（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-K096-M

Specification：48T(16 samples)/96T(40 samples)

Measuring instrument：Microplate reader（400-420 nm）

Detection range：34.34-1036.64 U

Elabscience®Glutathione Peroxidase (GSH-Px) Activity Assay Kit

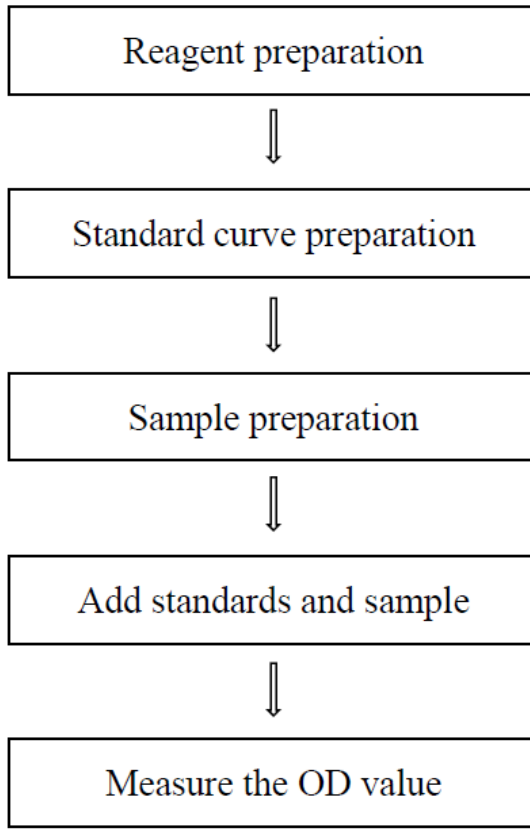
This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
Tell: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

Table of contents

Assay summary



Intended use

This kit can be used to measure GSH-Px activity of serum, plasma, tissue, cells, cell culture supernatant samples.

Detection principle

Glutathione peroxidase (GSH-Px) can promote the reaction of hydrogen peroxide (H_2O_2) and reduced glutathione to produce H_2O and oxidized glutathione (GSSG). The activity of glutathione peroxidase can be expressed by the rate of enzymatic reaction. The activity of glutathione can be calculated by measuring the consumption of reduced glutathione. Hydrogen peroxide (H_2O_2) and reduced glutathione can react without catalysis of GSH-Px, so the portion of GSH reduction by non-enzymatic reaction should be subtracted. GSH can react with dinitrobenzoic acid to produce 5-thio-dinitrobenzoic acid anion, which showed a stable yellow color. Measure the absorbance at 412 nm, and calculate the amount of GSH.

$$H_2O_2 + 2GSH \xrightarrow{\text{GSH-PX}} 2H_2O + GSSH$$

$$GSH + DTNB \longrightarrow GSSH + TNB$$

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Stock Solution	0.5 mL ×1 vial	0.5 mL ×1 vial	2-8℃, 12 months
Reagent 2	Acid Reagent	25 mL×1 vial	50 mL×1 vial	2-8℃, 12 months
Reagent 3	Phosphate	12 mL ×1 vial	12 mL ×1 vial	2-8℃, 12 months
Reagent 4	DTNB Solution	3.5 mL×1 vial	7 mL×1 vial	2-8℃, 12 months, shading light
Reagent 5	GSH Standard	3.07 mg ×1 vial	3.07 mg ×1 vial	2-8℃, 12 months
Reagent 6	GSH Standard Stock Diluent	1.5 mL ×1 vial	1.5 mL ×2 vials	2-8℃, 12 months
	Microplate	96 wells		No requirement
	Plate Sealer	2 pieces		

Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

Microplate reader (400-420 nm), Micropipettor, Incubator, Vortex mixer, Centrifuge

Reagents:

Double distilled water, Normal saline (0.9% NaCl), PBS (0.01 M, pH 7.4)

Reagent preparation

① Equilibrate all reagents to room temperature before use.

② The preparation of stock application solution：

Before testing, please prepare stock application solution according to the test wells. For example, prepare 500 μL of stock application solution (mix well 5 μL of stock solution and 495 μL of double distilled water). The stock application solution should be prepared on spot. Store at 2-8 ℃ for 3 days.

③ The preparation of GSH standard stock diluent application solution：

Before testing, please prepare sufficient GSH standard stock diluent application solution. For example, prepare 13 mL of GSH standard stock diluent application solution (mix well 1.3 mL of GSH standard stock diluent and 11.7 mL of double distilled water).The GSH standard stock diluent application solution should be prepared on spot. If the GSH standard stock diluent is formed into ice, please dissolve it at 65 ℃.

④ The preparation of 1 mmol/L GSH standard solution：

Dissolve one vial of GSH standard with 10 mL of GSH standard stock diluent application solution, mix well to dissolve. The 1 mmol/L GSH standard solution should be prepared on spot. Aliquoted storage at -20 ℃ for 1 month.

⑤ The preparation of 100 μmol/L GSH standard solution：

Dilute 110 μL of 1 mmol/L GSH standard solution with 990 μL of GSH standard stock diluent application solution, mix well. The 100 μmol/L GSH standard solution should be prepared on spot. Store at 2-8 ℃ for 7 days.

⑥ The preparation of standard curve：

Always prepare a fresh set of standards. Discard working standard dilutions after use.

Dilute 100 μmol/L GSH standard solution with GSH standard stock diluent application solution to a serial concentration. The recommended dilution gradient is as follows: 0, 10, 20, 40, 50, 60, 80, 100 μmol/L.

Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (μmol/L)	**0**	**10**	**20**	**40**	**50**	**60**	**80**	**100**
100 μmol/L GSH standard solution (μL)	0	30	60	120	150	180	240	300
GSH standard stock diluent application solution (μL)	300	270	240	180	150	120	60	0

Sample preparation

① Sample preparation

Serum and plasma: detect directly. If not detected on the same day, the serum or plasma can be stored at -80℃ for a month.

Tissue sample:

① Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).

② Wash tissue in cold PBS (0.01 M, pH 7.4).

③ Homogenize 20 mg tissue in 180 μL normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4) with a dounce homogenizer at 4℃.

④ Centrifuge at 10000×g for 10 min at 4℃ to remove insoluble material. Collect supernatant and keep it on ice for detection.

⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

Cell (adherent or suspension) samples:

① Harvest the number of cells needed for each assay (initial recommendation 1×10^6 cells).

② Wash cells with PBS (0.01 M, pH 7.4).

③ Homogenize 1×10^6 cells in 300-500 μL normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4) with a ultrasonic cell disruptor at 4℃.

④ Centrifuge at 10000×g for 10 minutes at 4℃ to remove insoluble material.

Collect supernatant and keep it on ice for detection.

⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

② Inhibition ratio of sample

The Inhibition ratio can be detected by this kit is 10-50%, the optimal inhibition ratio is 25-45%. When the inhibition ratio is 25-45%, the corresponding sampling dilution factor is the optimal sampling dilution factor. If inhibition ratio > 50%, need to dilute the sample or decrease the sampling volume than take the test. If inhibition ratio < 10%, need to increase the concentration of sample or increase the sampling volume.

$$\text{Inhibition ratio}=\frac{OD_{\text{Non-enzyme}}-OD_{\text{Enzyme}}}{OD_{\text{Non-enzyme}}}\times 100\%$$

③ Dilution of sample

The recommended dilution factor for different samples is as follows (for reference only):

Sample type	Dilution factor
Human serum	1
Mouse serum	3-5
Rat serum	5-8
10% Mouse brain tissue homogenate	1
10% Rat liver tissue homogenate	30-60
HepG2 cells (5 mgprot/mL)	1
10% Epipremnum aureum leaves tissue homogenate	1
10% Chinese cabbage leaves tissue homogenate	3-5

Note: The diluent is normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4). For the dilution of other sample types, please do pretest to confirm the dilution factor

The key points of the assay

① The supernatant after centrifugation after adding acid reagent in enzymatic reaction must be clarified.

② Determine optimal dilution factor of samples before formal experiment. It is recommended to choose the optimal dilution factor when inhibition ratio in the range of 25%~45%.

③ Stock application solution should be preheated at 37℃ for 5 min in advance.

Operating steps

Enzymatic reaction

① Non-enzyme tube: take 20 μL of 1 mmol/L GSH standard into 1.5 mL EP tube.
Enzyme tube: take 20 μL of 1 mmol/L GSH standard, 20 μL of sample into 1.5 mL EP tube and mix fully.

② Preheat the tubes at 37℃ water bath for 5 min. Preheat stock application solution at 37℃ for 5 min at the same time.

③ Add 10 μL of stock application solution to the tubes and mix fully. React at 37 ℃ for 5 min accurately.

④ Non-enzyme tube: add 200 μL of acid reagent and 20 μL of sample to the tubes.
Enzyme tube: add 200 μL of acid reagent to the tubes.

⑤ Mix fully with a vortex mixer and centrifuge at 3100×g for 10 min, and take 100 μL of the supernatant for chromogenic reaction.

Chromogenic reaction

① Non-enzyme well: Take 100 μL of supernatant of Non-enzyme tubes to the wells.
Enzyme well: Take 100 μL of supernatant of Enzyme tubes to the wells.
Standard wells: Take 100 μL of GSH standard solution with different concentrations to the wells

② Add 100 μL of phosphate to each well.

③ Add 50 μL of DTNB solution to each well.

④ Oscillate for 10 s with microplate reader and stand for 5 min. Measure the OD values at 412 nm with microplate reader.

Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean OD value of the blank (Standard #①) from all standard readings. This is the absoluted OD value.

3. Plot the standard curve by using absoluted OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (**y = ax + b**) with graph software (or EXCEL).

The sample:

1. Serum (plasma) sample:

Definition: The amount of GSH-Px in 0.1 mL of sample that catalyze the consumption of 1 μmol/L GSH with deducting the effect of non-enzyme reaction at 37℃ for 5 minute is defined as 1 unit

$$\text{GSH-Px activity (U)} = (\Delta A_{412} - b) \div a \times \frac{0.23+V}{0.03+V} \times \frac{0.1^*}{V} \times f$$

2. Tissue and cells sample:

Definition: The amount of GSH-Px in 1 mg of protein that catalyze the consumption of 1 μmol/L GSH with deducting the effect of non-enzyme reaction at 37℃ for 5 minute is defined as 1 unit.

$$\text{GSH-Px activity (U/mgprot)} = (\Delta A_{412} - b) \div a \times \frac{0.23+V}{0.03+V} \div (V \times C_{pr}) \times f$$

[Note]

ΔA_{412}: The absolute OD value of sample ($OD_{\text{Non-enzyme tube}}$-$OD_{\text{Enzyme tube}}$).

(0.23+V)/(0.03+V): Dilution factor of sample in enzymatic reaction.

0.1*: The volume of sample in definition.

V: The volume of sample added to the reaction system.

f: Dilution factor of sample before tested.

C_{pr}: Concentration of protein in sample (mgprot/mL).

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay (CV = Coefficient of Variation).

Parameters	Sample 1	Sample 2	Sample 3
Mean (U)	88.50	264.50	856.40
%CV	2.6	2.3	2.3

Intwe-assay Precision

Three human serum samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (U)	88.50	264.50	856.40
%CV	8.4	9.0	8.7

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 104%.

	Standard 1	Standard 2	Standard 3
Expected Conc. (μmol/L)	15.5	43.5	74.3
Observed Conc. (μmol/L)	15.7	46.1	78.0
Recovery rate (%)	101	106	105

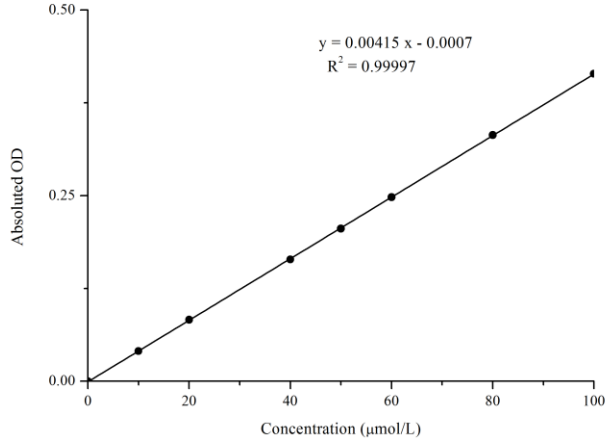
Sensitivity

The analytical sensitivity of the assay is 34.34 U. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

2. Standard curve:

As the OD value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), so the standard curve and data are provided as below for reference only:

Concentration (μmol/L)	**0**	**10**	**20**	**40**	**50**	**60**	**80**	**100**
Average OD	0.068	0.109	0.151	0.232	0.274	0.316	0.400	0.482
Absoluted OD	0	0.041	0.083	0.164	0.206	0.248	0.332	0.414



Appendix II Example Analysis

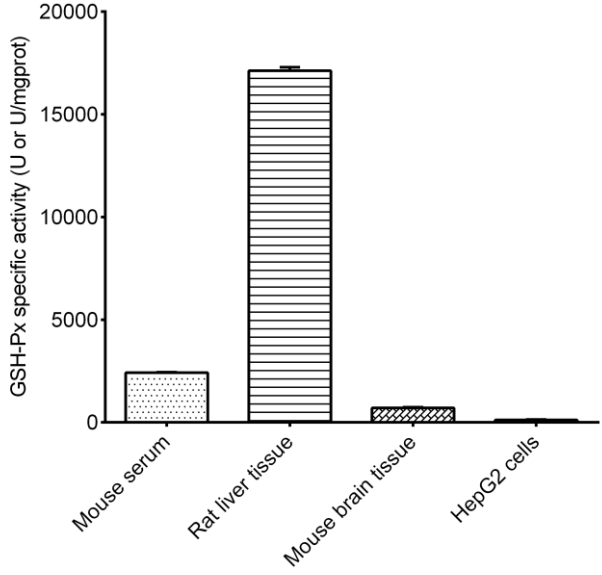
Example analysis:

Dilute mouse serum with normal saline (0.9% NaCl) for 4 times, take 20 μL of diluted sample and carry the assay according to the operation steps. The results are as follows:

Standard curve: y= 0.00415x-0.0007, the average OD value of the non-enzyme well is 0.381, the average OD value of the enzyme well is 0.263, and the calculation result is:

$$\text{GSH-Px activity (U)} = (0.381 - 0.263 + 0.0007) \div 0.00415 \times 5 \times 5 \times 4 = 2860.24\ \text{U}$$

Detect mouse serum (dilute for 4 times), 10% rat liver tissue homogenate (the concentration of protein in sample is 14.05 mgprot/mL, dilute for 30 times), 10% mouse brain tissue homogenate (the concentration of protein in sample is 4.20 mgprot/mL), HepG2 cells (the concentration of protein in sample is 5.02 mgprot/mL) according to the protocol, the result is as follows:



Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.