DATA SHEET

NWLSSTM Thioredoxin-1 ELISA

Product NWK-TRX01

For Research Use Only

Introduction:
Thioredoxins(Trx) are small, multi-functional proteins with oxido-reductase activity and are ubiquitous in essentially all living cells. Trx contains a redoxactive disulfide/dithiol group within the conserved Cys-Gly-Pro-Cys active site. The two cysteine residues in the conserved active centers can be oxidized to form intramolecular disulfide bonds. Reduction of the active site disulfide in oxidized Trx is catalyzed by Thioredoxin reductase (TR) with NADPH as the electron donor. The reduced Trx is a hydrogen donor for ribonucleotide reductase, the essential enzyme for DNA synthesis, and a potent general protein disulfide reductase with numerous functions in growth and redox regulations. Specific protein disulfide targets for reduction by Trx include protein disulfide–isomerase (PDI) and a number of transcription factors such as p53, NF-kB and AP-1 (T1-151). Trx is also capable of removing H_2O_2, particularly when it is coupled with either methionine sulfoxide reductase or several isoforms of peroxiredoxins. To summarize, Trx is a multifunctional protein with anti-inflammatory and antiapoptotic effects, as well as antioxidative effects. Accordingly, Trx is a well-recognized biomarker of various diseases involving oxidative stress and is a useful biomarker for research focused in this area.
Intended Use:
The NWLSS™ Thioredoxin -1 (human Trx1) ELISA kit is to be used for the in vitro quantitative determination of human Trx1 in serum, plasma, cell lysate and other biological samples. The assay will recognize both native and recombinant human Trx1.
Test Principle:
The NWLSS™ Thioredoxin I (Trx1) 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 Trx1. This stationary phase antibody binds sample or standard Trx1 while nonbound proteins are removed by washing. Next, bound Trx1 is tagged with a biotin-conjugated monoclonal antibody specific for Trx1 followed by Avidin conjugated to Horseradish Peroxidase (HRP). Subsequent addition of TMBsubstrate solution causes blue color (650 nm) development proportional to the amount of Trx1 originally captured by the stationary phase antibody. Finally, addition of a sulfuric acid solution stops the reaction resulting in a yellow color product measured at450nm. Sample Trx1 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-I
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 1.
1 vial rHu-Thioredoxin Standard (lyophilized) (1 Vial)
1 bottle Sample/Standard Dilution Buffer (25mL)
1 vial 100X Secondary Antibody (Lyophilized) (1 Vial)
(Biotin Labeled anti-huTrx-1)

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1 bottle Reagent Dilution Buffer (25mL)
1 vial 100X Avidin-HRP Conjugate (150 · L)
1 bottle Assay Preparation Buffer (30 mL)
1 bottle TMB Substrate (20 mL)
1 bottle Stop Solution (1 N Sulfuric Acid, H_2SO_4) (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 · L). 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 ☐Ž. Any unused reconstituted standard should be discarded or frozen at -70 ☐Ž. 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 oC.

Reagent Preparation:
Assay Preparation Buffer
The Assay Preparation Buffer is provided ready to use.
Secondary Antibody
1. Reconstitute *100X Secondary Antibody* by adding 150 · L *Reagent Dilution Buffer to the vial.*
2. Equilibrate *100X Secondary Antibody* to room temperature, mix gently.
3. Mix 20· l 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· l 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 Trx1 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 dilution schemes however here are some basic guidelines:
Plasma:
Average normal plasma thioredoxin levels have been reported in the range of 25-50 ng/mL. Therefore, a starting dilution of 5X to 10X will yield data in the mid part of the curve. Samples from oxidative stress or known inflammatory model systems would be expected to contain significantly elevated levels of Trx1 such that higher dilutions may be necessary in some situations.
Tissue:
Thioredoxin 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 Trx1 standard to 1· g/ml by adding 1ml of *Sample/Standard Dilution Buffer* into the standard protein glass vial containing lyophilized human Trx1 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 · L Sample/Standard Dilution Buffer to tube 1 and 500 · L Sample/Standard Dilution Buffer to each tube 2-8.

3. Add 25 · L Reconstituted 1 · g/mL Standard to tube 1 and mix well.

4. Make a serial dilution by transferring 500 · L of 25 ng/mL Standard into tube 2 mixing thoroughly then 500 · L of resulting 12.5 ng/mL to tubes 3 and so on to create all Standards down to 0.39 ng/mL.

Assay Protocol:

1. Add 300· l 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 · L *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 100· l of *Diluted Standards* to the appropriate microtiter wells and 100ul of *Sample Dilution Buffer* to zero wells.

5. Add 100· l 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 100· l 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 100· l *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 100· l 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.

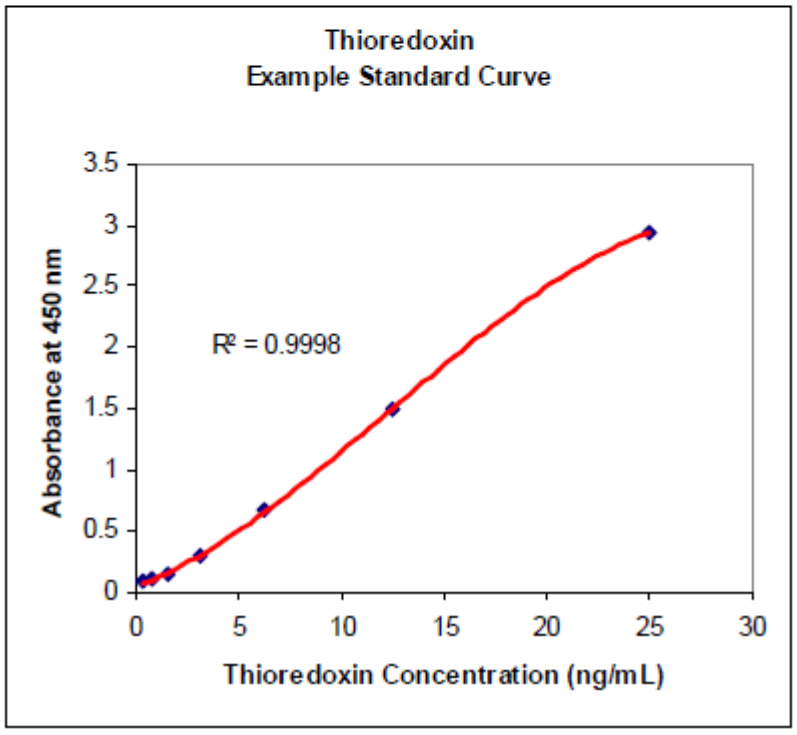
Data Analysis:

1. Plot the mean absorbance at 450 nm for each standard versus the Thioredoxin 1 concentration. A 4-parameter curve fit is recommended. This can typically be done using the software provided with most plate readers. An example curve is shown below.

2. Sample Thioredoxin 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.



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Performance Details:

Specificity

The following substances were tested and found to have no cross-reactivity: human Trx2, mouse Trx1 and rat Trx1.

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 = 5.6 %
Inter-assay = 5.3 %

Accuracy:

Recovery on addition from 99.3-100.4%, Average = 100.2%
Recovery on dilution from 81-103.0%, Average = 91.9%

Overall mean recovery = 96.05%

References

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