Technical Manual

Glutathione Peroxidase (GSH-Px) Activity Assay Kit

- Catalogue Code: MAES0084
- Size: 96T
- Research Use Only

1. Key features and Sample Types

Detection method:

Colorimetric method

Specification:

96T

Range:

34.34-1036.64 U

Sensitivity:

34.34 U

Storage:

2-8°C for 6 months

Expiry:

See Kit Label

Experiment Notes:

This kit is for **research use only.**

Instructions should be strictly followed. Changes of operation may result in unreliable results.

The validity of kit is 6 months.

Do not use components from different batches of kit.

2. 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.

3. Intended Use

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

4. 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 412nm, and calculate the amount of GSH.

5. Kit components & storage

Item	Specification	Storage
Stock Solution	0.5 mL × 1 vial	2-8°C, 6 months
Acid Reagent	50 mL× 1 vial	2-8°C, 6 months
Phosphate	12 mL × 1 vial	2-8°C, 6 months
DTNB Solution	7 mL× 1 vial	2-8°C, 6 months, avoid direct sunlight
GSH Standard	3.07 mg × 1 vial	2-8°C, 6 months
GSH Standard Stock Diluent	1.5 mL × 2 vials	2-8°C, 6 months
Microplate	96 wells	No requirement
Plate Sealer	2 pieces	

Materials required but not supplied

- Micropipettor
- Incubator
- Centrifuge
- Microplate Reader (400-420 nm)
- Tips (10 μL, 200 μL, 1000 μL)
- EP tubes (1.5 mL, 2 mL)
- Double distilled water
- Normal Saline (0.9% NaCl)
- PBS (0.01 M, pH 7.4)

6. Assay Notes:

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°C for 5 min in advance.

7. Reagent preparation:

1. Bring all reagents to room temperature before use.

2. **Stock application solution:** Dilute the stock solution 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.

3. **Standard diluent:** Dilute the GSH standard stock diluent with double distilled water for 10 times. Prepare the fresh solution before use. If the GSH standard stock diluent is formed into ice, please dissolve it at 65°C. The prepared solution can be stored at 2-8°C for 7 days.

4. **GSH standard solution (1 mmol/L):** Dissolve a vial of GSH standard with standard diluent 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. **GSH standard solution (100 μmol/L):** Dilute GSH standard solution (1 mmol/L) with standard diluent for 10 times and mix fully. Prepare fresh solution before use. The prepared solution can be stored at 2-8°C for 7 days.

8. Sample Preparation

1. Serum sample:
Fresh blood should be incubated at 25°C for 30 min to clot the blood. Centrifuge the sample 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.

2. Plasma sample:
Place the fresh blood sample into a tube of anticoagulant and centrifuge at 700-1000g 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.

3. Cell sample:
Collect the cells 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 (2×10^6): 10 mM Tris-HCl (pH 7.4), including 10 mM NaCl, 10 mM sucrose, 0.1 mM EDTA (μL) =1: 300. Sonicate the sample on an ice water bath. Centrifuge at 10000 g for 10 min, then take the supernatant and preserve it on ice for detection. If not detected on the same day, the cells sample (without homogenization) can be stored at -80°C for a month.

4. Tissue sample:
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 10 mM Tris-HCl (pH 7.4), including 10 mM NaCl, 10 mM sucrose, 0.1 mM EDTA (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. If not detected on the same day, the tissue sample (without homogenization) can be stored at -80°C for a month.

Sample Notes:

The Inhibition ratio can be detected by this kit is 20-60%, the optimal inhibition ratio is 45-55%.

If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.

Dilution of Samples:

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.

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

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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 homogenate	20-40

Note: The diluent is normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4);

9. Assay Protocol

Ambient Temperature: 25-30°C

Optimum detection wavelength: 412 nm

10. Operation Steps

The preparation of standard curve

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

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 stock application solution at 37°C for 5 min at the same time.

3. Add 10 μL of stock application solution to the tubes and mix fully. React at 37°C 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 reagnt 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 well: 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.

Operation Table

Enzymatic reaction

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

Chromogenic reaction

	Standard well	Non-enzyme well	Enzyme well
Standard solution with different concentration (μL)	100		
Supernatant (μL)		100	100
Phosphate (μL)	100	100	100
DTNB solution (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.			

11. Calculations

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

1. Serum (plasma) sample:

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

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

2. Tissue and cells sample:

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

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

y: $OD_{Standard} - OD_{Blank}$ (OD_{Blank} is the OD value when the standard concentration is 0).
x: The concentration of standard.
a: The slope of standard curve .
b: The intercept of standard curve.
(0.23+V)/(0.03+V): Dilution factor of sample in enzymatic reaction
0.1*: The volume of sample in definition
ΔA_{412}: $OD_{Non\text{-}enzyme\ tube} - OD_{Enzyme\ tube}$.
V: The volume of sample added to the reaction system
f: Dilution factor of sample before test.
C_{pr}: Concentration of protein in sample, mgprot/mL.

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12. Performance Characteristics

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

Analysis

Dilute mouse serum with normal saline (0.9% NaCl) for 4 times, take 20 μL of diluted sample, 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:

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

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Safety Notes

Some of the reagents in the kit contain dangerous substances. Prevent touching 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.

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

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Notes:

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