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Northwest
Life Science Specialties, LLC

Premier Products for Superior Life Science Research

NWLSS™ Peroxiredoxin 3 ELISA

Product NWK-PRX03
For Research Use Only

Simple ELISA kit for quantification of human Peroxiredoxin 3 (Prx3) in biological samples.

nwkprx03rev031109

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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 Prx1, Prx2, prx4, Prx5, Prx6, mouse Prx3 and rat Prx3.

Sensitivity

The minimal detectable dose of human Prx3 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 = 5.0%
Inter-assay = 2.93%

Accuracy:

Recovery on addition from 92.9-103.6%, Average = 98.2%
Recovery on dilution from 85-108.1, Average = 98.1%

Overall mean recovery = 98.15%

References

1. Sue Goo Rhee et al. (2005) *Free Radic Biol Med.* 38 (12):1543-52.

2. Fujii J, Ikeda Y. (2002) *Redox Rep.* 7 (3):123-30.

3. Sue Goo Rhee et al. (2001) *IUBMB Life* 52 (1-2):35-41.

4. Tong-Shin Chang et al. (2004) *J Biol Chem.* 279 (40):41975-84.

5. Larisa Nonn et al. (2003) *Mol Cancer Res*. 1 (9):682-9.

6. Zachary A. Wood et al. (2003) *TIBS* 28 (1): 32-40.

Introduction:

Organisms living under aerobic conditions have developed various anti-oxidative mechanisms to protect them from damage by reactive oxygen species (ROS). Peroxiredoxin (Prx) is a growing anti-oxidative protein family that has been identified six members in mammals. They share a common reactive Cys residue in the N-terminal region, and are capable of serving as a peroxidase and involve thioredoxin and/or glutathione as the electron donor. Prx1 to Prx4 have an additional Cys residue in the conserved C-terminal region, and are cross members as judged by the amino acid sequence similarity. Prx5 also contains an additional Cys in its C-terminal region that is less conserved. On the other hand, Prx6 has only one unique Cys. These Prx family members are distributed in subcellular localization, Prx1, 2, and 6 in cytosol, Prx3 in mitochondria, Prx4 in ER and secretion, Prx5 showing complicated distribution including peroxisome, mitochondria and cytosol, all of which are potential sites of ROS production. In addition to their role as a peroxidase, however, a body of evidence has accumulated to suggest that individual members also serve divergent functions, which are associated with various biological processes such as the detoxification of oxidants, cell proliferation, differentiation and gene expression. It would be expected that these functions might not necessarily depend on peroxidase activity and, therefore, it seems likely that the divergence is due to unique molecular characteristics intrinsic to each member. The specific localization of Prx3 in mitochondria together with the identification of its mitochondria-specific electron suppliers, namely thioredoxin 2 and thioredoxin reductase 2, suggest that these three proteins might provide a primary line of defense against H_2O_2 produced by the mitochondrial respiratory chain. Furthermore, Prx3 expression is induced by oxidants in the cardiovascular system and is thought to play a role in the antioxidant defense system and homeostasis within mitochondria.

Intended Use:

The NWLSS™ Peroxiredoxin 3 (Prx3) ELISA kit is to be used for the in vitro quantitative determination of human Prx3 in serum, plasma, cell lysate and other biological samples. The assay will recognize both native and recombinant human Prx3.

Test Principle:

The NWLSS™ Peroxiredoxin 3 (Prx3) 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 Prx3. This stationary phase antibody binds sample or standard Prx3 while non-bound proteins are removed by washing. Next, bound Prx3 is tagged with a biotin-conjugated monoclonal antibody specific for Prx3 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 Prx3 originally captured by the stationary phase antibody.

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 Peroxiredoxin 3	
Sensitivity:	2 ng/mL	
Range:	2 ng/mL—32ng/mL	

Kit Contents:

1 Foil Pouch	96 well microplate precoated with anti-hu Peroxiredoxin 3.	
1 vial	rHu-Peroxiredoxin 3 Standard (lyophilized)	
1 bottle	Sample Dilution Buffer	(25mL)
1 vial	100X Secondary Antibody (Biotin labeled anti-hu Prx1)	(150 uL)
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	(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 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.

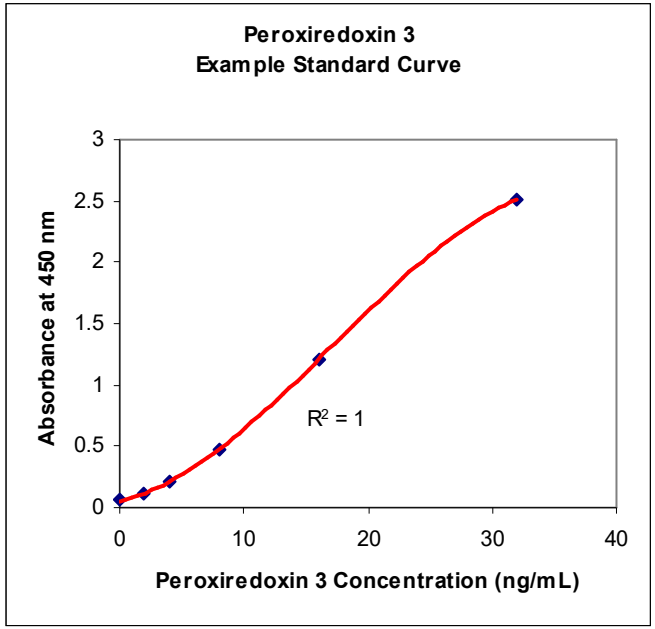
Data Analysis:

1. Plot the mean absorbance at 450 nm for each standard versus the Peroxiredoxin 3 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 Peroxiredoxin 3 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 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 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.

All reagents containing Sodium Azide also contain Thimerosal as a preservative. Thimerosal contains Hg thus should be handled with great care.

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. Equilibrate to room temperature, mix gently.

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

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

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

2. Mix 20ul 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 Prx3 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 peroxiredoxin 3 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 Prx3 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 Prx3 standard to 1ug/ml by adding 1ml of *Sample Dilution Buffer* into the glass vial containing lyophilized human Prx1 protein. Swirl or mix gently, and allow to sit for 5 minutes to ensure complete reconstitution.

1. Label tubes 1-8 tubes as:
32, 16, 8, 3.4, 2, 1 0.5 and zero (0) ng/mL.

2. Add 968 uL Standard Dilution Buffer to tube 1 and 500 uL Standard Dilution Buffer to each tube 2-8.

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

4. perform a serial dilution by transferring 500 uL of 32 ng/mL Standard into tube 2 mixing thoroughly then 500 uL of resulting 16 ng/mL to tube 3 and so on through tube 7 to create all standards down to 0.5 ng/mL.