Glutathione Peroxidase (GSH-Px) Activity Assay Kit

Catalog No: E-BC-K096-S

Method: Colorimetric method

Specification: 100 Assays (Can detect 48 samples without duplication)

Instrument: Spectrophotometer

Sensitivity: 12.65 U

Detection range: 12.65-387 U

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

General information

▲Intended use

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

▲Background

Glutathione Peroxidase (GSH-Px) is an important enzyme that catalyzes decomposition of hydrogen peroxide. GSH specifically catalyze the reaction between GSH and hydrogen peroxide, protecting cell membrane structure and keeping membrane function integrity. Se-cysteine is the active center of the GSH-Px. Determination of GSH-Px activity in organism can be an indicator of selenium level as Se is essential section of GSH-Px.

$$ROOH + 2GSH \xrightarrow{\text{GSH-Px}} GSSG + ROH + H_2O$$

▲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.

$$H_2O_2 + 2GSH \xrightarrow{\text{GSH-Px}} 2H_2O + GSSG$$

$$GSH + DTNB \longrightarrow GSSG + TNB$$

▲Kit components & storage

Item	Component	Specification	Storage
Reagent 1	Stock Solution	2 mL × 1 vial	2-8℃，6 months
Reagent 2	Acid Reagent	60 mL× 4 vials	2-8℃，6 months
Reagent 3	Phosphate	Powder × 2 vials	2-8℃，6 months
Reagent 4	DTNB Solution	30 mL× 1 vial	2-8℃，6 months, shading light
Reagent 5	Salt Reagent	Powder × 4 vials	2-8℃，6 months, shading light
Reagent 6	GSH Standard	3.07 mg × 2 vials	2-8℃，6 months
Reagent 7	GSH Standard Stock Diluent	6 mL × 1 vial	2-8℃，6 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.

▲Materials prepared by users

Instruments

Spectrophotometer (412 nm), Micropipettor, Incubator, Vortex mixer, Centrifuge

Consumptive material

Tips (10 μL, 200 μL, 1000 μL), EP tubes (1.5 mL, 2 mL, 5 mL, 10 mL)

Reagents:

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

▲Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the skin and clothing. Wash immediately with plenty of water if touching it carelessly. All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

▲Precautions

Before the experiment, please read the instructions carefully, and wear gloves and work clothes.

▲The key points of the assay

1. The supernatant after centrifugation after adding reagent 2 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%~55%.
3. Reagent 1 application solution should be preheated at 37℃ for 5 min in advance.

Pre-assay preparation

▲ Reagent preparation

1. Reagent 1 application solution
   Dilute the reagent 1 with double distilled water for 100 times. Prepare the fresh solution before use. The prepared solution can be stored at 2-8℃ for 3 days.
2. Reagent 3 application solution
   Dissolve a vial of reagent 3 with 60 mL double distilled water. The prepared solution is saturated solution, take the supernatant for experiment if the crystal is appeared.
3. Reagent 5 application solution
   Dissolve a vial of powder with 10 mL double distilled water. The prepared solution can be stored at 2-8℃ for 1 month with shading light.
4. GSH standard application solution
   Dilute the reagent 7 with double distilled water for 10 times. Prepare the fresh solution before use. If the reagent 7 is formed into ice, please dissolve it at 65°C.
5. 1 mmol/L GSH standard solution
   Dissolve a vial of reagent 6 with GSH standard application solution to a final volume of 10 mL before use and mix fully. Prepare the fresh solution before use. The prepared solution can be stored at 2-8℃ for 3 days.
6. 20 μmol/L GSH standard solution
   Dilute 1 mmol/L GSH standard solution with GSH standard application solution for 50 times. Prepare the fresh solution before use.

▲ Sample preparation

The samples should be prepared as conventional methods. Also please refer to appendix II.

Sample requirements

The samples should not contain SDS, Tween20, NP-40, Triton X-100 and other detergents, and should not contain DTT, 2-merhydryl ethanol and other reducing reagents.

Determination of optimal sampling volume

1. The optimal sampling volume are different for different species, it is recommended to take 2~3 samples to do a pre-experiment to determining optimal sampling volume before formal experiment.
2. 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 volume is the optimal sampling volume.

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

3. 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.

Assay protocol	
Ambient temperature	25-30°C
Optimum detection wavelength	412 nm

Instructions for the use of transferpettor:

(1) Equilibrate the pipette tip in that reagent before pipetting each reagent.

(2) Don't add the liquid outside the tips into the reaction system when pipetting each reagent.

Assay protocol

▲Operating steps

1. 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 reagent 1 application solution at 37℃ for 5 min at the same time.

(3) Add 0.1 mL of reagent 1 application solution to the tubes and mix fully. React at 37 ℃ for 5 min accurately.

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

2. Chromogenic reaction

(1) Non-enzyme tube: Take 1 mL of supernatant of Non-enzyme tubes to 5 mL EP tube.
Enzyme tube: Take 1 mL of supernatant of Enzyme tubes to 5 mL EP tube.
Blank tube: Take 1 mL of GSH standard application solution to 5 mL EP tube.
Standard tube: Take 1 mL of 20 μmol/L GSH standard solution to 5 mL EP tube.

(2) Add 1 mL of Reagent 3 application solution, 0.25 mL of Reagent 4, 0.05 mL of Reagent 5 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.

▲Operation table

1. Enzymatic reaction

	Non-enzyme tube	Enzyme tube
1 mmol/L GSH standard solution (mL)	0.2	0.2
Sample (mL)		A*
Preheat the tubes at 37℃ water bath for 5 min (Preheat reagent 1 application solution at 37℃ for 5 min at the same time).		
Reagent 1 application solution (mL)	0.1	0.1
React at 37℃ for 5 min accurately.		
Reagent 2 (mL)	2	2
Sample (mL)	A*	
Mix fully with vortex mixer, centrifuge at 3100 g for 10 min, and take 1 mL of the supernatant for chromogenic reaction.		

2. Chromogenic reaction

	Blank tube	Standard tube	Non-enzyme tube	Enzyme tube
GSH standard application solution (mL)	1			
20 μmol/L GSH standard solution (mL)		1		
Supernatant (mL)			1	1
Reagent 3 application solution (mL)	1	1	1	1
Reagent 4 (mL)	0.25	0.25	0.25	0.25
Reagent 5 application solution (mL)	0.05	0.05	0.05	0.05
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

1. Serum (plasma) and other liquid 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.

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

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.

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

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.

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

Note:

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

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.

▲Notes

1. This kit is for research use only.
2. Instructions should be followed strictly, changes of operation may result in unreliable results.
3. The validity of kit is 6 months.
4. Do not use components from different batches of kit.

Appendix I Performance characteristics

Appendix I Performance characteristics			
Detection range	12.65-387 U	Average intra-assay CV (%)	4.9
Sensitivity	12.65 U	Average inter-assay CV (%)	9.3
Average recovery rate (%)	105		

▲Inter-assay CV

Take three kits of different batches to detect the samples of high concentration, middle concentration and low concentration respectively. The concentrations should be within the detection range. The samples of each concentration were tested 3 times (n=3) parallelly with each kit. Then calculate the corresponding values according to the following formula. The average Inter-assay CV is 9.3%.

$$\overline{x}_T = \frac{\overline{x}_1 + \overline{x}_2 + \overline{x}_3}{3}$$

$$R = \frac{\overline{x}_{max} - \overline{x}_{min}}{\overline{x}_T} \times 100\%$$

$$\overline{R} = \frac{R_1 + R_2 + R_3}{3} \quad (n=3)$$

$\overline{x}_{max}$--- The max values of $\overline{x}_i$

$\overline{x}_{min}$--- The min values of $\overline{x}_i$

$\overline{x}_T$---The average values of $\overline{x}_i$

R_i---The value of each batch number kit

▲Intra-assay CV

Take one kit to detect the samples of high concentration, middle concentration and low concentration respectively. The concentrations should be within the detection range. The samples of each concentration were tested 6 times (n=6) parallelly, and the average Intra-assay CV is 4.9%, which was calculated according to the following formula.

$$CV = \frac{S}{\bar{x}} \times 100\%$$

S--- Standard deviation

▲Sensitivity

The volume of sample added to reaction is 0.1 mL, 20 non-enzymatic tubes were measured according to the operation table. Calculate the three standard deviations of OD value for non-enzymatic tubes (ΔA_1) according to the formula of IUPAC, calculate the sensitivity according to the formula in calculation is 12.65 U.

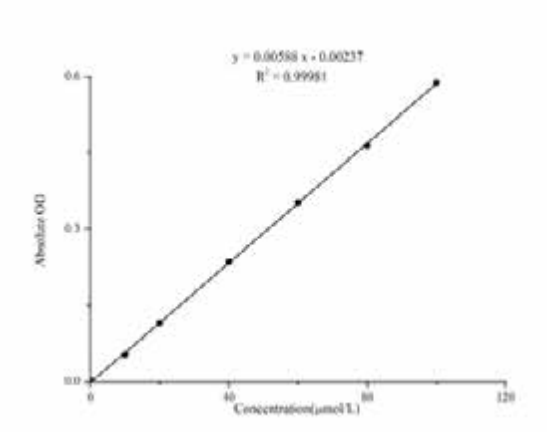
▲Recovery rate

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 3 times parallelly to get the average recovery rate of 105%.

▲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



▲Example analysis

Take 0.2 mL of 2% pakchoi leaf tissue homogenate, carry the assay according to the operation table.

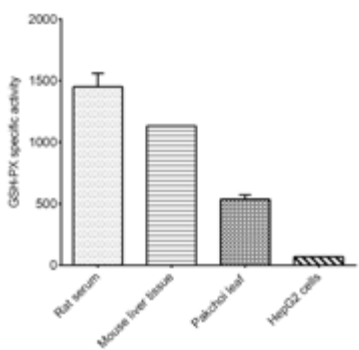
The results are as follows:

TThe 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:

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

$$= 533 \text{ U/mgprot}$$

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:



Appendix II Sample preparation

The following sample pretreatment methods are for reference only.

▲Serum

Collect fresh blood and stand at 25℃ for 30 min to clot the blood. Then centrifuge at 2000 g for 15 min at 4℃ . Take the serum (which is the upper light yellow clarified liquid layer) to preserve it on ice for detection. If not detected on the same day, the serum can be stored at -80℃ for a month.

▲Plasma

Take fresh blood into the tube which has anticoagulant (heparin is recommended), centrifuge at 1000-2000 g for 10 min at 4℃ . Take the plasma (which is the upper light yellow clarified liquid layer, don't take white blood cells and platelets in the middle layer) to preserve it on ice for detection. If not detected on the same day, the plasma can be stored at -80℃ for a month.

▲Cell culture supernatant

Detect directly. If there is turbidity, centrifuge at 3100 g for 10 min. Take the supernatant to preserve it on ice for detection. If not detected on the same day, it can be stored at -80℃ for a month.

▲Tissue sample

Take 0.02-1g fresh tissue to wash with PBS (0.01 M, pH 7.4) at 2-8℃ . Absorb the water with filter paper and weigh. Homogenize at the ratio of the volume of homogenized medium (2-8℃) (mL): the weight of the tissue (g) =9:1, then centrifuge the tissue homogenate for 10 min at 10000 g at 4℃ . Take the supernatant to preserve it on ice for detection. Meanwhile, determine the protein concentration of supernatant (E-BC-K168-S). If not detected on the same day, the tissue sample (without homogenization) can be stored at -80℃ for a month.

▲Cells

Collect the cells (For adherent cells, the cell scraper rather than trypsin is recommended) and wash the cells with PBS (0.01 M, pH 7.4) for 1~2 times. Centrifuge at 1000 g for 10 min and then discard the supernatant and keep the cell sediment. Add homogenization medium at a ratio of cell number (10^6): homogenization medium (μL) =1: 300-500. Sonicate or grind with hand-operated in ice water bath. Centrifuge at 1500 g for 10 min, then take the supernatant and preserve it on ice for detection. Meanwhile, determine the protein concentration of supernatant (E-BC-K168-S). If not detected on the same day, the cells sample (without homogenization) can be stored at -80℃ for a month.

Note:

1. Homogenized medium: 10 mM Tris-HCl (pH 7.4), including 10 mM NaCl, 10 mM sucrose, 0.1 mM EDTA.

2. Homogenized method:

(1) Hand-operated: Weigh the tissue and mince to small pieces (1 mm^3), then put the tissues pieces to glass homogenized tube. Add homogenized medium into homogenized tube, place the tube into the ice bath with left hand, and insert the glass tamping rod vertically into the homogenized tube with the right hand to grind up and down for 6-8 min.

Or put the tissue into the mortar, and add liquid nitrogen to grind fully. Then add the homogenized medium to homogenize.

(2) Mechanical homogenate: Weigh the tissue to EP tube, add the homogenized medium to homogenize the tissue with homogenizer instrument (60 Hz, 90s) in the ice bath. (For samples of skin, muscle and plant tissue, the time of homogenization can be properly prolonged.)

(3) Ultrasonication: Treat the cells with ultrasonic cell disruptor (200 W, 2 s/time, interval for 3 s, the total time is 10 min).

▲ Note for sample

1. If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.
2. If a lysis buffer is used to prepare tissue or cell homogenates, there is a possibility of causing a deviation due to the introduced chemical substance.

Appendix III References

1. Mannervik B. Glutathione Peroxidase. Methods Enzymol, 1985, 77(5): 490-495
2. Ursini F, Maiorino M. Glutathione Peroxidases.Bioenergetics, 2013,399-404.
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4. Mohammedi K, Patente T A, Bellili-Munoz N, et al. Glutathione peroxidase-1 gene (GPX1) variants, oxidative stress and risk of kidney complications in people with type 1 diabetes. Metabolism-clinical & Experimental, 2016, 65(2): 12-19.
5. Lubos E, Loscalzo J, Handy D E. Glutathione peroxidase-1 in health and disease: from molecular mechanisms to therapeutic opportunities. Antioxidants & Redox Signaling , 2011, 15(7): 1957-1997.