Northwest
Life Science Specialties, LLC

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NWLSS™ Methionine Sulfoxide Reductase B ELISA

Product NWK-MSRB01

For Research Use Only

ELISA kit for quantification of human methionine sulfoxide reductase B in biological samples.

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Introduction:

The oxidation of methionine at the sulfur atom leads to alternative epimers of methionine sulfoxide (MetO R form and S form). Methionine sulfoxide reductase B (MsrB), also known as SelX, is a selenoprotein that can reduce the R form of both free and protein-incorporated MetO to methionine. It has a crucial role in protecting cells against oxidative damages. MsrA reduces only the S epimer of MetO and MsrB reduces the R epimer of MetO in proteins. Although the catalytic mechanisms of MsrA and MsrB are similar, the two Msrs have no sequence identity and no structural similarity.

Intended Use:

The NWLSS™ MsrB ELISA kit is intended to be used for the in vitro quantitative determination of Methionine sulfoxide reductase A (MsrB) in human serum, plasma, cell lysates and cell culture supernatants.

Test Principle:

The NWLSS™ Methionine Sulfoxide Reductase B ELISA is a sandwich format Enzyme-Linked Immunosorbent Assay (ELISA). The microtiter plate provided in this kit has been pre-coated with a monoclonal antibody specific to human MsrB. Samples are pipetted into these wells. Nonbound MsrB and other components of the sample are removed by washing, then monoclonal antibody specific to MsrB added. In order to quantitatively determine the amount of MsrB present in the sample, Streptavidin Horseradish Peroxidase (HRP) is added to each microplate well. After another wash step, a TMB-substrate solution added to each well. Finally, a sulfuric acid stop solution is added and the resulting yellow colored product is measured at 450nm. The amount od MsrB in the sample can be determined by direct comparison with the standard curve generated in the assay.

Specifications:

Format:	1 X 96 well ELISA presented as 12 X 8 well (12 X 8 well) strips in frame.	
Number of tests:	Triplicate =	24
	Duplicate =	40
Specificity:	Human MsrB	
Sensitivity:	0.13 ng/mL	
Range:	0.13 ng/mL—5 ng/mL	

Kit Contents:

1 Foil Pouch	96 well microplate precoated with anti-hu MsrB	
1 vial	Human MsrB Standard (lyophilized)	(1 Vial)
1 Bottle	Sample/Standard Dilution Buffer	(25mL)
1 vial	100X Secondary Antibody (Lyophilized) (rabbit anti-hu MsrB)	(1 Vial)
1 Bottle	Reagent Dilution Buffer	(25mL)
1 vial	100X Anti-rabbit IgG-HRP Conjugate	(150 μL)
1 Bottle	TMB Substrate Solution	(15 mL)
1 Bottle	Stop Solution (1 N Sulfuric Acid)	(15 mL)
2 Bottle	20X Concentrated Wash Buffer	(25 mL ea)
2	Adhesive Plate Covers	(2)

Required Materials Not Provided:

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

Polypropylene tubes.

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 ℃. 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 microtiter 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:

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.

Anti Rabbit IgG-HRP Conjugate

1. Equilibrate to room temperature, mix gently.

2. Mix 20µL of *100X Anti Rabbit IgG-HRP Conjugate* with 2mL *Reagent Dilution Buffer* for each16-well strip to be assayed. Label as **"Working Conjugate Solution".**

3. Return the unused *100X Anti-Rabbit IgG-HRP Conjugate* to the refrigerator.

Wash Buffer

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

2. Mix 0.5 volume *20X Wash Buffer* with 9.5 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 human MsrB in various matrices has not been fully investigated. It is beyond the scope of this publication to comment on specific sample processing protocols except to recommend that serum or plasma samples be separated by centrifugation and separated from coagulated or packed cells as soon as possible. For best results, serum and plasma samples should be diluted 40X prior to assay.

Cell lysates can be made by isolating cell samples followed by mechanical homogenization or sonication. Homogenates should be clarified by centrifugation prior to assay.

Standard Curve Preparation:

Reconstitute the human MsrB standard to 1 μg/mL by adding 1mL of *Standard/Sample Dilution Buffer* into the standard protein glass vial containing lyophilized human MsrB protein. Swirl or mix gently, and allow to sit for 5 minutes to ensure complete reconstitution.

1. Label tubes 1-8 tubes as:
15, 7.5, 3.75, 1.88, 0.94, 0.47, 0.23 and zero (0) μg/mL.

2. Add 985 μL **Standard Dilution Buffer** to tube 1 and 500 μL **Standard Dilution Buffer** to each tube 2-8.

3. Add 15 μL **Reconstituted 1 ng/mL Standard** to tube 1 and mix well.

4. Make a serial dilution by transferring 500 μL of 15 μg/mL Standard into tube 2 mixing thoroughly then 500 μL of resulting 7.5 μg/mL to tubes 3 and so on to create all Standards down to 78.13 μg/mL. Sufficient for 1 X 16 well strip (1 set of standards in duplicate).

Assay Protocol:

1. Add 100µL of ***Diluted Standards*** to the appropriate microtiter wells and 100ul of ***Sample Dilution Buffer*** to zero wells.

2. Add 100µL of ***Sample*** to each well according to plan.

3. Cover the plate with the plate cover and incubate for 2 hours at 37 °C.

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

5. Add 100µL of ***Working Secondary Antibody*** to each well.

6. Cover the plate with the plate cover and incubate for 1 hour at 37 °C.

7. Aspirate or decant the solution from the wells then wash the wells 3 times as previously described in step 4.

8. Add 100µL ***Working Conjugate Solution*** to each well.

9. Cover the plate with the plate cover and incubate for 1 hour at 37 °C.

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

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

12. Incubate the plate at room temperature for approximately 7-12 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):

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

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

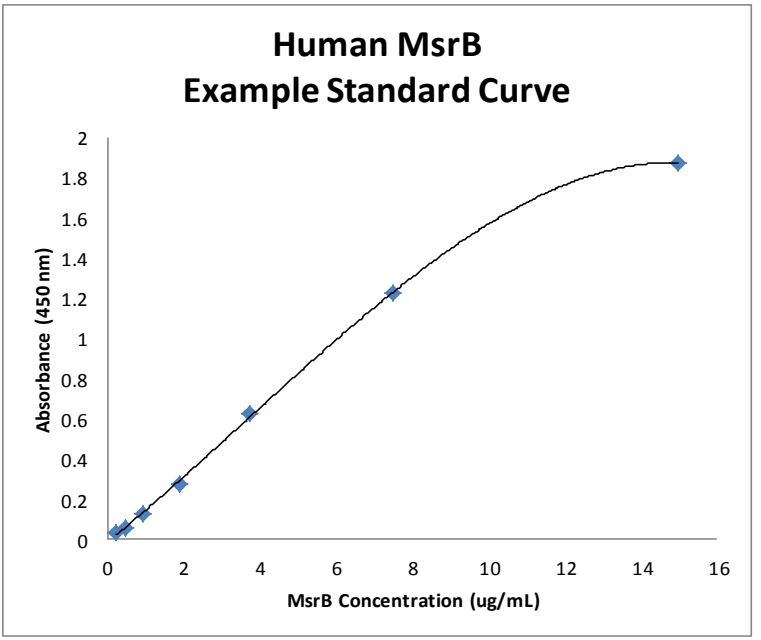
Data Analysis:

1. Plot the mean absorbance at 450 nm for each standard versus the MsrA 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 MsrB is determined by comparing their absorbance 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:
The following substances were tested and found to have no cross-reactivity: human MsrA.

Sensitivity
The minimal detectable dose of human MsrA was calculated as the mean of 12 zero standard replicates plus 3 standard deviations. And was found to be 10 ng/mL.

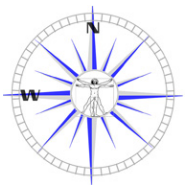
Precision
Intra-assay = 7.87 %
Inter-assay = 5.96 %

Accuracy:
Recovery on addition is 96.8~104.5% (Average 100.8%)

References:
1. Zheung, D. et al. (2003) J. Biomol. NMR, 27, 183-184.
2. Weissbach, H. (2002) Arch.Biochem.Biophys. 397(2), 172-178.
3. Kryukov, G. (2002) Proc.Natl.Acad.Sci.U.S.A. 99(7), 4245-4250.

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:



Northwest
Life Science Specialties, LLC

5131 NE 94th Avenue, Suite 201
Vancouver, WA 98662
Phone 360-449-3091 or Toll Free: 888-449-3091
Fax 360-449-3092
E-mail: sales@nwlifescience.com