

IK096-M Glutathione Peroxidase (GSH-Px) Activity Assay Kit

Method: Colorimetric method

Specification: 96T (Can detect 40 samples without duplication) Instrument: Microplate reader

Sensitivity: 34.34 U

Detection range: 34.34-1036.64 U

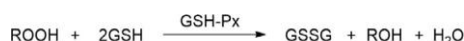
General information

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.



Detection principle

Item	Component	Specification	Storage
Reagent 1	Stock Solution	0.5 mL × 1 vial	2-8°C , 6 months
Reagent 2	Acid Reagent	50 mL × 1 vial	2-8°C , 6 months
Reagent 3	Phosphate	12 mL × 1 vial	2-8°C , 6 months
Reagent 4	DTNB Solution	7 mL × 1 vial	2-8°C , 6 months, shading light
Reagent 5	GSH Standard	3.07 mg × 1 vial	2-8°C , 6 months
Reagent 6	GSH Standard Stock Diluent	1.5 mL × 2 vials	2-8°C , 6 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.

Glutathione peroxidase (GSH-Px) can promote the reaction of hydrogen peroxide (H₂O₂) and reduced glutathione to produce H₂O 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 (H₂O₂) 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 412nm, and calculate the amount of GSH.



**▲ Kit components & storage****▲ Materials prepared by users****🔬 Instruments**

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

📦 Consumptive material

Tips (10 μ L, 200 μ L, 1000 μ L), EP tubes (1.5 mL, 2 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 25%~45%.
3. Reagent 1 application solution should be preheated at 37°C 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°C for 3 days.
2. Reagent 6 application solution:
Dilute the reagent 6 with double distilled water for 10 times. Prepare the fresh solution before use. If the reagent 6 is formed into ice, please dissolve it at 65°C. The prepared solution can be stored at 2-8°C for 7 days.
3. 1 mmol/L GSH standard solution:
Dissolve a vial of reagent 5 with reagent 6 application solution to a final volume of 10 mL before use and mix fully. Prepare fresh solution before use. The prepared solution can be stored at 2-8°C for 7 days.
4. 100 μ mol/L GSH standard solution:
Dilute 1 mmol/L GSH standard solution with reagent 6 application solution for 10 times and mix fully. Prepare fresh solution before use. The prepared solution can be stored at 2-8°C for 7 days.

▲ 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.

▲ Dilution of sample

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 10-50%, the optimal inhibition ratio is 25-45%. When the inhibition ratio is 25-45%, the corresponding sampling volume is the optimal sampling volume.

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

3. 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.

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)

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

▲ Plate set up

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S1	S1'	S9	S9'	S17	S17'	S25	S25'	S33	S33'
B	B	B	S2	S2'	S10	S10'	S18	S18'	S26	S26'	S34	S34'
C	C	C	S3	S3'	S11	S11'	S19	S19'	S27	S27'	S35	S35'
D	D	D	S4	S4'	S12	S12'	S20	S20'	S28	S28'	S36	S36'
E	E	E	S5	S5'	S13	S13'	S21	S21'	S29	S29'	S37	S37'
F	F	F	S6	S6'	S14	S14'	S22	S22'	S30	S30'	S38	S38'
G	G	G	S7	S7'	S15	S15'	S23	S23'	S31	S31'	S39	S39'
H	H	H	S8	S8'	S16	S16'	S24	S24'	S32	S32'	S40	S40'

Note: A-H, standard wells; S1-S40, Non-enzyme wells; S1'-S40', Enzyme wells.

▲ Operating steps

The preparation of standard curve

Dilute 100 µmol/L GSH standard solution with reagent 6 application solution to a serial concentration. The recommended dilution gradient is as follows: 0, 10, 20, 40, 50, 60, 80, 100 µmol/L.

The measurement of samples

1. 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°C water bath for 5 min. Preheat reagent 1 application solution at 37°C for 5 min at the same time.

3) Add 10 µL of reagent 1 application solution to the tubes and mix fully. React at 37 °C for 5 min accurately.

4) **Non-enzyme tube:** add 200 µL of reagent 2 and 20 µL of sample to the tubes.

Enzyme tube: add 200 µL of reagent 2 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.

2. Chromogenic reaction

1) **Non-enzyme wells:** Take 100 µL of supernatant of Non-enzyme tubes to the wells.

Enzyme wells: 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 reagent 3 to each well.

3) Add 50 µL of reagent 4 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.

▲ Operation table

1. Enzymatic reaction

	Non-enzyme tube	Enzyme tube
1mmol/L GSH (µL)	20	20
Sample (µL)		20
Preheat the tubes at 37°C water bath for 5 min (Preheat reagent 1 application solution at 37°C for 5 min at the same time).		
Reagent 1 application solution (µL)	10	10
React at 37°C for 5 min accurately.		
Reagent 2 (µL)	200	200
Sample (µL)	20	
Mix fully with a vortex mixer, centrifuge at 3100 g for 10 min, and take 100 µL of the supernatant for chromogenic reaction.		

2. Chromogenic reaction

	Standard well	Non-enzyme well	Enzyme well
Standard solution with different concentration(µL)	10 0		
Supernatant (µL)		10 0	10 0
Reagent 3 (µL)	10 0	10 0	10 0
Reagent 4 (µL)	50	50	50
Oscillate for 10 s at microplate reader and stand for 5 min. Measure the OD values at 412 nm with microplate reader.			

▲ Calculation

Plot the standard curve by using OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve with graph software (or EXCEL). The concentration of the sample can be calculated according to the formula based on the OD value of sample. If the sample have been diluted, the concentration calculated from the standard curve must be multiplied by the dilution factor. The actual concentration is the calculated concentration multiplied by dilution factor.

The standard curve is: $y = ax + b$.

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°C for 5 minute is defined as 1 unit.

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

xf

2. Tissue and cells ample:

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°C for 5 minute is defined as 1 unit.

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

Note:

y: The absolute OD value of standard; x: The concentration of standard;

a: The slope of standard curve.

b: The intercept of standard curve.

ΔA_{412} : The absolute OD value of sample (OD_{Non-enzyme tube} - OD_{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)

▲ 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	34.34-1036.64 U	Average intra-assay CV (%)	2.4
Sensitivity	34.34 U	Average inter-assay CV (%)	8.7
Average recovery rate (%)	104		

▲ 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 table. 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:

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

Appendix II Sample preparation

The following sample pretreatment methods are for reference only.

▲ Serum

Collect fresh blood and stand at 25°C for 30 min to clot the blood. Then centrifuge at 2000 g for 15 min at 4°C. 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°C for a month.

▲ Plasma

Take fresh blood into the tube which has anticoagulant (Heparin is recommended as anticoagulant), centrifuge at 1000-2000 g for 10 min at 4°C. 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°C 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°C for a month.

▲ Tissue

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

▲ Notes 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.