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

Catalog No：E-BC-K096-S

Specification：50 assays(24 samples)/100 assays(48 samples)

Measuring instrument：Spectrophotometer（412 nm）

Detection range：12.65-387 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.

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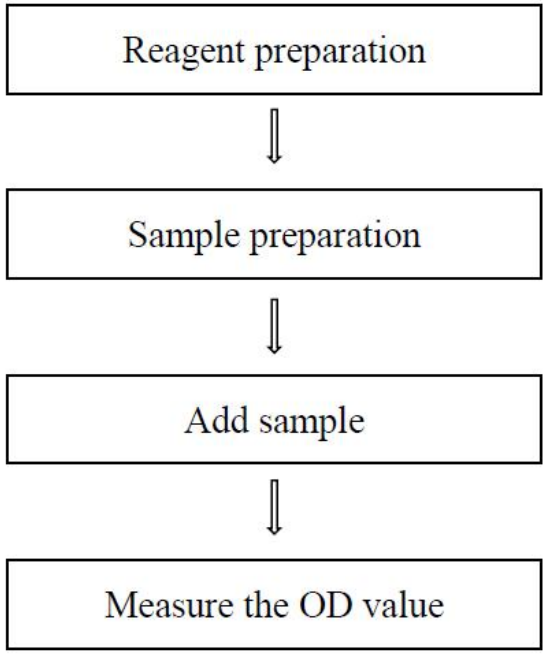
Assay summary

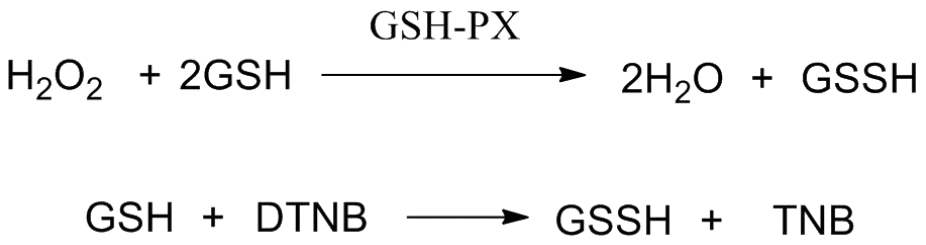


Intended use

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.



Kit components & storage

Item	Component	Size 1 (50 assays)	Size 2 (100 assays)	Storage
Reagent 1	Stock Solution	1 mL × 1 vial	2 mL × 1 vial	2-8℃, 12 months
Reagent 2	Acid Reagent	60 mL× 2 vials	60 mL× 4 vials	2-8℃, 12 months
Reagent 3	Phosphate	Powder×1 vial	Powder ×2 vials	2-8℃, 12 months
Reagent 4	DTNB Solution	15 mL× 1 vial	30 mL× 1 vial	2-8℃, 12 months, shading light
Reagent 5	Salt Reagent	Powder×2 vials	Powder×4 vials	2-8℃, 12 months, shading light
Reagent 6	GSH Standard	3.07 mg × 1 vial	3.07 mg × 2 vials	2-8℃, 12 months
Reagent 7	GSH Standard Stock Diluent	3 mL× 1 vial	6 mL× 1 vial	2-8℃, 12 months

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:

Spectrophotometer (412 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：

For each well， prepare 100 μL of stock application solution (mix well 1 μL of stock solution and 99 μL of double distilled water). The stock application solution should be prepared on spot. Store at 2-8°C for 12 h.

③ The preparation of phosphate application solution：

Dissolve one vial of phosphate with 60 mL of double distilled water, mix well to dissolve. Store at 2-8°C for 6 months. The phosphate application solution is saturated solution, take the supernatant for experiment if the crystal is appeared.

④ The preparation of salt application solution：

Dissolve one vial of salt reagent with 10 mL of double distilled water, mix well to dissolve. Store at 2-8°C for 1 month protected from light.

⑤ The preparation of GSH standard stock diluent application solution：

Before testing, please prepare sufficient GSH standard stock diluent application solution. For example, prepare 12 mL of GSH standard stock diluent application solution (mix well 1.2 mL of GSH standard stock diluent and 10.8 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°C.

⑥ 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. Aliquoted storage at -20°C for 1 month.

⑦ The preparation of 20 μmol/L GSH standard solution：

For each well，prepare 1000 μL of 20 μmol/L GSH standard solution (mix well

20 μL of 1 mmol/L GSH standard solution and 980 μL of GSH standard stock diluent application solution). The 20 μmol/L GSH standard solution should be prepared on spot.

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 30 mg tissue in 270 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 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).

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 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 20-60%, the optimal inhibition ratio is 45-55%. When the inhibition ratio is 45-55%, the corresponding sampling dilution factor is the optimal sampling dilution factor. If inhibition ratio > 60%, need to dilute the sample or decrease the sampling volume than take the test. If inhibition ratio < 20%, 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	1-3
Mouse plasma	4-8
Rat serum	5-8
10% Mouse liver tissue homogenization	20-40

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 45%~45%.

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

Operating steps

Enzymatic reaction

① Non-enzyme tube: take 0.2 mL of 1 mmol/L GSH standard solution into 5 mL EP tube.

Enzyme tube: take 0.2 mL of 1 mmol/L GSH standard solution, A* mL of sample into 5 mL EP tube and mix fully.

(For serum or plasma, A* is 0.1 mL. For tissue, cell, cell culture supernatant, A* is 0.2 mL.)

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

③ Add 0.1 mL of stock application solution to the tubes and mix fully. React at 37 ℃ for 5 min accurately.

④ Non-enzyme tube: add 2 mL of acid reagent and A* mL of sample to the tubes.

Enzyme tube: add 2 mL of acid reagent to the tubes.

⑤ Mix fully with a vortex mixer and centrifuge at 3100×g for 10 min, and take 1 mL of the supernatant for chromogenic reaction. (If the supernatant contains some sediment, transfer the supernatant to a new EP tube and centrifuge again).

Chromogenic reaction

① Non-enzyme well: Take 1 mL of supernatant of Non-enzyme tubes to 5 mL EP tube.

Enzyme well: Take 1 mL of supernatant of Enzyme tubes to 5 mL EP tube.

Blank wells: Take 1 mL of GSH standard application solution to 5 mL EP tube.

Standard wells: Take 1 mL of 20 μmol/L GSH standard solution to 5 mL EP tube.

② Add 1 mL of phosphate application solution, 0.25 mL of DTNB solution, 0.05 mL of salt application solution to each tube.

③ Mix fully and stand for 15 min at room temperature. Set the spectrophotometer

to zero with double distilled water and measure the OD values of each tube at 412 nm with 1 cm optical path cuvette.

Calculation

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

$$\text{GSH-Px activity (U)} = \frac{\Delta A_1}{\Delta A_2} \times c \times f_1 \times f$$

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.

$$\text{GSH-Px activity (U/mgprot)} = \frac{\Delta A_1}{\Delta A_2} \times c \times f_2 \times f \div (V \times C_{pr})$$

3. Cell culture supernatant sample:

Definition: The amount of GSH-PX in 0.1 mL of cell culture supernatant 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.

$$\text{GSH-Px activity (U)} = \frac{\Delta A_1}{\Delta A_2} \times c \times f_2 \times f \div 2^*$$

[Note]

ΔA_1: $OD_{\text{Non-enzyme tube}}$-$OD_{\text{Enzyme tube}}$.

ΔA_2: OD_{Standard}-OD_{Blank}.

c: the concentration of standard, 20 μmol/L.

f: dilution factor of sample before tested.

f_1: dilution factor of serum/plasma in enzymatic reaction, 6 times.

f_2: dilution factor of tissue, cells or cell culture supernatant in enzymatic reaction, 5 times.

*: the volume of cell culture supernatant in the definition is 0.1 mL and the volume of cell culture supernatant in operation step is 0.2 mL.

V: the volume of sample added into the reaction, mL.

C_{pr}: the 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)	24.60	135.70	264.50
%CV	5.3	4.8	4.6

Inter-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)	24.60	135.70	264.50
%CV	9.8	9.4	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 105%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (U)	75.5	184.2	244.5
Observed Conc. (U)	77.0	198.9	256.7
recovery rate (%)	102	108	105

Sensitivity

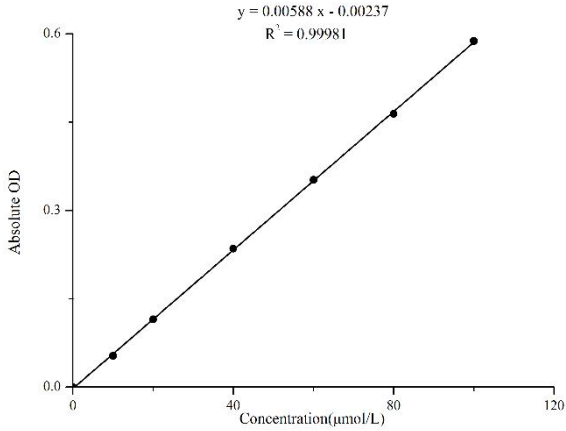
The analytical sensitivity of the assay is 12.65 U. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

2. Standard curve:

(It doesn't need to prepare the standard curve for this kit and the provided standard curve is for reference only)

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**	**60**	**80**	**100**
Average OD	0.072	0.125	0.187	0.307	0.424	0.536	0.660
Absoluted OD	0	0.053	0.115	0.235	0.352	0.464	0.588



Appendix II Example Analysis

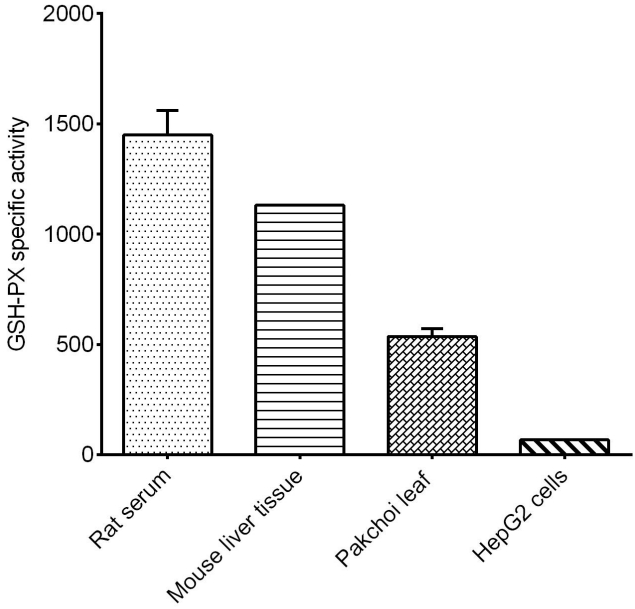
Example analysis：

Take 0.2 mL of 2% pakchoi leaf tissue homogenate, carry the assay according to the operation steps. The results are as follows:

The average OD value of the blank is 0.043, the average OD value of the standard is 0.154, the average OD value of the non-enzymatic tube is 0.461, the average OD value of the enzymatic tube is 0.277, the concentration of standard is 20 μmol/L, the concentration of 2% protein homogenate in sample is 1.56 mgprot/mL, and the calculation result is:

$$\text{GSH-Px acitivity (U/mgprot)} = \frac{0.461-0.277}{0.154-0.043} \times 20 \times 5 \div (0.2 \times 1.556) = 533 \text{ U/mgprot}$$

Detect rat serum (dilute for 8 times, A*= 0.1 mL), 5% mouse liver tissue homogenate (the concentration of protein in sample is 8.34 mgprot/mL, dilute for 10 times, A*= 0.2 mL), 2% pakchoi leaf tissue homogenate (the concentration of protein in sample is 1.56 mgprot/mL, A*= 0.2 mL), HepG2 cells (the concentration of protein in sample is 3.26 mgprot/mL, A*= 0.2 mL) according to the protocol, the result is as follows：


2000
1500
1000
500
0
GSH-PX specific activity
Rat serum
Mouse liver tissue
Pakchoi leaf
HepG2 cells

Appendix III Publications

1. Wang J, Pu X, Zhuang H, et al. Astragaloside IV alleviates septic myocardial injury through DUSP1-Prohibitin 2 mediated mitochondrial quality control and ER-autophagy[J]. Journal of Advanced Research, 2024.
2. Lim T W, Lim R L H, Pui L P, et al. Studies on the antioxidant mechanisms of betacyanins from improved fermented red dragon fruit (Hylocereus polyrhizus) drink in HepG2 cells[J]. Sustainable Materials and Technologies, 2024, 41: e01086.
3. Dumitraş D A, Dreanca A I, Pall E, et al. Inhibition of tumor growth and modulation of antioxidant activity of rhodoxanthin isolated from Taxus baccata aril against B16F10 murine malignant melanoma[J]. Antioxidants, 2022, 11(11): 2264.
4. Li Y, Yang F, Liu J, et al. Protective effects of sodium butyrate on fluorosis in rats by regulating bone homeostasis and serum metabolism[J]. Ecotoxicology and Environmental Safety, 2024, 276: 116284.
5. Zhao Y, Wang T, Li P, et al. Bacillus amyloliquefaciens B10 can alleviate aflatoxin B1-induced kidney oxidative stress and apoptosis in mice[J]. Ecotoxicology and Environmental Safety, 2021, 218: 112286.
6. Wanas H, Elbadawy H M, Almikhlafi M A, et al. Combination of niclosamide and pirfenidone alleviates pulmonary fibrosis by inhibiting oxidative stress and MAPK/Nf-κB and STATs regulated genes[J]. Pharmaceuticals, 2023, 16(5): 697.

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.