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Ver.240707

Glutathione Peroxidase (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 (glutathione peroxidase, GSH-Px or GPX) is an important peroxidase widely existed in the body. GPX can catalyzes the formation of oxidized glutathione (GSSG) from reduced glutathione (GSH) and reduce toxic hydrogen peroxide to non-toxic hydroxyl compounds. GPX catalyzes the oxidation of GSH by hydrogen peroxide to produce GSSG. GSH can react with DTNB to form compounds with characteristic absorption peaks at 412 nm. The decrease of absorbance at 412 nm can reflect the activity of GPX.

Kit components

Reagent	Volume	Storage
Extract solution	30mL× 1	2-8°C
Reagent I	Powder×2	2-8°C
Add 1.65 mL of distilled water to dissolve when the solution will be used. Store for 2 weeks at 2-8°C.		
Preparation of Reagent I working solution : Before use, the samples is prepared according to the ratio of reagent I : diluent = 1 : 1 according to the number of samples.		
Reagent II	10 µL×1	2-8°C
Preparation of Reagent II working solution : Dilute reagent II with the ratio of 2µL reagent II and 10 mL distilled water before use.		
Reagent III	60mL× 1	2-8°C
If the bottom of the bottle is crystallized, it can be dissolved in water bath at 50°C. This solution is a saturated solution. If the bottom of the bottle is still crystallized, the supernatant can be absorbed and used.		
Reagent IV	30 mL×1	2-8°C
Reagent V	10mL×1	2-8°C
Standard	Powder×1 10 mg reduced glutathione (GSH).	2-8°C
Add 0.405 mL of distilled water to the standard solution of 80 µmol/mL when the solution will be used.		
Diluent	4 mL×1	2-8°C

Reagents and Equipment Required but Not Provided

Spectrophotometer, balance, table centrifuge, 1 mL glass cuvette, mortar/homogenizer, EP tube

Procedure

I. Sample preparation:

1. **Tissue:** Accordance ratio Tissue weight (g): Extract solution (mL)=1:5~10 (Suggested 0.05g of tissue with 1mL of Extract solution), homogenate on ice bath. Centrifuge at 5000 rpm at 4°C for 10 minutes, take the supernatant and place it on ice for test (If the supernatant is not clear, centrifuge for 3 minutes)

2. **Bacteria or cells :** Amount of cells (10^4): Extract solution (mL): 500~1000:1(Add 1mL of Extract solution to 5 million cells), ultrasonic with ice bath to break cells (300W,3second, interval 7second,total time 3 minutes). Centrifuged at 5000 rpm at 4°C for 10 minutes, take the supernatant and place it on ice for test (If the supernatant is not clear, centrifuge for 3 minutes)
3. **Serum sample:** Detect directly

II. Determination procedure:

1. Preheat spectrophotometer for 30 minutes, adjust wavelength to 412 nm, set zero with distilled water.
2. The standard solution of 80 $\mu\text{mol/mL}$ is diluted to 0.08 $\mu\text{mol/mL}$ with the distilled water.
3. Operation table: (1.5 mL centrifugal tube with the following reagents in turn)

Reagent Name(μL)	Test tube (T)	Control tube (C)
Sample Supernatant	100	-
Reagent I working solution	100	100
Preheat for 5 minutes at 37°C		
Reagent II working solution	50	50
Reaction for 5 minutes at 37°C		
Reagent III	1000	1000
Sample Supernatant	-	100

Centrifuge at 4000 rpm at room temperature for 10 minutes and take the supernatant into EP tube

Reagent Name(μL)	Test tube(T)	Control tube(C)	Standard tube(S)	Blank tube (B)
Distilled water	-	-	-	500
Supernatant	500	500	-	-
Standard solution	-	-	500	-
Reagent IV	500	500	500	500
Reagent V	125	125	125	125

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. Calculate $\Delta A_T = A_C - A_T$, $\Delta A_S = A_S - A_B$.

III. Calculation

Calculation of inhibition percentage: Inhibitory percentage = $(A_C - A_T) / (A_C - A_B) \times 100\%$

As far as possible, 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 the dosage and re-determine it. 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.

Calculation of GPX activity

1) Protein concentration:

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

$$\begin{aligned}\text{GPX (U/mg prot)} &= \Delta A_T \div (\Delta A_S \div C_S) \times 1000 \times V_{EV} \div (C_{pr} \times V_{SV}) \div T \\ &= 200 \times \Delta A_T \div \Delta A_S \div C_{pr}\end{aligned}$$

2) Sample weight

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

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

3) Cell amount

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the oxidation of 1nmol of GSH per minute in the reaction system every 10^4 cells.

$$\begin{aligned}\text{GPX(U/10}^4\text{cell)} &= \Delta A_T \div (\Delta A_S \div C_S) \times 1000 \times V_{EV} \div (N \times V_{SV} \div V_{TV}) \div T \\ &= 200 \times \Delta A_T \div \Delta A_S \div N\end{aligned}$$

4) Liquid volume:

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

$$\begin{aligned}\text{GPX(U/mL)} &= \Delta A_T \div (\Delta A_S \div C_S) \times 1000 \times V_{EV} \div V_S \div T \\ &= 200 \times \Delta A_T \div \Delta A_S\end{aligned}$$

C_S : Concentration of standard mixtures, 0.08 $\mu\text{mol/mL}$;

V_{EV}: Volume of enzymatic reaction system, 1.25mL;

V_{SV}: Sample volume contained in sample mixtures, 0.1 mL;

V_{TV}: Extraction solution volume, 1 mL;

C_{pr} : 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.