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Northwest
Life Science Specialties, LLC
Premier Products for Superior Life Science Research

NWLSS™ EcSOD ELISA

Product NWK-SOD03
For Research Use Only

Simple ELISA kit for quantification of human Extracellular Superoxide Dismutase (EcSOD or SOD3) in biological samples.

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Introduction:

Superoxide dismutase (SOD) is an antioxidative enzyme involved in the defense against reactive oxygen species (ROS). SOD catalyzes the dismutation of superoxide radical anion (O2-) to hydrogen peroxide, which is then catalyzed to innocuous O_2 and H_2O by glutathione peroxidase and/or catalase. Three unique and highly compartmentalized mammalian superoxide dismutases have been biochemically and molecularly characterized. SOD1, or CuZnSOD or SOD1 exists as a homodimer with Cu/Zn at its active site. Cu/ZnSOD is found almost exclusively in the intracellular cytosol. MnSOD or SOD2 exists as a tetramer with Mn at the active site. MnSOD is initially synthesized with a leader peptide which targets this enzyme exclusively to the mitochondrial spaces. EcSOD or SOD3 is the most recently characterized SOD. Although EcSOD also has Cu/Zn at its active site, it exists as a tetramer and contains a unique heparin-binding domain at its carboxy-terminus that enables its localization to the extracellular matrix. EcSOD is highly expressed in selected tissues including blood vessels, heart, lungs, kidney and placenta where it is found in the interstitial extracellular matrix. EcSOD is now known to play an important role in maintaining vascular tone, attenuating age-related cognitive decline, lung function, and the metabolism of NO.

Intended Use:

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

Test Principle:

The NWLSS™ EcSOD 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 EcSOD. This stationary phase antibody binds sample or standard EcSOD while nonbound proteins are removed by washing. Next, bound EcSOD is tagged with a biotin-conjugated monoclonal antibody specific for EcSOD 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 EcSOD 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 EcSOD 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 EcSOD	
Sensitivity:	2 ng/mL	
Range:	2 ng/mL-168 ng/mL	

Kit Contents:

1 Foil Pouch	96 well microplate precoated with anti-hu EcSOD.	
1 vial	rHu-EcSOD Standard	1 Vial
1 bottle	Sample/Standard Dilution Buffer	(25mL)
1 vial	100X secondary antibody (Lyophilized) (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.

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 °C. Any unused reconstituted standard should be discarded or frozen at -70 °C. 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 °C.

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 ecSOD in various matrices has not been investigated. It is beyond the scope of this publication to comment on specific sample processing protocols.

Dilutional Scheme:
ecSOD 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 EcSOD Standard to 1µg/ml by adding 1 mL of- *Sample/Standard Dilution Buffer* to the glass vial containing lyophilized human ecSOD protein. Swirl or mix gently, and allow to sit for 5 minutes to ensure complete reconstitution.

1. Label tubes 1-8 tubes as:
128, 64, 32, 16, 8, 4, 2 and zero (0) ng/mL.

2. Add 872 µL **Standard Dilution Buffer** to tube 1 and 500 µL **Standard Dilution Buffer** to each tube 2-8.

3. Add 128 µL **Reconstituted 1 µg/mL Standard** to tube 1 and mix well.

4. Make a serial dilution by transferring 500 µL of 128 ng/mL Standard into tube 2 mixing thoroughly then 500 µL of resulting 64 ng/mL to tubes 3 and so on to create all Standards down to 2 ng/mL.

Assay Protocol:

1. Add 300ul 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 100ul 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.

Assay Protocol: (continued):

16. After appropriate incubation time, add 100µl 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.

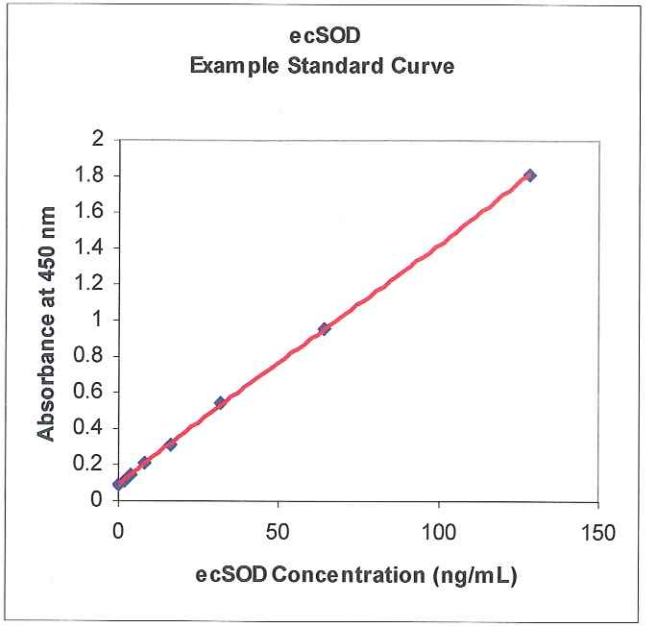
Data Analysis:

1. Plot the mean absorbance at 450 nm for each standard versus the EcSOD 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 EcSOD 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.



Performance Details:

Specificity

The following substances were tested and found to have no cross-reactivity: human SOD1, SOD2, SOD4.

Sensitivity

The minimal detectable dose of human EcSOD was calculated to be 2ng/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 = 1.45 %
Inter-assay = 2.53 %

Accuracy:

Recovery on addition is 95.0~101.9% (mean 98.4%)
Recovery on dilution is 97.0~105.3% (mean 100.9%)

Overall mean recovery = 99.65%

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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: