



StressMarq
Biosciences INC.

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StressXpress®

SOD Activity Kit

Catalog# SKT-214 (2 Plate kit)

Quantitative colorimetric measurement of superoxide dismutase activity

TABLE OF CONTENTS

GENERAL INFORMATION	3	Materials Supplied
	4	Precautions
	4	Storage
	4	Materials Needed but Not Supplied
INTRODUCTION	5	Background
	6	Assay Overview
PRE-ASSAY PREPARATION	7	Sample Types
	8	Sample Preparation
	9	Standard Preparation
ASSAY PROTOCOL	12	Assay Protocol
ANALYSIS	13	Calculation of Results
	13	Typical Data
	14	Validation Data
	16	Sample Values
RESOURCES	17	References
	18	Warranty and Contact Information
	19	Plate Template
	20	Notes

GENERAL INFORMATION

Materials Supplied

Catalog Number	Reagent	Quantity	Description
SKC-214A	Clear 96 well Half Area Plates	2 Plates	-
SKC-214B	Superoxide Dismutase Standard	1 vial	1 Unit/vial of bovine Erythrocyte Superoxide Dismutase lyophilized.
SKC-214C	Assay Buffer	50 mL	Buffer containing detergents, stabilizers and dye.
SKC-214D	Xanthine Oxidase Buffer	6 mL	Buffer containing detergents and stabilizers.
SKC-214E	Xanthine Oxidase Concentrate	225 µL	A 25X concentrated suspension of Xanthine Oxidase.
SKC-214F	Substrate Diluent	12 mL	Substrate buffer.
SKC-214G	Substrate Concentrate	1.1 mL	A 10X concentrate of the Detection Reagent.

If any of the items listed above are damaged or missing, please contact our Customer Service department at (250) 294-9065. We cannot accept any returns without prior authorization.



WARNING: Not for human or animal disease diagnosis or therapeutic drug use.

Precautions

As with all such products, this kit should only be used by qualified personnel who have had laboratory safety instruction. The complete booklet should be read and understood before attempting to use the product.

Storage

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.

Materials Needed But Not Supplied

- 2 mM Potassium Cyanide solution for inhibition of Cu/Zn and extracellular SOD if desired.
- Repeater pipet with disposable tips capable of dispensing 25 and 50 μ L.
- 96 well plate reader capable of reading optical absorption at 430 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.

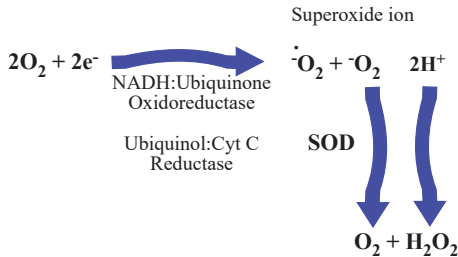
Please read this booklet completely prior to using the product.

FOR RESEARCH USE ONLY.

NOT FOR USE IN DIAGNOSTIC PROCEDURES.

Background

Short-lived and highly reactive oxygen species (ROS) such as $O_2^{\cdot-}$ (superoxide), $\cdot OH$ (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 (MnSOD, SOD2) and extracellular superoxide dismutase (EC-SOD, SOD3), all play critical roles in scavenging $O_2^{\cdot-}$. 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 Syndrome⁽³⁾ while decreased activity is seen in diabetes, Alzheimer's disease, rheumatoid arthritis, Parkinson's disease, uremic anemia, atherosclerosis, some cancers, and thyroid dysfunction⁽³⁻⁸⁾.

Assay Overview

The Superoxide Dismutase (SOD) Colorimetric 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 booklet 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 Sample Diluent 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 430 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.

Sample Types

Sample Types Validated:

Serum, Plasma, Tissues

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 tissues. Samples containing visible particulate should be centrifuged prior to using.

Some serum and plasma samples may contain significant hemoglobin concentrations and the optical density at 430 nm determined prior to running the assay. After addition of the Substrate solution to all the used wells the optical density at 430 nm should be read and subtracted from the optical density recorded at the end of the 20 minute incubation.

Process any cell pellet as described for Cell Lysates on page 8.

To measure cytosolic (SOD1, Cu/Zn) and/or mitochondrial SOD (SOD2, Mn) the sample supernatants prepared on page 8 should be centrifuged at 10,000 x g for 15 minutes 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 samples with 2 mM potassium cyanide. Addition of cyanide will inactivate other SOD enzymes.

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.

Sample Preparation

Samples should be kept on ice to maintain enzyme activity.

Cell Suspensions and Adherent Cells

1. Centrifuge $> 1 \times 10^6$ 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 microtube on ice. Centrifuge, discard supernatant, and place on ice.
3. Wash $> 1 \times 10^6$ 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.
5. Homogenize or sonicate the pellet in 0.5-1 mL of PBS per 100 mg of cells. Centrifuge at 1,500 x g for 10 minutes at 4°C.
6. Collect the supernatant and assay 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.

Tissue Samples

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

Plasma Samples

1. 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.
2. Plasma should be diluted at least 1:5 by taking one part of plasma and adding 4 parts of Assay Buffer prior to assaying. Further dilutions in Assay Buffer may be needed.

RBC/Erythrocytes

1. Eyrthrocytes 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.
3. 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.
4. RBCs should be diluted at least 1:100 prior to running in the assay. Take 10 µL of lysed RBCs and add to 990 µL of Assay Buffer prior to assaying. Further dilutions in Assay Buffer may be needed.

Serum Samples

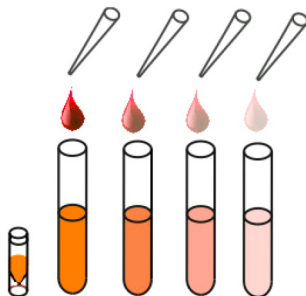
1. Collect blood in serum tubes. Centrifuge the sample at 700 x g for 15 minutes at 4°C. Aspirate off the serum supernatant.
2. Serum should be diluted at least 1:5 with the provided Assay Buffer prior to assaying. Take one part of serum and add 4 parts of Assay Buffer prior to assaying. Further dilutions in Assay Buffer may be needed.

Standard Preparation

Standard Preparation

Superoxide Dismutase Standards are prepared by labeling six tubes as #2 through #7. The lyophilized vial of SOD Standard is used for Standard 1. Add 250 µL of Assay Buffer to the vial, vortex and let stand at room temperature for 5 minutes. Pipet 75 µL of Assay Buffer into tubes #2 to #7. Carefully add 75 µL 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.



	Standard 1	Standard 2	Standard 3	Standard 4	Standard 5	Standard 6	Standard 7
Assay Buffer Volume (μL)	250	75	75	75	75	75	75
Addition	Stock	Standard 1	Standard 2	Standard 3	Standard 4	Standard 5	Standard 6
Volume 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 μL	100 μL	150 μL	200 μL
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

Assay Protocol

Use the plate layout sheet on the back page 21 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 430 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 430 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.

ANALYSIS

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 430 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.

Typical Data

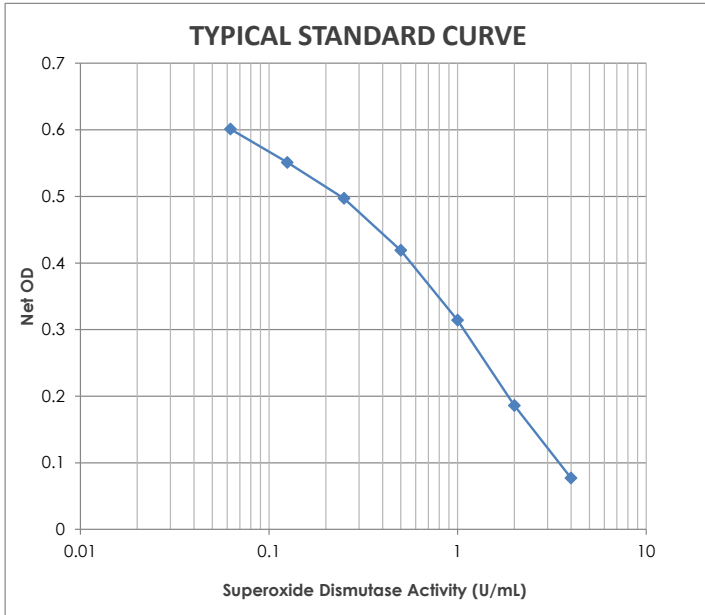
Sample	Mean OD	% Inhibition	SOD Activity (U/ mL)
Standard 1	0.055	8.8	4
Standard 2	0.085	13.6	2
Standard 3	0.157	25.0	1
Standard 4	0.235	37.5	0.5
Standard 5	0.362	57.7	0.25
Standard 6	0.479	76.4	0.125
Standard 7	0.563	89.8	0.0625
Zero	0.627	100	0
Sample 1	0.297	47.3	0.360
Sample 2	0.122	19.4	1.307

Always run your own standard curves for calculation of results.

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.

Typical Standard Curve



Validation Data

Sensitivity and Limit of Detection

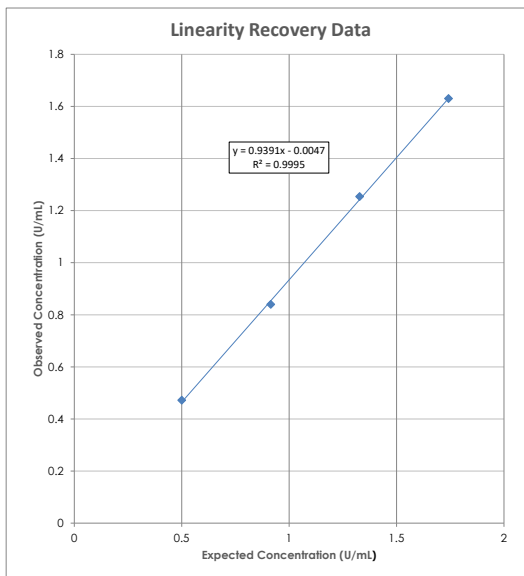
Sensitivity was calculated by comparing the ODs for twenty wells run for each of the zero and standard #7. The detection limit was determined at two (2) standard deviations from the zero along the standard curve.

Sensitivity was determined as 0.052 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	Observed Activity (U/mL)	Expected Activity (U/mL)	% Recovery
80%	20%	1.63	1.743	107%
60%	40%	1.254	1.329	106%
40%	60%	0.84	0.915	109%
20%	80%	0.472	0.501	106%
Mean Recovery				107%



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	1.270	7.4
2	2.430	2.1
3	1.693	1.3

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	3.603	4.1
2	3.520	5.4
3	3.128	16.6

Sample Values

Eight random adult human serum and five plasma samples were diluted in Assay Buffer and run in the assay. The serum samples ranged from 1.79 to 4.88 U/mL with an average of 3.67 U/mL. Sodium heparin plasma samples ranged from 5.01 to 7.25 U/mL with an average 6.54 U/mL.

References

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Warranty and Limitation of Remedy

StressMarq Biosciences Inc. makes **no warranty or guarantee** of any kind, whether written or oral, expressed or implied, including without limitation, any warranty of fitness for a particular purpose, suitability and merchantability, which extends beyond the description of the chemicals hereof. StressMarq **warrants only** to the original customer that the material will meet our specifications at the time of delivery. StressMarq will carry out its delivery obligations with due care and skill. Thus, in no event will StressMarq have **any obligation or liability**, whether in tort (including negligence) or in contract, for any direct, indirect, incidental or consequential damages, even if StressMarq is informed about their possible existence. This limitation of liability does not apply in the case of intentional acts or negligence of StressMarq, its directors or its employees.

Buyer's **exclusive remedy** and StressMarq's sole liability hereunder shall be limited to a refund of the purchase price, or at StressMarq's option, the replacement, at no cost to Buyer, of all material that does not meet our specifications.

Said refund or replacement is conditioned on Buyer giving written notice to StressMarq within thirty (30) days after arrival of the material at its destination. Failure of Buyer to give said notice within thirty (30) days shall constitute a waiver by Buyer of all claims hereunder with respect to said material.

For further details, please refer to our Warranty and Refund Policy located on our website and in our catalog.

Contact Information

Technical Service Contact Information

Phone: 250-294-9065

Fax: 250-294-9025

E-Mail: techsupport@stressmarq.com

Hours: M-F 9:00 AM to 5:00 PM PST

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

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NOTES

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