Technical Manual

Glutathione Peroxidase (GSH-Px) Activity Assay Kit

- Catalogue Code: MAES0085
- Size: 100 Assays
- Research Use Only

1. Key features and Sample Types

Detection method:

Colorimetric method

Specification:

100 Assays

Range:

12.65-387 U

Sensitivity:

12.65 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 (H2O2) 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	2 mL × 1 vial	2-8°C, 6 months
Acid Reagent	60 mL× 4 vials	2-8°C, 6 months
Phosphate	Lyophilized × 2 vials	2-8°C, 6 months
DTNB Solution	30 mL× 1 vial	2-8°C, 6 months, avoid direct sunlight
Salt Reagent	Lyophilized × 4 vials	2-8°C, 6 months, avoid direct sunlight
GSH Standard	3.07 mg × 2 vials	2-8°C, 6 months
GSH Standard Stock Diluent	6 mL × 1 vial	2-8°C, 6 months

Materials required but not supplied

- Micropipettor
- Incubator
- Centrifuge
- Spectrophotometer (412 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 12 h.

3. **Phosphate application solution:** Dissolve a vial of phosphate lyophilized with 60 mL double distilled water. The prepared solution can be stored at 2-8°C for 6 months. The prepared solution is saturated solution, take the supernatant for experiment if the crystal is appeared.

4. **Salt application solution:** Dissolve a vial of salt reagent with 10 mL double distilled water. The prepared solution can be stored at 2-8°C for 1 month with avoid direct sunlight.

5. **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.

6. **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 3 days.

7. **GSH standard solution (20 μmol/L):** Dilute GSH standard solution (1 mmol/L) with standard diluent for 50 times and mix fully. Prepare fresh solution before use.

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 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.

4. 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.

5. 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。 (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. If not detected on the same day, the tissue sample (without homogenization) can be stored at -80°C for a month.

Sample Notes:

The concentration should be determined before preforming the assay. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

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

Dilution of Samples:

Large variances in results may be seen when performing pre-experiments. Dilute the sample according to the result of the pre-experiment and the detection range (12.65-387 U).

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

Sample Type:	Dilution Factor
Human serum	1
293T supernatant	1
10% Rat heart tissue homogenization	50-100
10% Rat liver tissue homogenization	100-200
10% Rat spleen tissue homogenization	50-100
Mouse serum	1
10% Epipremnum aureum tissue homogenization	1-2
10% Rat lung tissue homogenization	50-100
10% Rat kidney tissue homogenization	50-100
10% Rat brain tissue homogenization	20-50

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

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, sample wells; S1'-S40', control wells.

10. Operation Steps

Enzymatic reaction

1. **Non-enzyme tube:** take 0.2 mL of 1 mmol/L GSH standard 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. 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 0.1 mL of stock application solution to the tubes and mix fully. React at 37°C 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 acid reagnt 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.

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 standard diluent to 5 mL EP tube.
   Standard tube: Take 1 mL20 μmol/L of 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.

Operation Table

Enzymatic reaction

	Non-enzyme tube	Enzyme tube
1 mmol/L GSH (mL)	0.2	0.2
Sample (mL)		A
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 (mL)	0.1	0.1
React at 37°C water bath for 5 min accurately.		
Acid reagent (mL)	2	2
Sample (mL)	A	
Mix fully and centrifuge at 3100 g for 10 min, then take 100 µL of supernatant for chromogenic reaction.		

Chromogenic reaction

	Blank tube	Standard tube	Non-enzyme tube	Enzyme tube
Standard diluent (mL)	1			
20 µmol/L GSH standard solution (mL)		1		
Supernatant (mL)			1	1
Phosphate application solution (mL)	1	1	1	1
DTNB solution (mL)	0.25	0.25	0.25	0.25
Salt application solution (mL)	0.05	0.05	0.05	0.05
Oscillate for 10 s at microplate reader and stand for 5 min. Measure the OD values at 412 nm with microplate reader.				

11. Calculations

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.

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

2. Tissue and cells sample:

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

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

3. Cell culture supernatant sample:

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

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

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

12. Performance Characteristics

Detection Range	12.65-387 U
Sensitivity	12.65 U
Average recovery rate (%)	105
Average inter-assay CV (%)	9.3
Average intra-assay CV (%)	4.9

Analysis

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

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:

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

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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