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: BC1195

Size: 100T/48S

Product Composition: Before use, please carefully check whether the volume of the reagent is consistent with the volume in the bottle. If you have any questions, please contact Solarbio staff in time.

Reagent name	Size	Preservation Condition
Extract solution	Liquid 60 mL×1	2-8℃
Reagent I	Powder×2	2-8℃
Reagent II	Liquid 10 μL×1	2-8℃
Reagent III	Liquid 30 mL×1	2-8℃
Reagent IV	Liquid 15 mL×1	2-8℃
Reagent V	Liquid 5 mL×1	2-8℃
Standard	Powder×1	2-8℃
Diluent	Liquid 4 mL×1	2-8℃

Solution Preparation:

1. Reagent I: Add 1.65 mL of distilled water to dissolve when the solution will be used. Store for 2 weeks at 2-8℃.
2. Reagent I working liquid: Before use, the samples is prepared according to the ratio of Reagent I: Diluent = 1:1 according to the number of samples.
3. Reagent II working liquid: Dilute reagent II with the ratio of 2μL Reagent II and 10 mL distilled water before use.
4. Reagent III: 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.
5. Reagent IV: If the bottom of the bottle is crystallized, it can be dissolved in water bath at 40℃.
6. Standard: 10 mg reduced glutathione (GSH). Add 0.405 mL of distilled water to the standard solution of 80 μmol/mL when the solution will be used.

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/cell ultrasonic crusher, 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 spectrophotometer counter to 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 (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 (μL)	Test tube (T)	Control tube (C)	Standard tube (S)	Black tube (B)
Distilled water	-	-	-	100
Supernatant	100	100	-	-
Standard solution	-	-	100	-
Reagent IV	100	100	100	100
Reagent V	25	25	25	25

Well mix, react 15min at room temperature. The absorbance at 412 nm is measured. The absorbance is recorded as A_T, A_C, A_S and A_B, respectively. Calculate $\Delta A_T = A_C - A_T$, $\Delta A_S = A_S - A_B$. Blank tube and standard tube only need to be measured once or twice.

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

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 activity (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, count by 10^4;

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 activity (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 activity (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

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