NWLSS™ Human Glutahione Peroxidase cGPx (GPX1) ELISA

Product NWK-GPX1H1
For Research Use Only

Simple ELISA kit for quantification of human cellular Glutathione Peroxidase (GPX1) in biological samples.

Table of Contents

Introduction:

Glutathione Peroxidase (GPx) is a family of ubiquitously expressed proteins which catalyze the reduction of hydrogen peroxides and organic hydroperoxides by glutathione. Several isoforms exist that differ in their primary structure and location. The classical cytosolic/mitochondrial Gpx1 (cGpx) is selenium dependent and the first of the Gpx family to be discovered. A gastrointestinal form (GiGpx or GPX2), is an intracellular enzyme expressed only in GI system epithelial cells. An extra-cellular form (plGpx or GPx3) is mainly expressed by the kidney where it is released into the blood circulation. Phospholipid hydroperoxide (PhGpx or GPx4) expressed in most tissues, can reduce many hydroperoxides including those integrated into membranes and hydroperoxy lipids in low density lipoprotein or thymine. Mammalian GPx family members, except for the recently described Cys containing GPx3 and epididymis-specific secretory GPx (eGpx or GPx5), possess selenocysteine at the active site. Because GPx1 plays a major role in the prevention of oxidative stress, it may also be an important anti-atherogenic enzyme. In mice, cGPx deficiency results in abnormal vascular and cardiac function and structure. From a clinical perspective, it has been reported that low erythrocyte GPx1 activity identifies patients with coronary artery disease who are at the high risk for cardiovascular events. The measurement of GPx1 activity therefore could provide additional information on risk and thus might be another useful biomarker in identifying patients who would benefit from preventive antioxidant treatment.

Intended Use:

The NWLSS™ cGPx ELISA kit is intended to be used for the in vitro quantitative determination of human cGPx in cell lysate and tissue homoge-nates. The assay will recognize both native and recombinant human cGPx.

Test Principle:

The NWLSS™ cGPx 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 cGPx. This stationary phase antibody binds sample or standard cGPxwhile nonbound proteins are removed by washing. Next, bound cGPx is tagged with a biotin conjugated monoclonal antibody specific for cGPx 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 cGPx 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 cGPx 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 cGPx

Sensitivity: 12.5 pg/mL

Range: 12.5 pg/mL—800 pg/mL

Kit Contents:

1 Foil Pouch	96 well microplate precoated with anti-hu cGPx.	
1 vial	rHu-cGPx Standard (lyophilized)	(1 Vial)
1 Bottle	Sample/Standard Dilution Buffer	(25mL)
1 vial	100X Secondary Antibody (Lyophilized) (biotin labeled anti-hu cGPx)	(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	(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.

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 ℃. 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. 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 20μL 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 cGPx (GPx1) 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 tissue or cell samples be homogenized in normal phosphate buffered saline and treated as necessary to appropriately disrupt the cell membranes in order to release cGPx into the homogenate. Assuming a relatively short half life for cGPx outside of the cell, homogenates should be kept as cold as possible and tested as soon as possible after creating the lysate or homogenate.

Dilutional Scheme:

cGPx 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 1/5, 1/10, 1/20 etc. 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 cGPx standard to 1μg/mL by adding 1mL of *Standard/Sample Dilution Buffer* into the standard protein glass vial containing lyophilized human cGPx protein. Swirl or mix gently, and allow to sit for 5 minutes to ensure complete reconstitution.

1. Label tubes 1-8 tubes as:
100, 50, 25, 12.5, 6.25, 3.13, 1.56 and zero (0) ng/mL.

2. Add 900 μL **Standard Dilution Buffer** to tube 1 and 500 μL **Standard Dilution Buffer** to each tube 2-8.

3. Add 100 μL **Reconstituted 1 μg/mL Standard** to tube 1 and mix well.

4. Make a serial dilution by transferring 500 μL of 100 ng/mL Standard into tube 2 mixing thoroughly then 500 μL of resulting 50 ng/mL to tubes 3 and so on to create all Standards down to 1.56 ng/mL. Sufficient for 1 X 16 well strip (1 set of standards in duplicate).

Assay Protocol:

1. Add 300µL 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 cGPx 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 cGPx 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.

Human cGPx Example Standard Curve. Absorbance at 450 nm versus cGPx Concentration (ng/mL).
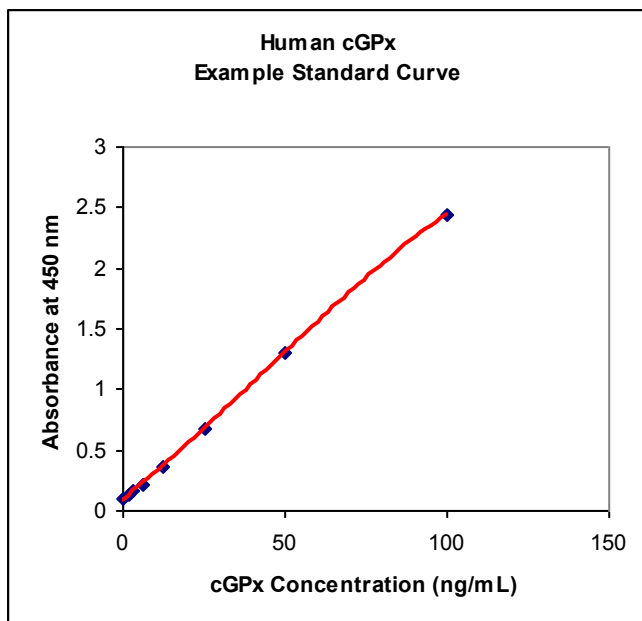
Performance Details:
The following substances were tested and found to have no cross-reactivity: human GPx3, human GPx4, Rat GPx1 and mouse GPx1.

Sensitivity
The minimal detectable dose of human cGPx was calculated to be 1.56 ng/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 = 3.65 %
Inter-assay = 6.48%

Accuracy:
Recovery on addition is 99.3~102.2%   (Average 101.2%)
Recovery on dilution is 97.9~103.4%   (Average 100.7%)

References:
1) Takebe, G.. et al. A comparative study on the hydroperoxide and thiol specificity of the glutathione peroxidase family and selenoprotein P (2002) *J. Biol. Chem*. **277**(43): 41254-41258

2) Avissar, N. et al. Human placenta makes extracellular glutathione peroxidase and secretes it into maternal circulation (1994) *Am. J. Physiol.* **267**: E68-76

3) Bao, Y. et al. Reduction of thymine hydroperoxide by phospholipid hydroperoxide glutathione peroxidase and glutathione transferases (1997) *FEBS Lett.* **410**: 210-212

4) Chambers, I. et al. The structure of the mouse glutathione peroxidase gene: the selenocysteine in the active site is encoded by the 'termination' codon, TGA (1986) *EMBO J.* **5**: 1221-1227

5) Perry, A. et al. Genetic evidence for an androgen-regulated epididymal secretory glutathione peroxidase whose transcript does not contain a selenocysteine codon (1992) *Biochem. J.* **285**: 863- 870

6) De Haan J. et al. An imbalance in antioxidant defense affects cellular function: the pathophysiological consequences of a reduction in antioxidant defense in the glutathione peroxidase-1 (Gpx1) knockout mouse (2003) *Redox Rep.* **8**(2): 69-79
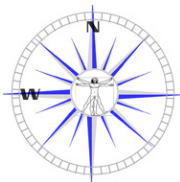
References (cont.):

7) Stefan Blankenberg, et al. Glutathione peroxidase 1 activity and cardiovascular events in patients with coronary artery disease (2003) N Eng J Med 349:1605-1613

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..

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