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

Note: Take two or three different samples for prediction before test**.**

Operation Equipment: Spectrophotometer/microplate reader

Cat No: AK0473-100T-48S

Components:

Extract solution: 60 mL×1, Storage at 4℃.

Reagent I: Powder×2. Storage at 4℃. Add 1.65 mL of Diluent to dissolve when the solution will be used. Store for 2weeks at 2-8℃

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. Storage at 4℃.

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: 30 mL×1. Storage at 4℃. If the bottom of the bottle is crystallized, it can be dissolved in water bath at 50℃. This solution is a saturated solution. If the bottom of the bottle is still crystallized, the supernatant can be absorbed and used.

Reagent IV: 15 mL×1, Storage at 4℃.

Reagent V: 5mL×1, Storage at 4℃.

Standard: Powder×1, Storage at 4℃. 10 mg reduced glutathione (GSH). Add 0.405 mL of Diluent to the standard solution of 80 μmol/mL when the solution will be used.

Diluent: 4 mL×1, Storage at 4℃.

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.

Reagents and Equipment Required but Not Provided:

Spectrophotometer/ microplate reader, balance, table centrifuge, micro glass cuvette/96-well plate, mortar/homogenizer, EP tube.

Procedure

I. Sample preparation:

1. Tissue: Accordance ratio tissue weight (g): Extract solution (mL)=1:5~10. (Suggest 0.05 g of tissue with 1 mL Extract solution), homogenate on ice bath, centrifuge at 5000 rpm at 4℃ for 10 min. The supernatant is placed on ice for test (If the supernatant is not clear, centrifuge for another 3 minutes).
2. Bacteria or cellsAmount of cells (10^4): Extractsolution(mL): 500~1000:1. (Add 1 mL Extract solution to 5 million cells), ultrasonic with ice bath to break cells (300 w, 3 s, interval 7 s, total time 3 min), then centrifuged at 5000 rpm at 4℃ for 10 min, the supernatant placed on ice for test (If the supernatant is not clear, centrifuge for longer minutes).
3. Serum sample: Detect directly.

II. Determination procedure:

1. Preheat Spectrophotometer/microplate reader for 30 minutes, adjust wavelength to 412 nm, set zero with distilled water.

2. Dilute 80μmol /mL standard solution with diluent to 0.08μmol/mL. The standard solution is prepared when the solution will be used.

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	20	-
Reagent I working solution	20	20
Preheat for 5 minutes at 37 ℃		
Reagent II working solution	10	10
React for 5 minutes at 37℃		
Reagent III	200	200
Sample Supernatant	-	20

Centrifuge at 4000 rpm at room temperature for 5 minutes and take the supernatant into EP tube or 96-well plate.

Reagent Name (μL)	Test tube (T)	Control tube (C)	Standard tube (S)	Black tube (B)
Diluent	-	-	-	100
Supernatant	100	100	-	-
Standard solution	-	-	100	-
Reagent IV	100	100	100	100
Reagent V	25	25	25	25

Well mix, react 15min at 25℃ 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:

1. 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 redetermine 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.

2. 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 1 nmol of GSH per minute in the reaction system every milligram of protein.

GPX (U/mg prot) $=\Delta A_T \div (\Delta A_S \div C_S) \times 1000 \times V_{EV} \div (Cpr \times V_{SV}) \div T = 200 \times \Delta A_T \div \Delta A_S \div Cpr$

2) Sample weight

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

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

3) Cell amount

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 10^4 cells.

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

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.

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_S: Concentration of standard mixtures, 0.08 μmol/mL;

V_{EV}: Volume of enzymatic reaction system, 0.25 mL;

Vsv: Sample volume contained in sample mixtures, 0.02 mL;

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

Cpr: 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:

1. When the absorbance is greater than 1.5, it is suggested that the sample be determined after diluted with the Extract solution.

2. It is recommended that not to take too many samples at a time, to avoid the influence of too long testing time on color development, which may let the determination is not accurate.

Experimental instances：

1. Take 0.1g of mouse liver, add 1mL of extract solution, homogenate and grind. Take the supernatant, dilute it by 40 times and test according to the measured steps. Calculate A_T=0.152， A_C=0.278， A_S=0.370， A_B=0.064， $\Delta A_T=A_C-A_T$=0.126， $\Delta A_S=A_S-A_B$=0.306, calculate the enzyme activity according to sample weight:
   GPX (U/g weight)=200×ΔA_T÷ΔA_S÷W×40（dilution ratio）=32941 U/g weight.
2. Take 0.1g of poplar leaf, add 1mL of extract solution, homogenate and grind. Calculate A_T=0.199， A_C=0.259， A_S=0.370， A_B=0.064， $\Delta A_T=A_C-A_T$=0.060， $\Delta A_S=A_S-A_B$=0.306, calculate the enzyme activity according to sample weight:
   GPX (U/g weight)=200×ΔA_T÷ΔA_S÷W=392 U/g weight.

Recent Product citations

[1] Yang Yang,Li Jing,Wei Cong,et al. Amelioration of nonalcoholic fatty liver disease by swertiamarin in fructose-fed mice. Phytomedicine. June 2019; 59.(IF4.18)

[2] Xuejuan Xia,Yuxiao,Xing,Guannan Li,et al. Antioxidant activity of whole grain Qingke (Tibetan Hordeum vulgare L) toward oxidative stress in d-galactose induced mouse model. Journal of Functional Foods. June 2018;(IF3.197)

[3] Qilong Wang, Guosheng Xiao, Guoliang Chen,et al. Toxic effect of microcystin-LR on blood vessel development. Toxicological & Environmental Chemistry. Feb 2019;(IF3.547)

[4] Wang H, Li Y Y, Qiu L Y, et al. Involvement of DJ 1 in ischemic preconditioning induced delayed cardioprotection in vivo[J]. Molecular medicine reports, 2017, 15(2): 995-1001