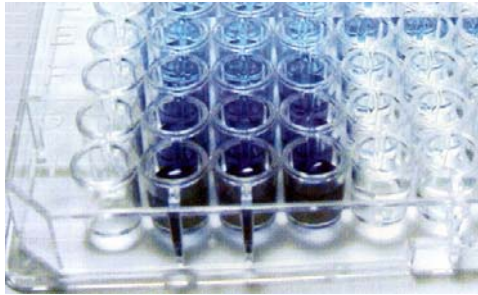


Human GPX3 ELISA Kit

Cat. No. G0631EK



Instruction Manual
Version 1. 0. 0

FOR RESEARCH USE ONLY
NOT FOR USE IN DIAGNOSTIC PROCEDURES



Table of Contents

Introduction	3
Assay Principles	4
Kit Components	4
Reagent Description	4
Storage of Reagents	5
Materials Required but not Supplied	5
Sample Collection and Storage	5
Flow Chart of Assay Procedure	6
Assay Procedure	7
Performance Characteristics	10
References	13
Plate Layout	14
Troubleshooting Guide	15

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 diagnosis of these metabolic diseases.

Assay Principles

This kit is an enzyme-linked immunosorbent assay (ELISA) for quantitative determination of GPX3 in human serum, plasma or cell culture supernatants.

Polyclonal antibody specific for human GPX3 has been pre-coated onto 96 well microplate. Standards and samples are pipetted into the wells and any GPX3 present is bound by immobilized antibody. Bound GPX3 is captured by biotinylated anti-human GPX3 polyclonal antibody. Streptavidin is added. After washing, a substrate solution is added. The colors develop in proportion to the bound GPX3 quantity. The color development is stopped and the intensity of color is measured.

Kit Components

- 1) Antibody coated 96-well plate, 12 X 8-well strips
- 2) 5x Wash concentrate, 100 ml
- 3) 5x Diluent, 50 ml
- 4) Secondary antibody, 12 ml
- 5) 100x Detector, 150 μ l
- 6) Standard, recombinant human GPX3 expressed by HEK 293 cells, 1 vial, lyophilized
- 7) QC sample = a positive control of recombinant human GPX3 protein, 1 vial, lyophilized (For actual concentrations of QC sample, see the 'Certificate of analysis' enclosed.)
- 8) Substrate I, 6 ml
- 9) Substrate II, 6 ml
- 10) Stop solution, 12 ml

Reagents Description

Antibody coated 96-well plate, 12 x 8-well strips, with absorbed polyclonal antibody against human GPX3
5x Wash concentrate, buffered detergent solution, supplied as a 5x concentrate
5x Diluent, for sample and reagent dilution

1x Secondary antibody, biotinylated polyclonal antibody against human GPX3
100x detector, Streptavidin
Standard, 64.0 ng, 1 vial each, recombinant human GPX3
QC sample, 1 vial each, human serum
Substrate I and II, chromogenic reagents
Stop solution, 1M H₃PO₄

Storage of Reagents

Reagent must be stored at 2-8°C when not in use. Reagents must be brought to room temperature before use. Do not expose reagents to temperatures greater than 25°C. Diluted wash solution may be stored at room temperature for up to one month.

Materials Required but not Supplied

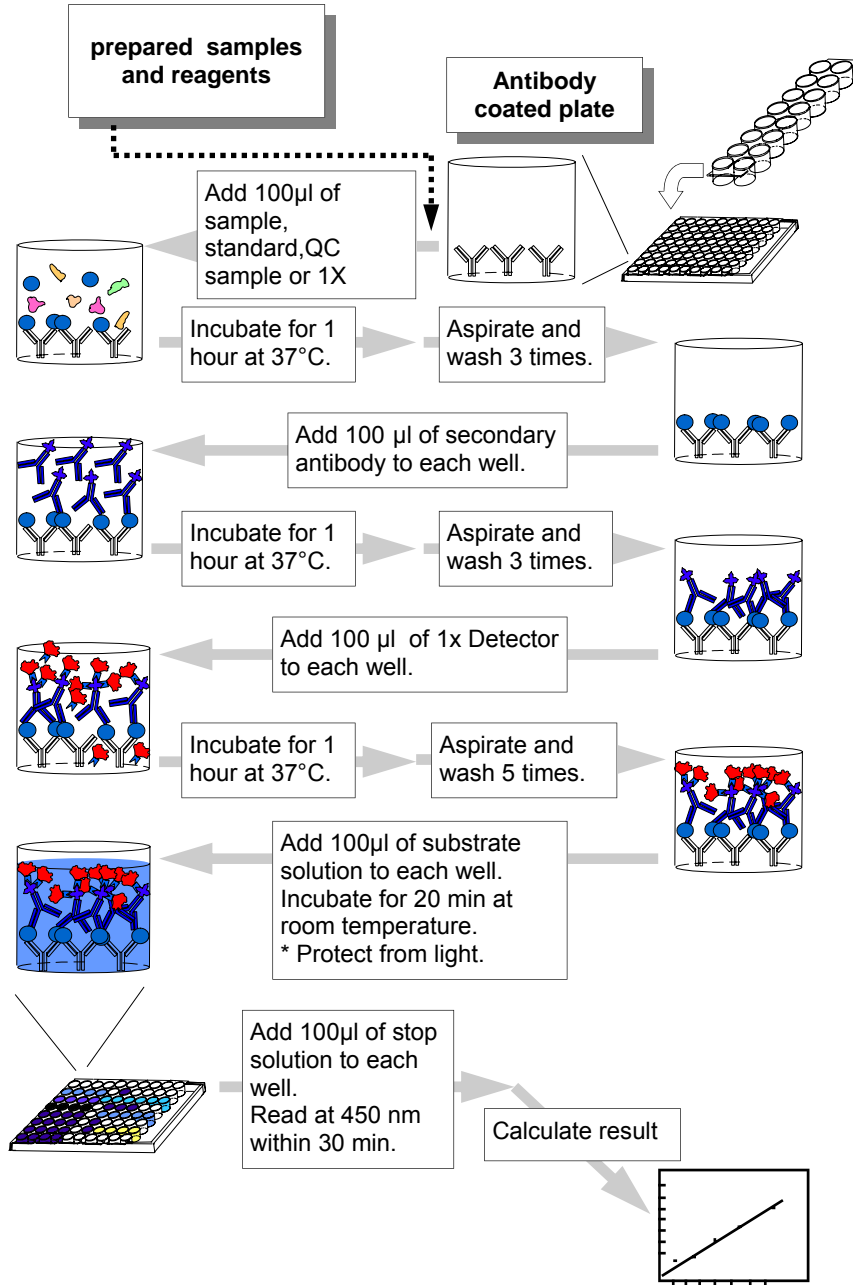
Precision single and multi-channel pipettes.
Disposable pipette tips.
Microtubes or equivalent for preparing dilutions.
Disposable plastic containers for preparing working detector antibody and substrate.
Reagent reservoirs.
Microwell or microstrip plate reader 450 nm
Deionized water

Sample Collection and Storage

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.

Flow Chart of Assay Procedure



Assay Procedure

1) Preparation of Working Reagents

1. Allow all samples and kit components to equilibrate to room temperature (20-25°C).
2. Plan the plate configuration and create a plate map. Calculate the amount of working reagents to use (See table below). It is recommended that standards and samples be run in duplicate.
3. Prepare **1X Wash Solution**. Dilute 5X Wash Concentrate 1:5 with deionized water (1 part 5X Wash Concentrate with 4 parts deionized water). The diluted 1X Wash Solution is stable for one month at room temperature.
4. Prepare **1X Diluent**. Dilute 5X Diluent 1:5 with deionized water (1 part 5X Diluent with 4 parts deionized water).
5. Prepare **1X Detector**. Dilute 100X Detector 1:100 with 1X Diluent (1 part 100X Detector with 99 parts 1X Diluent). Use the 1X Detector within one hour of preparation.
6. Freshly prepare just before use the **Substrate Solution** by adding one part Substrate I to one part Substrate II.

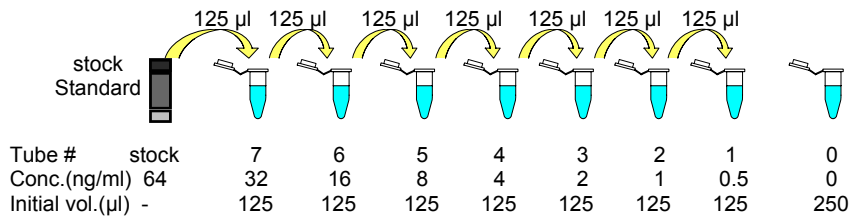
The amount of working reagents needed for 1 well				
Working reagents	Total volume needed	Stock solution added	Dilution solution added	Note
1X Wash Solution	2.8 ml	0.56 ml of 5X Wash Concentrate	2.24 ml of ddH ₂ O	Stable for 1 month at RT
1X Diluent	1 ml	0.2 ml of 5X Diluent	0.8 ml of ddH ₂ O	in the case of 10 µl plasma
1X Detector	110 µl	1.1 µl of 100X Detector	108.9 µl of 1X Diluent	Use within 1 hr.
Substrate Solution	110 µl	55 µl of Substrate I	55 µl of Substrate II	Freshly prepared just before use

7. Prepare working aliquots of the Standard as follows :
When opening the lyophilized Standard, remove cap gently as

the lyophilizate may have become dislodged during shipping. Add 1 ml of deionized water to the Standard vial to make a stock concentration of 64 ng/ml. Mix well.

A recommended dilution scheme is as follows :

- 1) Label 8 microcentrifuge tubes #0-7. Add 125 µl of the 1X Diluent to the microcentrifuge tubes # 0-7.
- 2) Add 125 µl of the stock Standard solution to tube #7 and vortex. This is Standard tube # 7 with a concentration of 32 ng/ml.
- 3) Standards # 6 to 1 are then prepared by performing a 1:2 dilution of the preceding standard. Do not add any standard to the tube # 0.

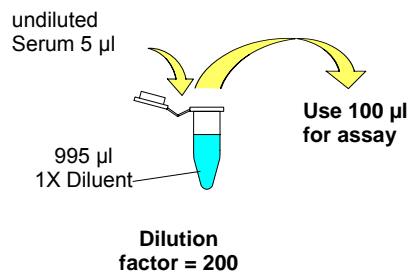


8. Reconstitute QC sample in 1 ml of deionized water.

2) Sample Preparation

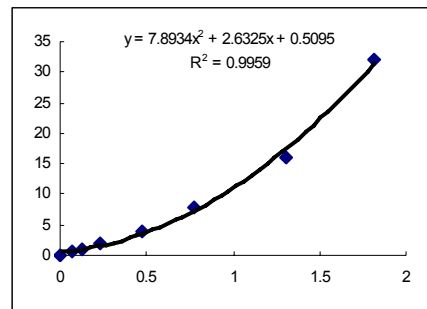
Dilute serum 1:200 with 1X Diluent (example, 5 µl serum plus 995 µl 1X Diluent; dilution factor=200) and mix well.

* If samples fall the outside range of assay, a lower or higher dilution may be required.



3) Experiment procedure

1. Remove the appropriate number of microwell strips from the sealed foil pouch.
2. Pipette 100 μ l of standard 0 to 7, the reconstituted QC sample and diluted serum sample into the antibody-coated plate according to the plate configuration. Use a new pipette tip for each standard or sample.
3. Incubate at 37°C for 1 hour.
4. Remove the solution and wash 3 times with 300 μ l of 1X Wash Solution to each well.
5. Add 100 μ l Secondary Antibody to each well.
6. Incubate at 37°C for 1 hour.
7. Remove the solution and wash 3 times with 300 μ l of 1X Wash Solution to each well.
8. Add 100 μ l 1X Detector to each well.
9. Incubate at 37°C for 1 hour.
10. Remove the solution and wash 5 times with 300 μ l of 1X Wash Solution to each well.
11. Add 100 μ l of the Substrate Solution to each well.
12. Incubate at room temperature for 20 min.
* Protect from light.
13. Using the multi-channel pipette, add 100 μ l Stop Solution to each well.
14. Read at 450 nm.
15. Subtract the absorbance of the blank from the readings for each standard and sample.
16. Construct a standard curve by plotting the known concentrations (Y) of standard versus the absorbances (X) of standard. A measurable range is typically shown between 0.5 ng/ml and 32 ng/ml.
17. Calculate the GPX3 concentrations of samples by interpolation of the regression curve formula as shown above in a form of a quadratic equation
18. The GPX3 concentrations calculated must be multiplied by dilution factor [see **2) Sample Dilution**] to obtain the concentrations of the undiluted samples (Dilution factor of lyophilized QC sample is 200).



Performance Characteristics

1) Sensitivity

The limit of detection: 100 pg/ml

2) Precision

a. Intra-Assay (precision within an assay)

8 samples of known concentration were tested 12 times on one plate to assess intra-assay precision.

Sample No.	Average (ng/ml)	CV(%)
1	423.68	5.04
2	544.73	4.77
3	654.51	5.03
4	93.34	8.46
5	131.11	8.35
6	73.28	9.64
7	1537.23	4.21
8	1011.34	4.24

b. Inter-Assay (precision between assay)

8 samples of known concentration were tested 8 times on separated plate to assess inter-assay precision.

Sample No.	Average (ng/ml)	CV(%)
1	403.75	4.83
2	549.24	4.77
3	643.32	4.17
4	94.19	4.09
5	32.09	4.66
6	64.68	1.12
7	1296.41	4.79
8	1516.90	5.04

3) Recovery

The recovery of GPX3 spiked to four different levels in six different human serum samples throughout the range of assay was evaluated.

Sample No.	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	89.1-99.4
6	92.70	87.2-98.3
7	94.08	87.8-97.8

4) Specificity

- a. Not cross reaction with rat and mouse sera
- b. Cross Reactivity with each protein

Analyte	Max. Conc. (ng/ml)	Cross Reactivity (%)	Analyte	Max. Conc. (ng/ml)	Cross Reactivity (%)
Human GPX3	32	100%	Human GPX3	32	100%
Human Adiponectin	600	N. R	Human 4-1BB ligand	2000	N. R
Human Resistin	2000	N. R	Human ANGPTL4	2000	N. R
Human FABP4	2000	N. R	Human Th-pok	2000	N. R
Human FABP3	2000	N. R	Human FABP3	2000	N. R
Human ACE2	2000	N. R	Human Foxp3	2000	N. R
Human IL-6	2000	N. R	Human AGF	2000	3.75
Human SOCS-3	2000	N. R	Human IDO	2000	N. R

N. R : No Cross-reactivity

6) Linearity

Effect of Serum Dilution

Sample No.	Serum Dilution	Expected (ug/ml)	Observed (ug/ml)	% Of Expected
1	1 : 100	354.52	354.52	100.0
	1 : 80	443.15	425.68	96.06
	1 : 120	295.44	289.16	97.87
2	1 : 100	104.86	104.86	100.0
	1 : 80	131.07	131.12	100.04
	1 : 120	87.38	81.91	93.74
3	1 : 100	1096.04	1096.04	100.0
	1 : 80	1370.05	1316.60	96.10
	1 : 120	913.36	943.44	103.29

% of expected = observed / expected x 100%

References

1. Chambers, I., Frampton, J., Goldfarb, P. et al. The structure of the mouse glutathione peroxidase gene: the selenocysteine in the active site is encoded by the 'termination' codon, TGA. EMBO J. 1986, 5: 1221-1227.
2. Takahashi, K., Akasaka, M., Yamamoto, Y. et al. Primary structure of human plasma glutathione peroxidase deduced from cDNA sequences. J. Biochem
3. Chu, F.-F., Esworthy, R. S., Doroshov, J. H. et al. Expression of glutathione peroxidase in human liver in addition to kidney, heart, lung, and breast in humans and rodents. Blood 1992, 79: 3233-3238.
4. Avissar N., Slemmon JR., Palmer IS, Cohen HJ. Partial sequence of human plasma glutathione peroxidase and immunologic identification of milk glutathione peroxidase as the plasma enzyme. J. Nutrition 1991, 121:1243-1249.

Troubleshooting Guide

Problem	Possible Cause	Solution
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 appropriate for the system.
	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 detector 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.
	Technique problem	Proper mixing of reagents and wash steps are critical.

AdipoGen Inc.
641-B, Graduate School of Life Science
and Biotechnology, Korea Univ.,
1, 5-ka, Anam-dong, Sungbuk-ku,
Seoul, Korea

© 2007 AdipoGen Inc. All right reserved.



TECHNICAL INFORMATION

Web www.adipogen.com

E-mail bsyoun@adipogen.com

Phone +82-02-927-1470

Fax +82-2-926-1670