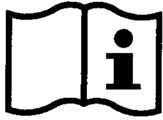
Product information



User´s Manual

GPX3 human ELISA

Enzyme immunoassay (ELISA) for the determination of human GPX3 in serum, plasma and cell culture supernatant.

REF IB99508

96

Storage: 2-8°C

RUO

For research use only, not for use in diagnostic procedures.

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1. Intended Use

The GPX3 human ELISA Kit is to be used for the measurement of human GPX3 in serum, plasma and cell culture supernatant. This ELISA Kit is for research use only.

2. Introduction

Glutathione peroxidase (GPX) catalyzes the reduction of hydrogen peroxide, organic hydroperoxide, and lipid peroxides by reduced glutathione and functions in the protection of cells against oxidative damage. This enzyme, found mainly in the cytosol of mammalian cells, is unusual in its content of a selenocysteine residue in its active site that is encoded by a TGA (stop codon) (1). The glutathione peroxidase found in plasma now called GPX3 is immunologically distinct from the erythrocyte and liver cytosolic enzymes. It also has some differences in physical and kinetic properties. Takahashi et al. (2) isolated cDNA clones coding for plasma GPX3. They found that the nucleotide sequence consisted of a 678-bp open reading frame coding for a 226-amino acid polypeptide with a molecular mass of 25,389. The amino acid sequence showed only 44% homology with other human cellular GPX family. Takahashi et al. (2) concluded that as the plasma enzyme contains 1 atom of selenium per subunit, the in-frame TGA observed at positions 217-219 could be assigned to selenocysteine and a tetramer of approximately 90-100 kDa where each of the 4 identical subunits contains an active site with the selenium atom in the form of selenocysteine residue (2). GPX3 is also found in human milk (3). Chu et al. (4) found that glutathione peroxidase-3 is expressed in kidney, lung, heart, breast, placenta, and, in the human but not the rodent, in liver as well. Since redox control has been implicated in the cause of metabolic dysfunction, plasma or serum measurement of GPX3 may give some benefits to the investigation of these metabolic diseases.

3. General References

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

(2) Primary structure of human plasma glutathione peroxidase deduced from cDNA sequences: K. Takahashi, et al.; J. Biochem. **108,** 145 (1990)

(3) Expression of glutathione peroxidase in human liver in addition to kidney, heart, lung, and breast in humans and rodents: F.F. Chu, et al.; Blood **79,** 3233 (1992)

(4) Partial sequence of human plasma glutathione peroxidase and immunologic identification of milk glutathione peroxidase as the plasma enzyme: N. Avissar, et al.; J. Nutrition **121,** 1243 (1991)

4. Assay Principle

This assay is a sandwich Enzyme Linked-Immunosorbent Assay (ELISA) for the measurement of human GPX3 in biological fluids. A polyclonal antibody specific for GPX3 has been pre-coated onto the 96-well microtiter plate. Standards and samples are pipetted into the wells for binding to the coated antibody. After extensive washing to remove unbound compounds, GPX3 is recognized by the addition of a biotinylated polyclonal antibody specific for GPX3 (Detection Antibody). After removal of excess biotinylated antibody, HRP labeled streptavidin (STREP-HRP) is added. Following a final washing, peroxidase activity is quantified using the substrate 3,3',5,5'-tetramethylbenzidine (TMB). The intensity of the color reaction is measured at 450 nm after acidification and is directly proportional to the concentration of GPX3 in the samples.

5. Handling & Storage

- Reagent must be stored at 2-8°C when not in use.
- Plate and reagents should be at room temperature before use.
- Do not expose reagents to temperatures greater than 25°C.

6. Kit Components

1 plate coated with human GPX3 Antibody	(6 x 16-well strips)	
2 bottles Wash Buffer 10X	(2 x 30 ml)	(Wash Buffer 10X)
2 bottles ELISA Buffer 10X	(2 x 30 ml)	(ELISA Buffer 10X)
1 vial Detection Antibody	(20 µl)	(DET)
1 vial HRP Labeled Streptavidin (lyophilized)	(2 µg)	(STREP-HRP)
1 vial human GPX3 Standard (lyophilized)	(64 ng)	(STD)
1 bottle TMB Substrate Solution	(12 ml)	(TMB)
1 bottle Stop Solution	(12 ml)	(STOP)
2 plate sealers (plastic film)		
2 silica Gel Minibags		

7. Materials Required but *Not* Supplied

- Microtiterplate reader at 450 nm
- Calibrated precision single and multi-channel pipettes. Disposable pipette tips
- Deionized water
- Microtubes or equivalent for preparing dilutions
- Disposable plastic containers for preparing working buffers
- Plate washer: automated or manual
- Glass or plastic tubes for diluting and aliquoting standard

8. General ELISA Protocol

8.1. Preparation and Storage of Reagents

NOTE: Prepare just the appropriate amount of the buffers necessary for the assay.

- **Wash Buffer 10X** has to be diluted with deionized water 1:10 before use (e.g. 50 ml Wash Buffer 10X + 450 ml water) to obtain Wash Buffer 1X.

- **ELISA Buffer 10X** has to be diluted with deionized water 1:10 before use (e.g. 20 ml ELISA Buffer 10X + 180 ml water) to obtain ELISA Buffer 1X.

- **Detection Antibody (DET)** has to be diluted to 1:1000 in ELISA Buffer 1X (10 µl DET + 10 ml ELISA Buffer 1X).

  NOTE: The diluted Detection Antibody is not stable and cannot be stored!

- **HRP Labeled Streptavidin (STREP-HRP)** has to be reconstituted with 100 µl of ELISA Buffer 1X.
  - After reconstitution of STREP-HRP, prepare aliquots and store them at -20°C. **Avoid freeze/thaw cycles**.
  - Dilute the reconstituted STREP-HRP to the working concentration by adding 50 µl in 10 ml of ELISA Buffer 1X (1:200).

  NOTE: The diluted STREP-HRP is not stable and cannot be stored!

- **Human GPX3 Standard (STD)** has to be reconstituted with 1 ml of deionized water.
  - This reconstitution produces a stock solution of 64 ng/ml. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 15 minutes. Mix well prior to making dilutions.

  NOTE: The reconstituted standard is aliquoted and stored at -20°C.

  - Dilute the standard protein concentrate (STD) (**64 ng/ml**) in ELISA Buffer 1X. A seven-point standard curve using 2-fold serial dilutions in ELISA Buffer 1X is recommended.
  - Suggested standard points are:

    32 , 16 , 8 , 4 , 2 , 1 , 0.5 and 0 ng/ml.

Dilute further for the standard curve:

To obtain	Add	Into
32 ng/ml	300 µl of GPX3 (64 ng/ml)	300 µl of ELISA Buffer 1X
16 ng/ml	300 µl of GPX3 (32 ng/ml)	300 µl of ELISA Buffer 1X
8 ng/ml	300 µl of GPX3 (16 ng/ml)	300 µl of ELISA Buffer 1X
4 ng/ml	300 µl of GPX3 (8 ng/ml)	300 µl of ELISA Buffer 1X
2 ng/ml	300 µl of GPX3 (4 ng/ml)	300 µl of ELISA Buffer 1X
1 ng/ml	300 µl of GPX3 (2 ng/ml)	300 µl of ELISA Buffer 1X
0.5 ng/ml	300 µl of GPX3 (1 ng/ml)	300 µl of ELISA Buffer 1X
0 ng/ml	300 µl of ELISA Buffer 1X	Empty tube

8.2. Sample Collection, Storage and Dilution

Serum : Use a serum separator tube. Let samples clot at room temperature for 30 minutes before centrifugation for 20 minutes at 1,000xg. Assay freshly prepared serum or store serum in aliquot at ≤ -20°C for later use. Avoid repeated freeze/thaw cycles.

Plasma : Collect plasma using heparin, EDTA, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000xg within 30 minutes of collection. Assay freshly prepared plasma or store plasma sample in aliquot at ≤ -20°C for later use. Avoid repeated freeze/ thaw cycles.

Serum, Plasma, or **Cell Culture Supernatant** have to be diluted in ELISA Buffer 1X. Samples containing visible precipitates must be clarified before use.

NOTE: *As a starting point, 1/200 dilution of serum or plasma is recommended! If sample values fall outside the detection range of the assay, a lower or higher dilution may be required!*

8.3. Assay Procedure (Checklist)

☐	1. Determine the number of 16-well strips needed for the assay and insert them in the frame for current use. The extra strips should be resealed in the foil pouch bag and stored at 4°C. ***NOTE:*** *Remaining 16-well strips coated with GPX3 antibody when opened can be stored at 4°C for up to 1 month.*
☐	2. Add 100 µl of the different standards into the appropriate wells in duplicate! At the same time, add 100 µl of diluted serum, plasma or cell culture supernatant samples in duplicate to the wells **(see 8.1. Preparation and Storage of Reagents and 8.2. Preparation of Samples)**.
☐	3. Cover the plate with plate sealer and incubate for **1 hour at 37°C.**
☐	4. Aspirate the coated wells and add 300 µl of Wash Buffer 1X using a multichannel pipette or auto-washer. Repeat the process for a total of three washes. After the last wash, complete removal of liquid is essential for good performance.
☐	5. Add 100 µl to each well of the Detection Antibody **(DET)**. **(see 8.1. Preparation and Storage of Reagents)**.
☐	6. Cover the plate with plate sealer and incubate for **1 hour at 37°C**.
☐	7. Aspirate the coated wells and add 300 µl of Wash Buffer 1X using a multichannel pipette or auto-washer. Repeat the process for a total of three washes. After the last wash, complete removal of liquid is essential for good performance.
☐	8. Add 100 µl to each well of the diluted HRP Labeled Streptavidin **(STREP-HRP)** **(see 8.1. Preparation and Storage of Reagents)**.
☐	9. Cover the plate with plate sealer and incubate for **1 hour at 37°C**.
☐	10. Aspirate the coated wells and add 300 µl of Wash Buffer 1X using a multichannel pipette or auto-washer. Repeat the process for a total of five washes. After the last wash, complete removal of liquid is essential for good performance.
☐	11. Add 100 µl to each well of TMB Substrate Solution **(TMB).**
☐	12. Allow the color reaction to develop **at room temperature (RT°C) in the dark for 20 minutes**.
☐	13. Stop the reaction by adding 100 µl of Stop Solution **(STOP).** Tap the plate gently to ensure thorough mixing. The substrate reaction yields a blue solution that turns yellow when Stop Solution **(STOP)** is added.
	! CAUTION: CORROSIVE SOLUTION!
☐	14. Measure the OD at 450 nm in an ELISA reader within 30 minutes.

9. Calculation of Results

- Average the duplicate readings for each standard, control and sample and subtract the average blank value (obtained with the 0 ng/ml point).
- Generate the standard curve by plotting the average absorbance obtained for each standard concentration on the horizontal (X) axis vs. the corresponding GPX3 concentration (ng/ml) on the vertical (Y) axis (see **10.** TYPICAL DATA).
- Calculate the GPX3 concentrations of samples by interpolation of the regression curve formula as shown above in a form of a quadratic equation.
- If the test samples were diluted, multiply the interpolated values by the dilution factor to calculate the concentration of human GPX3 in the samples.

10. Typical Data

The following data are obtained using the different concentrations of standard as described in this protocol:

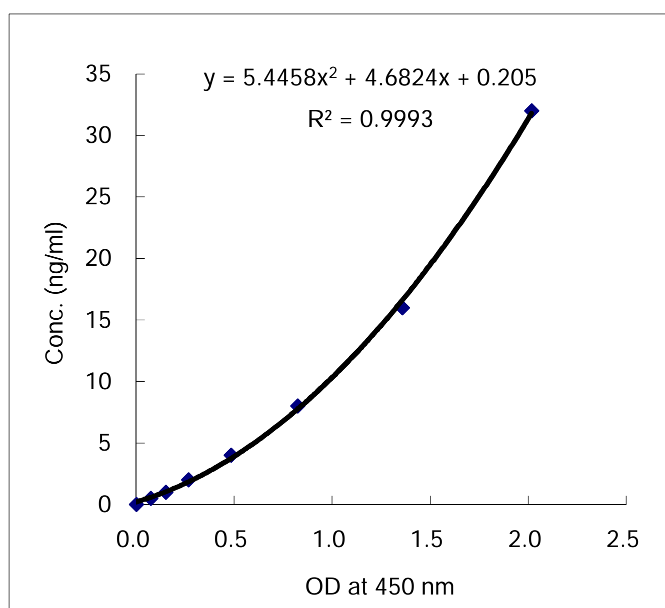

Figure: Standard curve

Standard hGPX3 (ng/ml)	Optical Density (mean)
32	2.017
16	1.356
8	0.823
4	0.483
2	0.266
1	0.151
0.5	0.074
0	0

11. Performance Characteristics

A. Sensitivity (Limit of detection):

The lowest level of GPX3 that can be detected by this assay is 100 pg/ml. ***NOTE:*** *The Limit of detection was measured by adding two standard deviations to the mean value of 50 zero standard.*

B. Assay range: 0.5 ng/ml – 32 ng/ml

C. Specificity:

This ELISA is specific for the measurement of natural and recombinant human GPX3. It does not cross-react with human adiponectin, human resistin, human FABP3, human FABP4, human ACE2, human IL-6, human SOCS-3, human 4-1BB ligand, human ANGPTL4, human Th-pok, human Foxp3, human ANGPTL6, human IDO.

D. Intra-assay precision:

Eight serum samples of known concentrations of human GPX3 were assayed in replicates 12 times to test precision within an assay.

Samples	Means (ng/ml)	SD	CV (%)	n
1	423.68	21.35	5.04	12
2	544.73	25.98	4.77	12
3	654.51	32.92	5.03	12
4	93.34	7.90	8.46	12
5	131.11	10.95	8.35	12
6	73.28	7.06	9.64	12
7	1,537.23	64.72	4.21	12
8	1,011.34	42.88	4.24	12

E. Inter-assay precision:

Eight samples of known concentrations of human GPX3 were assayed in 8 separate assays to test precision between assays.

Samples	Means (ng/ml)	SD	CV (%)	n
1	403.75	19.50	4.83	8
2	549.24	26.20	4.77	8
3	643.32	26.83	4.17	8
4	94.19	3.85	4.09	8
5	32.09	1.50	4.66	8
6	64.68	0.72	1.12	8
7	1,296.41	62.10	4.79	8
8	1,516.90	76.45	5.04	8

F. Recovery:

When samples (serum) are spiked with known concentrations of human GPX3, the recovery averages 92% (range from 88% to 94%).

Samples	Average recovery (%)	Range (%)
1	90.60	84.6-100.5
2	88.20	83.0-100.0
3	92.94	85.1-103.5
4	93.14	87.7-101.2
5	92.58	89.1-98.3
6	92.70	87.2-98.3
7	94.08	87.8-97.8

G. Linearity:

Different serum samples containing GPX3 were diluted several fold (1/80 to 1/120) and the measured recoveries ranged from 93% to 103%.

Samples	Sample Dilution	Expected (ng/ml)	Observed (ng/ml)	% of Expected
1	1 : 100	354.52	354.52	100
	1 : 80	443.15	425.68	96.06
	1 : 120	295.44	289.16	97.87
2	1 : 100	104.86	104.86	100
	1 : 80	131.07	131.12	100.04
	1 : 120	87.38	81.97	93.74
3	1 : 100	1,096.04	1,096.04	100
	1 : 80	1,370.05	1,316.60	96.10
	1 : 120	913.36	943.44	103.29

H. Expected values:

GPX3 levels range in plasma and serum from **100 to > 3,000 ng/ml** (from healthy donors).

12. Technical Hints and Limitations

- It is recommended that all standards, controls and samples be run in duplicate.
- Do not combine leftover reagents with those reserved for additional wells.
- Reagents from the kit with a volume less than 100 µl should be centrifuged.
- Residual wash liquid should be drained from the wells after last wash by tapping the plate on absorbent paper.
- Crystals could appear in the 10X solution due to high salt concentration in the stock solutions. Crystals are readily dissolved at room temperature or at 37°C before dilution of the buffer solutions.
- Once reagents have been added to the 16-well strips, DO NOT let the strips DRY at any time during the assay.
- Keep TMB Substrate Solution (TMB) protected from light.
- The Stop Solution (STOP) consists of sulfuric acid. Although diluted, the Stop Solution (STOP) should be handled with gloves, eye protection and protective clothing.

13. Troubleshooting

PROBLEM	POSSIBLE CAUSES	SOLUTIONS
No signal or weak signal	Omission of key reagent	Check that all reagents have been added in the correct order.
	Washes too stringent	Use an automated plate washer if possible.
	Incubation times inadequate	Incubation times should be followed as indicated in the manual.
	Plate reader settings not optimal	Verify the wavelength and filter setting in the plate reader.
	Incorrect assay temperature	Use recommended incubation temperature. Bring substrates to room temperature before use.
High background	Concentration of STREP-HRP too high	Use recommended dilution factor.
	Inadequate washing	Ensure all wells are filling wash buffer and are aspirated completely.
Poor standard curve	Wells not completely aspirated	Completely aspirate wells between steps.
	Reagents poorly mixed	Be sure that reagents are thoroughly mixed.
Unexpected results	Omission of reagents	Be sure that reagents were prepared correctly and added in the correct order.
	Dilution error	Check pipetting technique and double-check calculations.

14. Notes

Product Specific References:

1. Y.S. Lee, et al.; Mol. Endocrinol. **22,** 2176 (2008)
2. S.S. Chung, et al.; Mol. Cell Biol. **29,** 20 (2009)
3. J. Verhaeghe, et al.; Early Hum. Dev. **85,** 767 (2009)
4. J. Verhaeghe, et al.; Metabolism **60,** 71 (2011)
5. D. Agnani, et al.; J. Ovarian Res. **4,** 18 (2011)
6. G. Flehmig, et al.; PLos One **9,** e99785 (2014)

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