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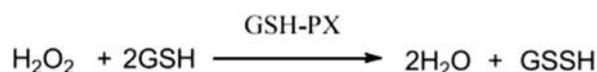
# **Glutathione Peroxidase (GSH-Px/GPX) Activity Assay Kit**

BC4403-01 (50 Tests/24 Samples)

**FOR RESEARCH USE ONLY, DO NOT USE IT IN CLINICAL DIAGNOSIS**

## Product Description

Glutathione Peroxidase (GSH-Px) can promote the reaction of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and reduced glutathione to produce H<sub>2</sub>O 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<sub>2</sub>O<sub>2</sub>) 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

Reagent	Volume	Storage
Extract Solution	30 mL	4°C
Reagent I	5 mL	4°C
Reagent II	20 µL	4°C
Reagent III	60 mL	4°C
If crystals are found in the bottom of the bottle, warm in a 50°C water bath till it dissolves. If crystals persists after heating, the supernatant can be used for the assay.		
Reagent IV	50 mL	4°C
If crystals are found in the bottom of the bottle, warm in a 40°C water bath till it dissolves.		
Reagent V	15 mL	4°C
Standard (10 mg reduced glutathione)	Powder	4°C
Add 0.405mL Diluent to get a standard concentration of 80 µmol/mL.		
Diluent	5 mL	4°C

## Reagents and Equipment Required but Not Provided

Constant temperature water bath, cooling centrifuge, spectrophotometer, 1.5ml microcentrifuge tube, 1ml glass cuvette and distilled water.

## Protocol

### I. Sample preparation

**Tissue:** Add 1mL Extract Solution to 0.05g tissue. Homogenate in ice and centrifuge at 5000 rpm at 4°C for 10 minutes. Take the supernatant and place it on ice for assay. (If the supernatant is not clear, centrifuge for 3 minutes more).

**Bacteria or cells:** Add 1mL Reagent I to 5 million cells. Subject to ultrasonication while keeping the samples in an ice bath (power 300W, sonication 3 seconds, interval 7 seconds for 3 minutes). Centrifuge at 5000 rpm at 4°C for 10 minutes. Take the supernatant and place it on ice for assay. (If the supernatant is not clear, centrifuge for 3 minutes more).

**Serum:** Use directly.

## II. Assay procedure

- Preparation of Reagent I working solution: Dilute Reagent I with Diluent in the ratio 1:1. Prepare according to the number of samples to be assayed.
- Preparation of Reagent II working solution: Dilute 3  $\mu\text{L}$  Reagent II with 10 mL distilled water. Prepare according to the number of samples to be assayed.
- Preheat the spectrophotometer for 30 minutes, adjust wavelength to 412 nm and set zero with distilled water.
- Dilute the Standard solution (80  $\mu\text{mol/mL}$ ) to 0.08  $\mu\text{mol/mL}$  with Diluent.
- Perform the subsequent assay in a 1.5ml micro centrifuge tube. Add the reagents in the order as mentioned in the table

Reagent	Test tube (T)	Control tube (C)
Sample (supernatant/serum)	100 $\mu\text{L}$	-
Reagent I working solution	100 $\mu\text{L}$	100 $\mu\text{L}$
Incubate for 5 minutes at 37°C		
Reagent II working solution	50 $\mu\text{L}$	50 $\mu\text{L}$
Incubate for 5 minutes at 37°C		
Reagent III	1000 $\mu\text{L}$	1000 $\mu\text{L}$
Sample	-	100 $\mu\text{L}$

- Centrifuge at 4000rpm at room temperature for 5 minutes and transfer the supernatant into a new 1.5mL micro centrifuge tube.
- Continue the assay as given in the table below

Reagent	Test tube (T)	Control tube (C)	Standard tube (S)	Blank tube (B)
Diluent	-	-	-	500 $\mu\text{L}$
Supernatant	500 $\mu\text{L}$	500 $\mu\text{L}$	-	-
Working standard	-	-	500 $\mu\text{L}$	-
Reagent IV	500 $\mu\text{L}$	500 $\mu\text{L}$	500 $\mu\text{L}$	500 $\mu\text{L}$
Reagent V	125 $\mu\text{L}$	125 $\mu\text{L}$	125 $\mu\text{L}$	125 $\mu\text{L}$

- Well mix. then placed at room temperature for 15 minutes, the absorbance at 412 nm is measured. The absorbance is recorded as  $A_T$ ,  $A_C$ ,  $A_S$  and  $A_B$ , respectively

$$\Delta A_T = A_C - A_T$$

$$\Delta A_S = A_S - A_B.$$

## Calculation

### 1. Calculation of inhibition percentage:

$$\text{Inhibitory percentage} = (A_C - A_T) / (A_C - A_B) \times 100\%$$

It is ideal to keep the inhibition percentage of the sample is within the range of 30 – 70%, and the closer it is to 50%, the more accurate it is. If inhibition percentage is less than 30% or more than 70%, it is usually necessary to adjust sample volume or sample preparation. If inhibition percentage is high, the sample should be diluted properly. If inhibition percentage is low, the sample with high concentration should be prepared again.

## 2. Calculation of GPx activity:

### a) Protein concentration

Unit definition: One unit of enzyme is defined as the amount of enzyme which catalyzes the oxidation of 1nmol of GSH in reaction system per minute for every milligram of protein.

$$\text{GPx (U/mg protein)} = \Delta A_T \div (\Delta A_S \div C_S) \times 1000 \times V_{EV} \div (C_{pr} \times V_{SV}) \div T$$
$$= 200 \times \Delta A_T \div \Delta A_S \div C_{pr}$$

### b) Sample weight:

Unit definition: One unit of enzyme is defined as the amount of enzyme which catalyzes the oxidation of 1nmol of GSH in reaction system per minute for every gram of sample.

$$\text{GPx (U/g weight)} = \Delta A_T \div (\Delta A_S \div C_S) \times 1000 \times V_{EV} \div (V_{SV} \div V_{TV} \times W) \div T$$
$$= 200 \times \Delta A_T \div \Delta A_S \div W$$

### c) Cells

Unit definition: One unit of enzyme is defined as the amount of enzyme which catalyzes the oxidation of 1nmol of GSH in reaction system per minute for every 10<sup>4</sup> cells.

$$\text{GPx (U/10}^4 \text{ cells)} = \Delta A_T \div (\Delta A_S \div C_S) \times 1000 \times V_{EV} \div (N \times V_{SV} \div V_{TV}) \div T$$
$$= 200 \times \Delta A_T \div \Delta A_S \div N$$

### d) Liquids (Serum/Plasma)

Unit definition: One unit of enzyme is defined as the amount of enzyme which catalyzes the oxidation of 1nmol of GSH in reaction system per minute for every millilitre of liquid.

$$\text{GPx (U/mL)} = \Delta A_T \div (\Delta A_S \div C_S) \times 1000 \times V_{EV} \div V_S \div T$$
$$= 200 \times \Delta A_T \div \Delta A_S$$

C<sub>S</sub> : Concentration of standard mixtures, 0.08 µmol/mL

V<sub>EV</sub>: Volume of enzymatic reaction system, 1.25mL

V<sub>SV</sub>: Sample volume contained in sample mixtures, 0.1 mL

V<sub>TV</sub>: Extraction solution volume, 1 mL

C<sub>pr</sub> : Supernatant protein concentration, mg/mL

T : Reaction time, 5 minutes

N : The amount of cells, tens of thousands

W : Sample weight, g

1000: 1 µmol=1000 nmol

## Note

- If initial absorbance is greater than 1.2, dilute the sample with Extract Solution and repeat the assay.
- It is recommended not to perform assay with too many samples, as increase in incubation time will result in inaccurate results.