Northwest
Life Science Specialties, LLC

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NWLSS™ Thioredoxin Reductase 1 ELISA

Product NWK-TRR01

For Research Use Only

Simple ELISA kit for quantification of human Thioredoxin Reductase 1 (TrxR1) in biological samples.

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Introduction:

The mammalian thioredoxin reductases (TrxRs) are a family of selenocysteine containing pyridine nucleotide-disulfide oxido-reductases. Three different TrxR isoenzymes, TrxR1 as cytosolic, TrxR2 as mitochondrial, and TrxR3 as testis-specific thiol regulator are known. All the mammalian TrxRs are homologous to glutathione reductase with respect to primary structure including the conserved redox catalytic site (-Cys-Val-Asn-Val-Gly-Cys-) but distinctively with a C-terminal extension containing a catalytically active penultimate selenocysteine(SeCys) residue in the conserved sequence(-Gly-Cys-SeCys-Gly). TrxR is homodimeric protein in which each monomer includes an FAD prosthetic group, a NADPH binding site and a redox catalytic site. Electrons are transferred from NADPH via FAD and the active-site disulfide to C-terminal SeCys-containing redox center, which then reduces the substrate like thioredoxin. The members of TrxR family are 55–58 kDa in molecular size and composed of three isoforms including cytosolic TrxR1, mitochondrial TrxR2, and TrxR3, also known as Trx and GSSG reductase (TGR). TrxR plays a key role in protection of cells against oxidative stress and redox-regulatory mechanism of transcription factors and various biological phenomena. Many tumor cells have elevated TrxR levels and TrxR has been shown to play a major role in drug resistance. Inhibition of TrxR and its related redox reactions may thus contribute to a successful single, combinatory or adjuvant cancer therapy. A great number of effective natural and synthetic TrxR inhibitors are now available possessing antitumor potential ranging from induction of oxidative stress to cell cycle arrest and apoptosis.

Intended Use:

The NWLSS™ Thioredoxin Reductase-1 (TrxR1) ELISA kit is to be used for the in vitro quantitative determination of human TrxR1 in human serum, human plasma, cell lysate and buffered solution. The assay will recognize both native and recombinant human TrxR1.

Test Principle:

The NWLSS™ Thioredoxin Reductase (TrxR1) 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 TrxR1. This stationary phase antibody binds sample or standard TrxR1 while nonbound proteins are removed by washing. Next, bound TrxR1 is tagged with a biotin-conjugated monoclonal antibody specific for TrxR1 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 TrxR1 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 450nm. Sample TrxR1 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 Thioredoxin Reductase 1	
Sensitivity:	0.4 ng/mL	
Range:	0.4 ng/mL—25ng/mL	

Kit Contents:

1 Foil Pouch	96 well microplate precoated with anti-hu Thioredoxin Reductase 1	
1 vial	rHu-Thioredoxin Reducatse 1 Standard (lyophilized)	
1 bottle	Sample Dilution Buffer	(25mL)
1 vial	100X Secondary Antibody (Lyophilized Biotin labeled anti-hu TrxR1)	(1 Vial)
1 bottle	Reagent Dilution Buffer	(25mL)
1 vial	100X Avidin-HRP Conjugate	(150 uL)
1 bottle	Assay Preparation Buffer	(30 mL)
1 bottle	TMB Substrate	(15 mL)
1 bottle	Stop Solution (1 N Sulfuric Acid)	(15 mL)
2 bottles	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.

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.

Standards and Secondary antibody each contain 0.01% sodium azide as preservative.

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 20 µL 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 20X Concentrated Wash Buffer to 19 volumes of *deionized water (1/20 dilution).* 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 TrxR1 in various matrices has not been properly investigated. Therefore, it is beyond the scope of this publication to comment on specific sample processing protocols or necessary sample dilutional schemes however here are some basic guidelines:

Plasma:

Average normal plasma thioredoxin reductase 1 levels have been reported in the range of 18 ng/mL. Therefore, a starting dilution of 2X to 5X is recommended for plasma from healthy (normal donors). Samples from oxidative stress or known inflammatory model systems would be expected may contain significantly elevated levels of TrxR1 such that higher dilutions may be necessary in some situations.

Tissue:

Thioredoxin Reductase 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.

Standard Curve Preparation:

Reconstitute the human TrxR1 standard to 1μg/mL by adding 1mL of *Sample Dilution Buffer* into the glass vial containing lyophilized human TrxR1 protein. Swirl or mix gently, and allow to sit for 5 minutes to ensure complete reconstitution.

1. Label tubes 1-8 tubes as:
25, 12.5, 6.25, 3.13, 1.56, 0.78, 0.39 and zero (0) ng/mL.

2. Add 975 μL **Standard Dilution Buffer** to tube 1 and 500 μL **Standard Dilution Buffer** to each tube 2-8.

3. Add 25 μL **Reconstituted 1 ug/mL Standard** to tube 1 and mix well.

4. Perform a serial dilution by transferring 500 μL of 25 ng/mL Standard into tube 2 mixing thoroughly then 500 μL of resulting 12.5 ng/mL to tube 3 and so on through tube 7 to create all standards down to 0.39 ng/mL. Sufficient for 1 X 16 well strip (1 set of standards in duplicate)

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 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.

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

5. Add 100uL 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 100uL 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 100uL ***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 100uL 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

Assay Protocol: (continued):

16. After appropriate incubation time, add 100ul 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.

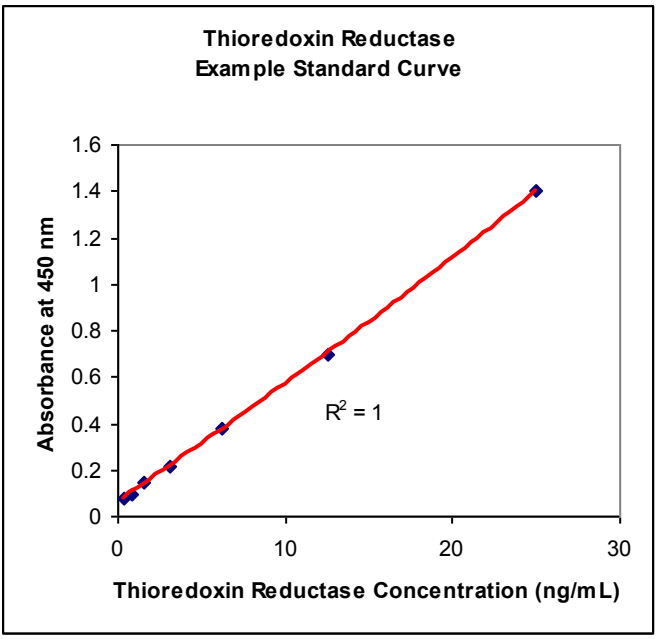
Data Analysis:

1. Plot the mean absorbance at 450 nm for each standard versus the Thioredoxin Reductase 1 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 Thioredoxin Reductase 1 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 TrxR2, mouse TrxR1 and rat TrxR1.

Sensitivity

The minimal detectable dose of human Trx1 was calculated to be 0.39ng/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 = 5.7 %
Inter-assay = 6.0 %

Accuracy:

Recovery on addition from 97.3-101.0%, Average = 98.6%
Recovery on dilution from 96.0-101.3, Average = 99.4%

References

1. Mustacich, D. and Powis,G. (2000) Biochem J. 15.346 Pt 1:1-8.

2. Nguyen, P. et al., (2006) Cancer Lett. 18.164-74.

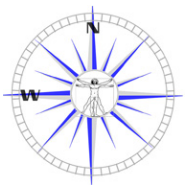
3. Urig, S. and Becker, K. (2006) Semin Cancer Biol. 16. 452-65.

4. Soderberg, A. et al., (2000) Cancer Research 60, 2281-2289

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:



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