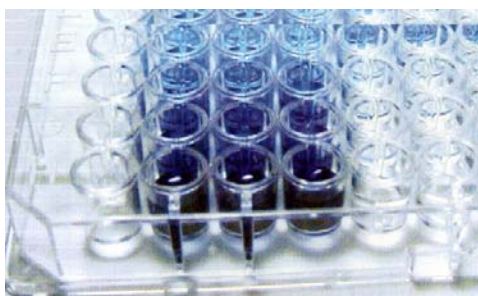


Mouse GPX3 ELISA Kit

Cat. No. G0832EK



Instruction Manual
Version 1.0.0

FOR RESEARCH USE ONLY
NOT FOR USE IN DIAGNOSTIC PROCEDURES



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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 mouse serum, plasma or cell culture supernatants.

A polyclonal antibody specific for mouse 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 purified anti-mouse GPX3 polyclonal antibody. HRP conjugated anti-guinea pig IgG is added. After washing, a substrate solution is added. The colors develop in proportion to the bounded 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 mouse GPX3 expressed by HEK 293 cells, 1 vial, lyophilized
- 7) QC sample = recombinant mouse GPX3 protein, 1 vial, lyophilized (For actual concentrations of QC sample, see the 'Certificate of analysis' enclosed.)
- 8) Substrate, 12 ml
- 9) Stop solution, 12 ml
- 10) Plate sealer, 3 sealers

Reagents Description

Antibody coated 96-well plate, 12 x 8-well strips, with absorbed polyclonal antibody against mouse GPX3
5x Wash concentrate, buffered detergent solution, supplied as a 5x concentrate

5x Diluent, for sample and reagent dilution
1x Secondary antibody, polyclonal antibody against mouse GPX3
100x detector, HRP conjugated guinea pig IgG
Standard, 64.0 ng, 1 vial each, recombinant mouse GPX3, lyophilized
QC sample, 1 vial each, recombinant mouse GPX3 protein
Substrate, 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.

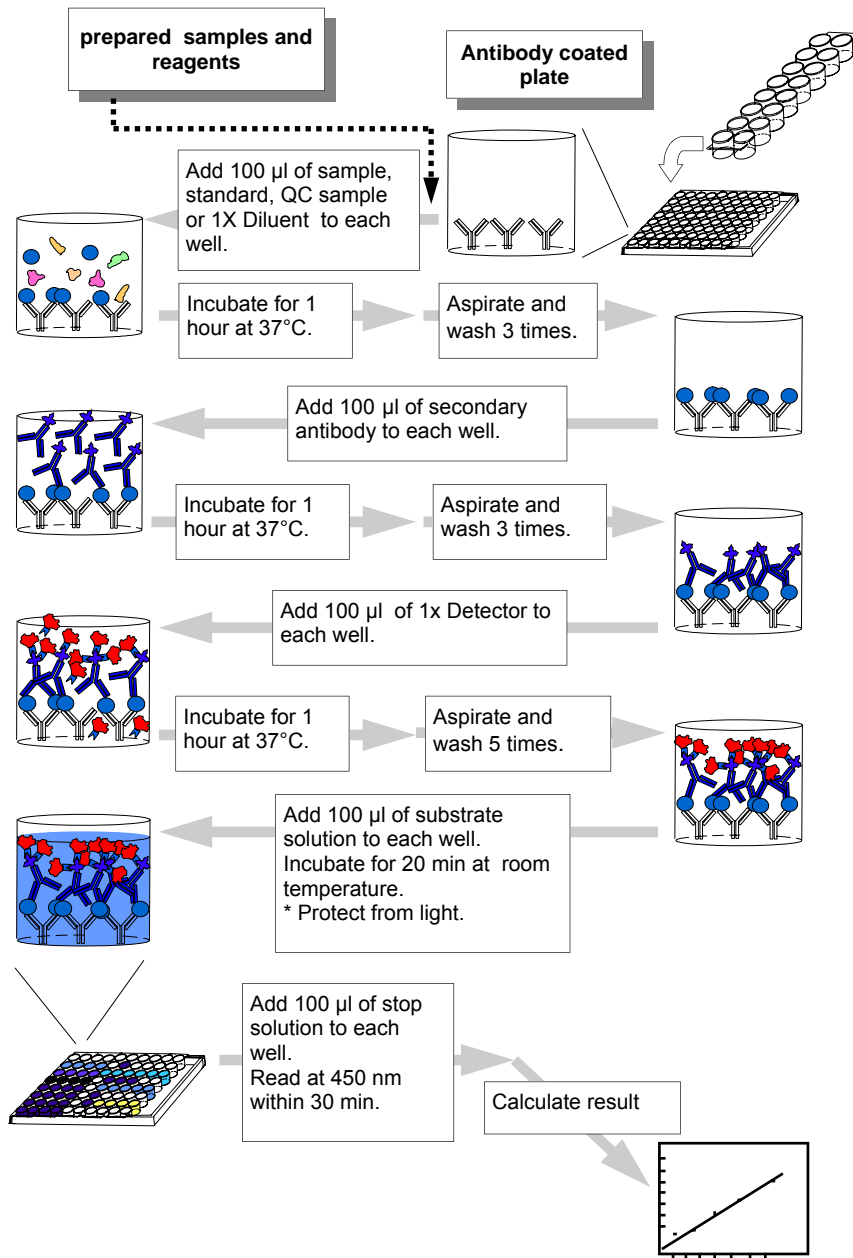
Materials Required but not Supplied

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

Sample Collection and Storage

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 ≤ -80°C for later use. Avoid repeated freeze/thaw cycles.

Flow Chart of Assay Procedure



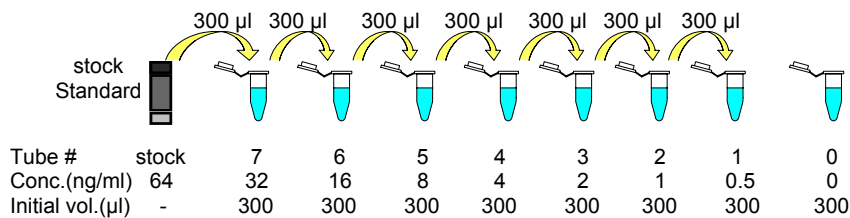
Assay Procedure

1. Preparation of 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.
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) Warm **Substrate Solution** to room temperature 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 :

- A) Label 8 microcentrifuge tubes #0-7. Add 300 μ l of the 1X Diluent to the microcentrifuge tubes # 0-7.
- B) Add 300 μ l of the stock Standard solution to tube # 7 and vortex. This is Standard tube # 7 with a concentration of 32 ng/ml.
- C) 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.



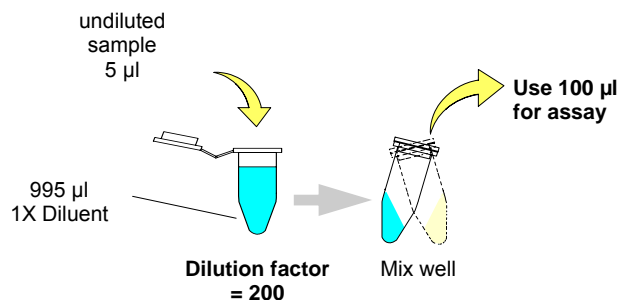
8) Reconstitute QC sample in 1 ml of deionized water. Mix well.

2. Sample Preparation

1) Dilute samples 1:200 with 1X Diluent (example, 5 μ l sample plus 995 μ l 1X Diluent; dilution factor=200) and mix well.

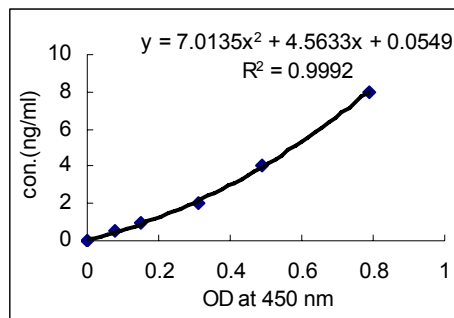
* If samples fall the outside range of assay, a lower or higher dilution may be required.

2) Use 100 μ l of the final diluted sample for ELISA.



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 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 Preparation**] to obtain the concentrations of the undiluted samples.



Performance Characteristics

1) **Sensitivity** : 150 pg/ml

2) Precision

a. Intra-Assay (precision within an assay)

4 samples were tested 4 times to assess intra-assay precision.

Sample	Mean (ng/ml)	SD (ng/ml)	CV (%)
1	1329.77	103.67	7.80
2	1124.71	90.49	8.05
3	900.31	63.74	7.08
4	293.67	23.89	8.13

b. Inter-Assay (precision between assays)

6 samples were tested 6 times to assess inter-assay precision.

Sample	Mean (ng/ml)	SD (ng/ml)	CV (%)
1	1255.21	70.71	5.63
2	1040.91	26.33	2.53
3	1336.42	46.50	3.48
4	1012.02	41.35	4.09
5	679.80	33.95	4.99
6	451.00	15.14	3.36

3) Specificity

- a. No cross reaction with human and rat sera
- b. Cross Reactivity

Analyte	Max. Conc. (ng/ml)	Cross Reactivity (%)
Mouse GPX3	10	100
Mouse Adiponectin	100	N. R.
Mouse Vaspin	100	N. R.
Mouse Visfatin	100	N. R.
Mouse Clusterin	100	N. R.
Mouse Progranulin	100	N. R.
Mouse RBP4	100	N. R.
Mouse ANGPTL3	100	N. R.
Mouse ANGPTL4	100	N. R.
Mouse IL33	100	N. R.
Mouse CD137	100	N. R.
Mouse Leptin	100	N. R.
Human Resistin	100	N. R.
Human GPX3	100	N. R.
Rat Resistin	100	N. R.

N. R. : No Cross-reactivity

4) Recovery

The recovery of GPX3 spiked to three different levels in three different mouse serum samples throughout the range of assay was evaluated.

Sample No.	Average recovery (%)	Range (%)
1	102.68	95-105
2	99.72	95-105
3	100.83	95-105

5) Linearity - Effect of Serum Dilution

Three serum samples were pre-treated as described in the protocol, resulting in the final dilution of x200 (labeled in the table below as dilution; 1).

Sample No.	Serum Dilution	Expected (ng/ml)	Observed (ng/ml)	% Of Expected
1	1	534.72	534.72	100
	1/2	267.36	248.45	92.93
	1/4	133.68	123.89	92.68
2	1	245.04	245.04	100
	1/2	122.52	112.71	91.99
	1/4	61.26	66.88	109.17
3	1	1038.64	1038.64	100
	1/2	519.32	495.58	95.43
	1/4	259.66	240.81	92.74

$$\% \text{ of expected} = \text{observed} / \text{expected} \times 100\%$$

References

1. Chambers, I., Frampton, J., Goldfarb, P. et al. 1986 The structure of the mouse glutathione peroxidase gene: the selenocysteine in the active site is encoded by the 'termination' codon, TGA. *EMBO J.* 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., Doroshow, J. H. et al. 1992 Expression of glutathione peroxidase in human liver in addition to kidney, heart, lung, and breast in humans and rodents. *Blood* 79:3233-3238.
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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.

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