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Northwest
Life Science Specialties, LLC
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NWLSS™ MnSOD ELISA

Product NWK-SOD04
For Research Use Only

Simple ELISA kit for quantification of human Manganese Superoxide Dismutase (SOD2 or MnSOD) in biological samples.

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Statement of Limited Warranty:

Northwest Life Science Specialties, LLC (NWLSS) makes no guarantee of any kind, expressed or implied, that extends beyond the description of the material in this kit, except that they will meet our specifications at the time of delivery. Customer's remedy and NWLSS' sole liability is limited to, at NWLSS' option, refund of the purchase price, or the replacement of material not meeting our specification. By acceptance of our product, customer assumes all liability and will indemnify and hold NWLSS harmless for the consequence of this product's use or misuse by the customer, its employees, or others. Refund or replacement is conditioned of customer notifying NWLSS within twenty-one (21) days of the receipt of product. Failure to give notice within 21 days shall constitute a waiver by the customer of all claims hereunder with respect to said product..

Notes:

Performance Details:

Specificity

The following substances were tested and found to have no cross-reactivity: human Cu/ZnSOD (SOD1), ecSOD (SOD3), SOD4 (CCS or copper chaperone), rat SOD2 and mouse SOD2.

Sensitivity

The minimal detectable dose of human MnSOD was calculated to be 25 pg/ml, by subtracting two standard deviations from the mean of 10 zero standard replicates (ELISA buffer, S0) and intersecting this value with the standard curve obtained in the same calculation.

Precision

Intra-assay = 5.35 %
Inter-assay = 6.28 %

Accuracy:

Recovery on addition is 98.6~99.6% (Average 99.1%)
Recovery on dilution is 98.8~104.5% (Average 100.9%)

Overall mean recovery = 100%

References

1. Edward Luk et al manganese Activation of Superoxide Dismutase 2 in the Mitochondria of Saccharomyces cerevisiae. (2005) *The Jounal of Biol. Chem.* 280(24):22715-22720

2. Macmillan-Crow LA et al Invited review: manganese superoxide dismutase in disease (2001) *Free radic Res* 34(4):325-336

3. Igor N. Zelko et al Superoxide dismutase multigene family: a comparison of the CuZn-SOD (SOD1), Mn-SOD(SOD2), and EC-SOD(SOD3) gene structures, evolution, and expression (2002*) Free Radical Biology & Medicine* 33(3):337-349

4. Ruby Leah B. et al The copper Chaperone CCS Directly Interacts with Copper/Zinc Superoxide Dismutase (1998) *The Journal of Biological Chemistry* 273(37):23625-23628

5. Tim D. Oury et al Human extracellular superoxide dismutase is a tetramer composed of two disulphide-linked dimmers: a simplified, high-yield purification of extracellular superoxide dismutase (1996 *Biochem .J* .317:51-57

6. Richard W. Strange et al The Structure of Holo and Metal-dificient Wild-

Introduction:

Superoxide dismutase (SOD) is an antioxidant enzyme involved in defense against reactive oxygen species (ROS). SOD catalyzes the dismutation of superoxide radical anion (O2-) to hydrogen peroxide, which is then catalyzed to O_2 and H_2O by glutathione peroxidase and/or catalase. Several classes of SOD have been discovered and can be differentiated according to the metal associated with their active site as well as their location within a cell or tissue. In mammals, Cu/ZnSOD (SOD1) is localized in the cytosol and ecSOD (SOD3) in the extracellular matrix of various tissues. MnSOD or SOD2 exists as a tetramer with individual subunit MW of approximately 23 kDa and Mn at its active site. It is initially synthesized with a leader peptide which targets this enzyme exclusively to the mitochondrial spaces. MnSOD, as the primary antioxidant enzyme for scavenging superoxide radicals in mitochondria, is essential for the survival of all aerobic organisms. Dysregulation of MnSOD in the form of under-expression is being investigated relative to various disease states as well as aging. Overexpression of MnSOD has been shown to protect against oxidative stress induced cell death and tissue injury. MnSOD has also been shown to play a major role in promoting cellular differentiation and tumorigenesis and in protecting against hyperoxia-induced pulmonary toxicity.

Intended Use:

The NWLSS™ MnSOD ELISA kit is intended to be used for the in vitro quantitative determination of human MnSOD in cell lysate and tissue homogenates. The assay will recognize both native and recombinant human MnSOD.

Test Principle:

The NWLSS™ MnSOD Assay is based on a sandwich Enzyme-Linked Immunosorbent Assay (ELISA). The microtiter plate provided in this kit has been pre-coated with a monoclonal antibody specific to human MnSOD. This stationary phase antibody binds sample or standard MnSOD while nonbound proteins are removed by washing. Next, bound MnSOD is tagged with a biotin-conjugated monoclonal antibody specific for MnSOD followed by Avidin conjugated to Horseradish Peroxidase (HRP). Subsequent addition of TMB-substrate solution causes blue color (650 nm) development proportional to the amount of MnSOD originally captured by the stationary phase antibody. Finally, addition of a sulfuric acid solution stops the reaction resulting in a yellow color product measured at 450 nm. Sample MnSOD concentration is determined by comparing the 450 nm absorbance of sample wells to the absorbance of known standards.

Specifications:

Format::	1 X 96 well ELISA presented as 6 X 16 well (2 X 8 well) Strips in frame.	
Number of tests:	Triplicate =	24
	Duplicate =	40
Specificity:	Human MnSOD	
Sensitivity:	25 pg/mL	
Range:	25 pg/mL—1600 pg/mL	

Kit Contents:

1 Foil Pouch	96 well microplate precoated with anti-hu MnSOD.	
1 vial	rHu-MnSOD Standard (lyophilized)	(1 Vial)
1 Bottle	Sample/Standard Dilution Buffer	(25mL)
1 vial	100X Secondary Antibody (Lyophiloized) (biotin labeled anti-hu MnSOD)	(1 Vial)
1 Bottle	Reagent Dilution Buffer	(25mL)
1 vial	100X Avidin-HRP Conjugate	(150 µL)
1 Bottle	Assay Preparation Buffer	(30 mL)
1 Bottle	TMB Substrate Solution	(20 mL)
1 Bottle	Stop Solution (1 N Sulfuric Acid)	(20 mL)
1 Bottle	10X Concentrated Wash Buffer	(100 mL)
3	Adhesive Plate Covers	(3)

Required Materials Not Provided:

Adjustable micropipettes with disposable tips (5-1000 µL). Multi-channel pipettes are useful and help to reduce intra-sample variability.

Serological pipettes.

Deionized water.

Automatic plate washer or other aspiration devices are optional.

Assay Protocol: (continued):

16. After appropriate incubation time, add 100 µl of *Stop Solution* to each well. The solution in the wells should change from blue to yellow.

17. Read and record the absorbance of each well at 450nm within 20 minutes of adding the Stop Solution.

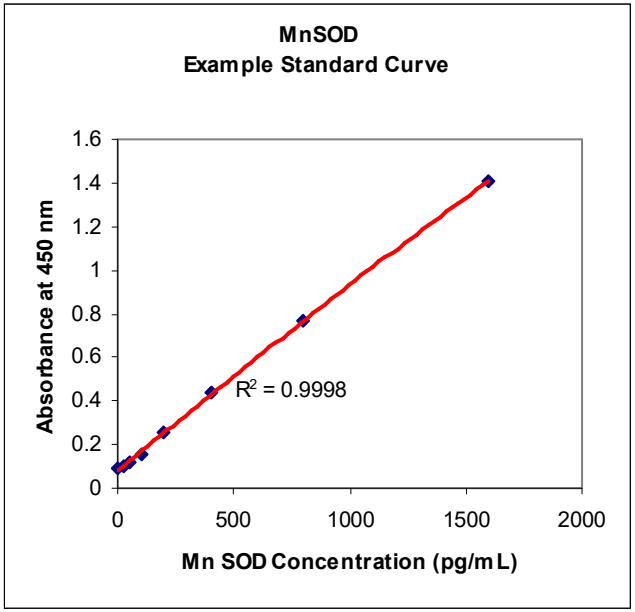
Data Analysis:

1. Plot the mean absorbance at 450 nm for each standard versus the MnSOD concentration. Select the best possible fit for the curve obtained (4–parameter is recommended). This can typically be done using the software provided with most plate readers. An example curve is shown below.

2. Sample MnSOD is determined by comparing their absorbance measurements at 450 with those of the standard curve.

3. Sample data as read from the standard curve must be multiplied by the dilution factor used.

Note: Samples with an ABS_{450} exceeding that of the highest standard should be additionally diluted with Sample Dilution Buffer and re-assayed in order to avoid erroneous results.



Assay Protocol:

1. Add 300µl of *Assay Prep Buffer* to all wells and incubate the plate for 5 minutes at room temperature.

2. Thoroughly aspirate or decant the solution from the wells.

3. Wash wells 2 times as follows: Dispense 300 µL *Working Wash Solution* to each well and allow to soak for 1-3 minutes before decanting or aspirating the remaining solution from the wells.

4. Add 100µl of *Diluted Standards* to the appropriate microtiter wells and 100µl of *Sample Dilution Buffer* to zero wells.

5. Add 100µl of *Sample* to each well according to plan.

6. Cover the plate with the plate cover and incubate for 2 hours at room temperature.

7. Aspirate or decant the solution from the wells then wash the wells 3 times as previously described in step 3.

8. Add 100µl of *Working Secondary Antibody* to each well.

9. Cover the plate with the plate cover and incubate for 1 hour at room temperature (20-25 °C).

10. Aspirate or decant the solution from the wells then wash the wells 3 times as previously described in step 3.

11. Add 100µl *Working Conjugate Solution* to each well.

12. Cover the plate with the plate cover and incubate for 30 minutes at room temperature (20-25 °C).

13. Thoroughly aspirate or decant the solution from the wells. Wash the wells 3 times previously described in step 3.

14. Add 100µl of *TMB Substrate* to each well. The liquid in the wells should begin to turn blue.

15. Incubate the plate at room temperature for approximately 10-15 minutes.
Note: The incubation time for the TMB substrate is dependent on ambient conditions as well as the specific microtiter plate reader in use. The user should adjust this time as necessary by monitoring the development of blue color at 650 nm and stopping when the high standard has reached maximal absorbance level.

Required Instrumentation:

Plate reader with 450 nm capability (650 nm is required for optional monitoring of color development prior to stopping the reaction).

Warnings, Precautions & Limitations:

Reagents are intended for research use only and are not for use in diagnostic or therapeutic procedures.

Individual components may be harmful if swallowed, inhaled or absorbed through the skin. Contact should be minimized through the use of gloves and standard good laboratory practices. If contact with skin or eyes occurs, rinse the site immediately with water and consult a physician.

Substrate solutions must be at room temperature prior to use. Avoid contact of substrate solutions with oxidizing agents and metal.

Improper or insufficient washing at any stage of the procedure will result in either false positive or false negative results. Completely empty wells before dispensing fresh Wash Buffer, fill with Wash Buffer as indicated for each wash cycle and do not allow wells to sit uncovered or dry for extended periods.

Storage Instructions:

All kit components of this kit are stable at 2 to 8 ℃. Any unused reconstituted standard should be discarded or frozen at -70 ℃. Standard can be frozen and thawed one time only without loss of immunoreactivity.

Assay Preparation:

1. Determine the number of wells required to assay standards, samples and controls for the appropriate number of replicates. It is recommended that testing be performed in duplicate or triplicate if possible.

2. Create an assay template showing positioning of standards, controls and samples.

3. Bring all samples and reagents to room temperature before use.

4. To avoid condensation, do not open foil pouches containing the micro-titer strips until after they have reached room temperature. Next remove the required number of strips and place in the frame supplied.

Return unused wells to the storage bag with desiccant, seal and store at 2-8 ℃.

Reagent Preparation:

Assay Preparation Buffer
The Assay Preparation Buffer is provided ready to use.

Secondary Antibody
1. Reconstitute *100X Secondary Antibody* by adding 150 µL *Reagent Dilution Buffer to the vial.*

2. Equilibrate *100X Secondary Antibody* to room temperature, mix gently.

3. Mix 20µl of *100X Secondary Antibody* with 2ml *Reagent Dilution Buffer* for each 16 well strip to be assayed. Label as "Working Secondary Antibody Solution".

4. Return the unused *100X Secondary Antibody* to the refrigerator.

AVIDIN-HRP Conjugate
1. Equilibrate to room temperature, mix gently.

2. Mix 20 µl of *100X AVIDIN-HRP Conjugate* with 2ml *Reagent Dilution Buffer* for each16-well strip to be assayed. Label as "Working Conjugate Solution".

3. Return the unused *100X AVIDIN-HRP Conjugate* to the refrigerator.

Wash Buffer
1. Equilibrate to room temperature, mix to re-dissolve any precipitated salt.

2. Mix 1 volume *10X Wash Buffer* with 9 volumes of *deionized water.* Label as "Working Wash Solution".

3. Store both the remaining concentrated Wash Buffer and the Working Wash Solution at 4°C in the refrigerator.

TMB Substrate
The TMB Substrate is provided ready to use.

Stop Solution
The Stop Solution is provided ready to use

Sample Handling/Preparation
The rate of degradation of native human MnSOD in various matrices has not been investigated. It is beyond the scope of this publication to comment on specific sample processing protocols except to recommend that tissue or cell samples (eg samples where mitochondria and thus MnSOD would be present) be homogenized in normal phosphate buffered saline and treated as necessary (sonication) to appropriately disrupt the mitochondria in order to release MnSOD into the homogenate. Assuming a relatively short half life for MnSOD outside of the mitochondria, homogenates should be kept as cold as possible and tested as soon as possible after disruption of the mitochondrial membrane.

Dilutional Scheme:
MnSOD levels are expected to vary greatly in various tissue types such that proper dilutional schemes for tissue homogenates must be experimentally determined by the end user. We recommend starting with a homogenate that is as concentrated as possible then making dilutions such as 1:1, 1:4, 1: 9 as necessary to see at what point the best data is generated for a given sample type or model system.

Standard Curve Preparation:
Reconstitute the human MnSOD standard to 10ng/ml by adding 1ml of *Sample/Standard Dilution Buffer* into the standard protein glass vial containing lyophilized human MnSOD protein. Swirl or mix gently, and allow to sit for 5 minutes to ensure complete reconstitution.

1. Label tubes 1-8 tubes as:
1600, 800, 400, 200, 100, 50, 25 and zero (0) pg/mL.

2. Add 840 µL Standard Dilution Buffer to tube 1 and 500 µL Standard Dilution Buffer to each tube 2-8.

3. Add 160 µL Reconstituted 10 ng/mL Standard to tube 1 and mix well.

4. Make a serial dilution by transferring 500 µL of 1600 pg/mL Standard into tube 2 mixing thoroughly then 500 µL of resulting 800 pg/mL to tubes 3 and so on to create all Standards down to 25 pg/mL.