Superoxide Dismutase Standard should be aliquoted and stored at -20°C.

OTHER MATERIALS REQUIRED

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

Protease inhibitors (optional) such as phenylmethylsulfonyl fluoride (PMSF) to supplement step 5 in sample preparation.

Repeater pipet with disposable tips capable of dispensing 25 μ L and 50 μ L.

96 well plate reader capable of reading optical absorption at 450 nm.

Software for converting optical density (OD) readings from the plate reader and carrying out four parameter logistic curve (4PLC) fitting. Contact your plate reader manufacturer for details.

PRECAUTIONS

As with all such products, 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.

SAMPLE TYPES AND PREPARATION

Iron containing SODs (FeSOD) are found in some bacteria and plants and have similar properties to MnSOD (SOD2). Extracellular SOD (SOD3) is obtained from serum, plasma, ascites or synovial fluid fluids.

Samples that need to be stored after collection should be stored at -70°C or lower, preferably after being frozen in liquid nitrogen. This assay has been validated for serum, plasma and erythrocyte lysates. Samples containing visible particulate should be centrifuged prior to using.

Samples should be kept on ice to maintain enzyme activity.

Plasma and Serum Samples

- 1a. Collect plasma in heparin or EDTA tubes. Centrifuge the sample at 700 x g for 15 minutes at 4°C . Aspirate off the pale yellow supernatant. Assay immediately or freeze at -80°C .
- b. Collect blood in serum tubes. Centrifuge the sample at 700 x g for 15 minutes at 4°C . Aspirate off the serum supernatant. Assay immediately or freeze at -80°C .
2. Plasma and serum should be diluted at least 1:5 by taking one part of sample and adding 4 parts of Assay Buffer prior to assaying. Further dilutions in Assay Buffer may be needed.

NOTE: Some serum and plasma samples may contain significant hemoglobin concentrations and the optical density at 450 nm determined prior to running the assay. After addition of the Substrate 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.



RBC/Erythrocytes

1. Erythrocytes can be lysed by taking the pelleted RBCs from the Plasma step above and adding 4 volumes of ice cold deionized water.
2. Centrifuge at 10,000 x g for 15 minutes at 4°C to remove debris. Assay immediately or store at -80°C.
3. RBCs should be diluted at least 1:100 prior to running in the assay. Take 10 µL of lysed RBCs and add to 990 mL of Assay Buffer prior to assaying. Further dilutions in Assay Buffer may be needed.

NOTE: Lysed RBCs will exhibit high background color. After adding the Detection Substrate solution read the blank OD at 450 nm prior to addition of Xanthine Oxidase reagent.

Cell Suspensions and Adherent Cells

1. Centrifuge $\geq 1 \times 10^6$ cells in suspension at 250 x g for 10 minutes at 4°C. Discard the supernatant.
2. Resuspend the cell pellet in ice-cold PBS and transfer to a micro-centrifuge tube on ice. Centrifuge, discard supernatant, and place on ice.
3. Wash $\geq 1 \times 10^6$ adherent cells with PBS prior to being harvested by gentle trypsinization. Transfer to a tube on ice and centrifuge at 250 x g for 10 minutes at 4°C and discard the supernatant.
4. Wash the pellet with ice-cold PBS and centrifuge at 250 x g for 10 minutes at 4°C. Discard the supernatant.
5. Homogenize or sonicate the pellet in 0.5-1 mL of PBS (+ protease inhibitors if desired) per 100 mg of cells. Centrifuge at 1,500 x g for 10 minutes at 4°C. Collect the supernatant.
- 6a. Assay the supernatant immediately or store at -80°C. Dilute at least 1:4 in Assay Buffer prior to measuring SOD activity. A 1:4 dilution of the sample is made by adding 3 parts of Assay Buffer to 1 part of supernatant.

OR

- 6b. To measure cytosolic (SOD1, Cu/Zn) and/or mitochondrial SOD (SOD2, Mn) the supernatants collected in Step 5 should be centrifuged at 10,000 x g for 15 minutes at 4°C. The resulting supernatants will contain the cytosolic SOD and the remaining cell pellets will contain mitochondrial SOD. To determine Mn SOD (SOD2) activity, homogenize the pellet in cold buffer containing a final concentration of 2 mM potassium cyanide. Addition of cyanide will inactivate Cu/Zn SOD enzymes. Assay immediately or store at -80°C.

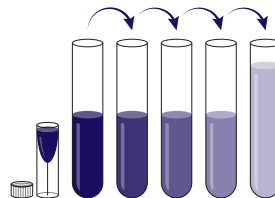
Tissue Samples

1. Wash tissue thoroughly with ice cold PBS prior to processing.
2. Homogenize and continue with supernatant as described in steps (5) and (6) for Cell Suspensions above.

STANDARD PREPARATION

Standard Preparation

Superoxide Dismutase Standards are prepared by labeling tubes as #2 through #7. The lyophilized vial of SOD Standard is used for Standard 1. Add 250 μ L of Assay Buffer to the vial, vortex and let stand at room temperature for 5 minutes. Pipet 75 μ L of Assay Buffer into tubes #2 to #7. Carefully add 75 μ L of the SOD Standard from the vial to tube #2 and vortex completely. Repeat this for tubes #2 through #7. The activity of SOD in the SOD Standard vial and tubes 2 through 7 will be 4, 2, 1, 0.5, 0.25, 0.125 and 0.0625 U/mL.



Use all Standards within 2 hours of preparation. Aliquot the reconstituted SOD vial and freeze at -20°C.

	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6	Std 7
Assay Buffer Vol (μL)	250	75	75	75	75	75	75
Addition	Stock	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6
Vol of Addition (μL)	-	75	75	75	75	75	75
Final Activity (U/mL)	4	2	1	0.5	0.25	0.125	0.0625

Xanthine Oxidase Preparation

Vortex the suspension of Xanthine Oxidase prior to pipetting. Pipet from the base of the tube.

	1/2 Plate	1 Plate	1.5 Plates	2 Plates
Xanthine Oxidase	50 μ L	100 μ L	150 μ L	200 μ L
Xanthine Oxidase Buffer	1.2 mL	2.4 mL	3.6 mL	4.8 mL
Final Mixture	1.25 mL	2.5 mL	3.75 mL	5 mL

Substrate Preparation*

Vortex the vial of Concentrate prior to pipetting.

	1/2 Plate	1 Plates	1.5 Plates	2 Plates
Substrate Concentrate	250 μ L	500 μ L	750 μ L	1 mL
Substrate Diluent	2.25 mL	4.5 mL	6.75 mL	9 mL
Final Mixture	2.5 mL	5 mL	7.5 mL	10 mL

*Substrate Diluent and prepared Substrate must be kept Tightly Capped



ASSAY PROTOCOL

Use the plate layout sheet on the back page to aid in proper sample and standard identification.

The Assay Buffer contains detergents. When pipetting samples or standards into the wells CAREFULLY add the sample slowly down the side of the well. Use Reverse Pipetting* to avoid bubbles!

1. Pipet 10 μL of samples or appropriate standards into duplicate wells in the plate.
2. Pipet 10 μL of Assay Buffer into duplicate wells as the Zero standard.
3. Add 50 μL of the Substrate Preparation to each well using a repeater pipet. **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 Preparation to each well using a repeater pipet.
5. Incubate at room temperature for 20 minutes.
6. Read the optical density at 450 nm.

* Reverse pipetting involves pressing the plunger down to the blow-out prior to picking up standards and samples. The selected volume of liquid plus an excess is pulled into the pipette tip. To dispense, the plunger is pressed only down to the first position, leaving some liquid in the tip. This way, liquid remains inside the tip when dispensing, minimizing bubble formation.

CALCULATION OF RESULTS

Average the duplicate OD readings for each standard and sample.

If your sample was visibly colored, then subtract the pre-read optical density at 450 nm from the subsequent Xanthine Oxidase reaction optical density after 20 minutes.

Inhibition values are sometimes quoted for SOD activity. Inhibition values can be obtained by dividing the measured Mean OD for the standard or samples by the Mean OD for the Zero standard (No SOD) and multiplying the result by 100. Create a standard curve by reducing the data using computer software capable of generating a four-parameter logistic curve (4PLC) fit. The sample activities obtained should be multiplied by the dilution factor to obtain neat sample values.

Or use the online tool from MyAssays to calculate the data:

www.myassays.com/arbor-assays-superoxide-dismutase-colorimetric-activity-kit.assay

TYPICAL DATA

Sample	Mean OD	% Inhibition	SOD Activity (U/mL)
Standard 1	0.075	10.5	4
Standard 2	0.110	15.4	2
Standard 3	0.169	23.7	1
Standard 4	0.262	36.7	0.5
Standard 5	0.359	50.4	0.25
Standard 6	0.480	67.3	0.125
Standard 7	0.568	79.7	0.0625
Zero	0.713	100	0
Sample 1	0.231	32.4	0.605
Sample 2	0.168	23.6	1.030

Always run your own standard curves for calculation of results. Do not use these data.

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.



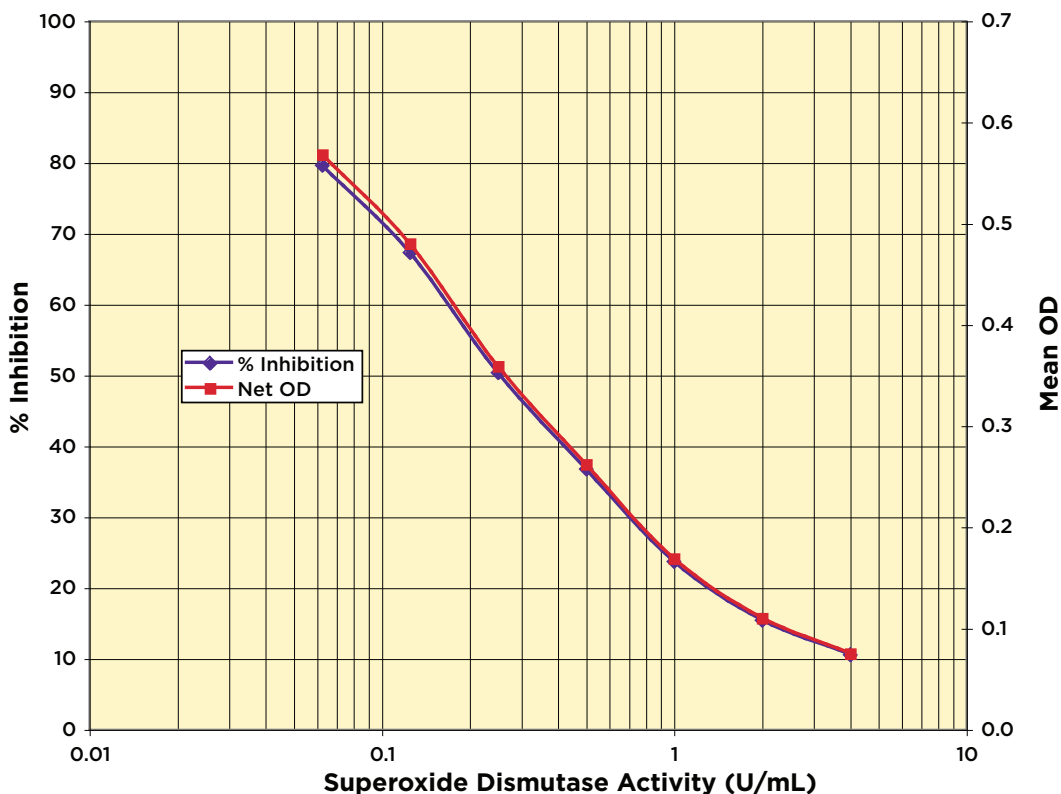
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EXPECT ASSAY ARTISTRY™

Typical Standard Curve



Always run your own standard curves for calculation of results. Do not use this data.

VALIDATION DATA

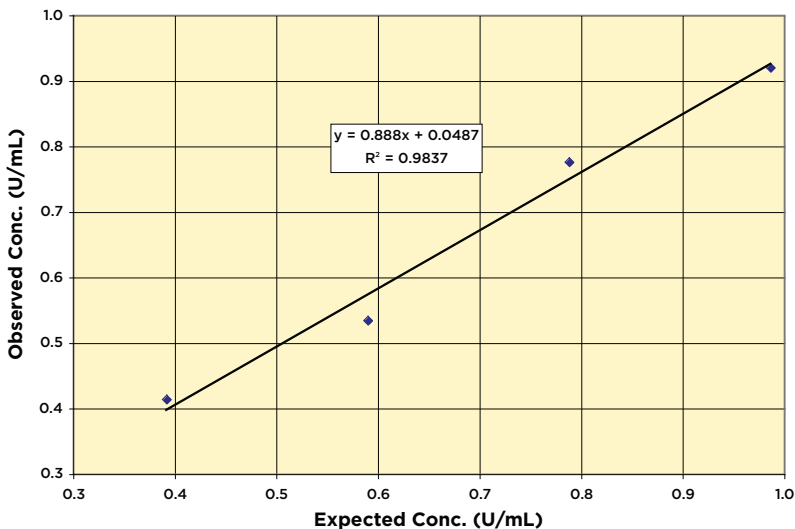
Sensitivity

Sensitivity was calculated by comparing the ODs for twenty wells run for each of the zero and standard #7. The detection limit was determined at two (2) standard deviations from the zero along the standard curve. **Sensitivity was determined as 0.044 U/mL.**

Linearity

Linearity was determined by taking two samples, one with a high known SOD activity and the other with a lower SOD activity and mixing them in the ratios given below. The measured activities were compared to the expected values based on the ratios used.

High Sample	Low Sample	Expected Activity (U/mL)	Observed Activity (U/mL)	% Recovery
80%	20%	0.987	0.920	93.2
60%	40%	0.789	0.776	98.4
40%	60%	0.590	0.534	90.4
20%	80%	0.392	0.414	105.6
Mean Recovery				96.9%



Intra Assay Precision

Three samples diluted in Assay Buffer were run in replicates of 20 in an assay. The mean and precision of the calculated concentrations were:

Sample	SOD Activity (U/mL)	%CV
1	0.407	4.6
2	0.726	7.3
3	1.203	16.8

Inter Assay Precision

Three samples diluted in Assay Buffer were run in duplicates in sixteen assays run over multiple days by four operators. The mean and precision of the calculated concentrations were:

Sample	SOD Activity (U/mL)	%CV
1	0.356	10.5
2	0.653	6.1
3	1.277	13.8

SAMPLE VALUES

Five random adult human serum and plasma samples were diluted in Assay Buffer and run in the assay. The serum samples ranged from 1.95 to 4.60 U/mL with an average of 3.44 U/mL. EDTA plasma samples ranged from 2.24 to 3.56 U/mL with an average 2.89 U/mL. Five samples of RBC from EDTA were normalized to hemoglobin levels using the DetectX® Hemoglobin Detection kit, K013-H1. The RBC activities ranged from 748 to 1,507 U/g Hgb with an average of 1052 U/g Hgb.



LIMITED WARRANTY

Arbor Assays warrants that at the time of shipment this product is free from defects in materials and workmanship. This warranty is in lieu of any other warranty expressed or implied, including but not limited to, any implied warranty of merchantability or fitness for a particular purpose.

We must be notified of any breach of this warranty within 48 hours of receipt of the product. No claim shall be honored if we are not notified within this time period, or if the product has been stored in any way other than outlined in this publication. The sole and exclusive remedy of the customer for any liability based upon this warranty is limited to the replacement of the product, or refund of the invoice price of the goods.

CONTACT INFORMATION

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