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

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

Operation Equipment: Spectrophotometer

Cat No: BC1190

Size: 50T/24S

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 30 mL×1	2-8℃
Reagent I	Powder×2	2-8℃
Reagent II	Liquid 10 μL×1	2-8℃
Reagent III	Liquid 60 mL×1	2-8℃
Reagent IV	Liquid 30 mL×1	2-8℃
Reagent V	Liquid 10 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, 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℃ for 10minutes, 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,3s, interval 7s, total time 3minutes). Centrifuged at 5000 rpm at 4℃ for 10minutes, take the supernatant andplace 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 30minutes,adjust wavelength to 412 nm,set zero with distilled water.
2. The standard solution of 80 μmol/mL is diluted to 0.08 μmol/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 5minutes at 37℃		
Reagent II working solution	50	50
Reaction for 5 minutes at 37℃		
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)	Black 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 = $(A_C-A_T)/(A_C-A_B)\times100\%$

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.

1. Calculation of GPX activity

1) Protein concentration:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzesthe oxidation of1nmolof GSH per minute in the reaction system every milligram of protein.

GPX (U/mg prot) $=\Delta A_T\div(\Delta A_S\div C_S)\times1000\times V_{EV}\div(Cpr\times V_{SV})\div T=200\times\Delta A_T\div\Delta A_S\div Cpr$

2) Sample weight

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

GPX (U/g weight) $=\Delta A_T\div(\Delta A_S\div C_S)\times1000\times V_{EV}\div(V_{SV}\div V_{TV}\times W)\div T=200\times\Delta A_T\div\Delta A_S\div W$

3) Cell amount

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the oxidation of1nmolof GSH per minute in the reaction system every 10^4 cells.

GPX(U/10^4cell) $=\Delta A_T\div(\Delta A_S\div C_S)\times1000\times V_{EV}\div(N\times V_{SV}\div V_{TV})\div T=200\times\Delta A_T\div\Delta A_S\div N$

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)$=\Delta A_T\div(\Delta A_S\div C_S)\times1000\times V_{EV}\div V_S\div T=200\times\Delta A_T\div\Delta A_S$.

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

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

Vsv: Sample volume contained in sample mixtures, 0.1 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 1.2, it is suggested that the sample bedetermined after diluted with the extraction 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=0.108, A_C=0.303, A_S=0.491, A_B=0.033, $\Delta A_T=A_C-A_T$=0.195, $\Delta A_S=A_S-A_B$=0.458, calculate the enzyme activity:

GPX (U/g weight)=200×$\Delta A_T \div \Delta A_S \div$W×40（dilution ratio）=34061 U/g.

2. Take 0.1g of poplar leaf, add 1mL of extract solution, homogenate and grind. Calculate A_T=0.220, A_C=0.318, A_S=0.491, A_B=0.033, $\Delta A_T=A_C-A_T$=0.098, $\Delta A_S=A_B-A_B$=0.458, calculate the enzyme activity :

GPX (U/g weight)=200×$\Delta A_T \div \Delta A_S \div$W=428 U/g.

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

Related products:

BC1170/BC1175	Reduced Glutathione (GSH) Assay Kit
BC1180/BC1185	Oxidized Glutathione(GSSG)Assay Kit
BC1150/BC1155	Oxidized Thioredoxin Reductase (TrxR) Assay Kit
BC1210/BC1215	γ-glutamate-cysteine ligase (GCL) Assay Kit