(FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS!)

Glutathione Peroxidase (GSH-Px) Activity Assay Kit (DTNB Method)

Catalog No.: BC00016

Size: 100T

Please read the instructions carefully before use. If you have any questions or need further help during experiment, please don't hesitate to contact us through the following methods:

✉ Email (Sale) order@enkilife.com

✉ Email (Techsupport) techsupport@enkilife.com

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Shelf life: Please refer to the label on the outer package.

Techsupport: In order to provide you with better service, please inform us the lot number on the label of the outer package.

Basic Information

Product Name	Glutathione Peroxidase (GSH-Px) Activity Assay Kit (DTNB Method)
Detection Method	Colorimetric
Sample Type	Tissue, Cells
Assay Type	Enzyme activity
Detection Instrument	Microplate reader (412 nm)

Product Introduction

GPx is an important antioxidant enzyme that can eliminate harmful substances such as hydrogen peroxide in the human body, protect cells from free radical damage, and maintain the stability of the intracellular environment. Lipids in cells are prone to react with free radicals to produce lipid peroxides. Glutathione Peroxidase can use reduced glutathione (GSH) to reduce lipid peroxides, thereby eliminating the toxic effects of free radicals. Glutathione Peroxidase is distributed in almost all tissues. In some pathological conditions,the activity of Glutathione Peroxidase will be significantly up-or down-regulated. Most GPx in cells contain selenium, and selenium is a component of the active center of the enzyme. There is also a small amount of GPx that does not contain selenium in cells. This kit detects the content of the most common selenium-containing GPx.

Product Features

- The organic peroxide reagent (t-Bu-OOH) provided in this kit does not react with GS in the absence of Glutathione Peroxidase, nor is it catalyzed and decomposed by cellular catalase. Therefore, it can relatively specifically detect the activity of Glutathione P eroxidase.

Principle

The total glutathione peroxidase activity assay kit (DTNB method) is a colorimetric method for detecting the activity of glutathione peroxidase (GPx). When GSH is relatively sufficient and there is a sufficient amount of organic peroxide, GPx can catalyze GSH to produce GSSG. The remaining GSH can react with the chromogenic substrate DTNB to produce GSSG and yellow TNB. At this time, the level of GPx activity determines the amount of remaining GSH, thereby determining the amount of yellow TNB formed. Therefore, the

amount of TNB formed is negatively correlated with the activity of GPx. The activity of glutathione peroxidase can be calculated by measuring the absorbance of yellow TNB.

$$2GSH + R - OOH \xrightarrow{GPx} GSSG + R - OH + H_2O$$

$$2GSH + DTNB \rightarrow GSSG + 2TNB$$

Since t-Bu-OOH cannot be catalyzed by glutathione peroxidase that does not contain selenium, this kit can be used to quantitatively detect the most common selenium-containing glutathione peroxidase more specifically.

Components

Components	Size(100T)	Storage
Sample Homogenate	100ml	-20℃
Glutathione Peroxidase Detection Buffer	50ml	-20℃
Reduced Glutathione (GSH)	4.5mg	-20℃, prepare into solution, store at -20° C after aliquoting.
DTNB	4.5mg	-20℃, prepare into solution, store at -20° C after aliquoting.
DMSO	1.5ml	-20℃
Peroxide Reagent(t-Bu-OOH)	200μl	-20℃
Microplate(96 wells)	1 plate	RT
Plate Sealer	2 pieces	RT

Storage

Unopened kits can be stored at -20℃ for 12 months.

Experimental Preparation

- Sample processing

1.Preparation of cell samples: For adherent cells, trypsin should be avoided because they will be used for enzyme activity determination. Cells can be treated with EDTA or collected with a cell spatula or cell scraper. Cells are washed once with PBS or saline. You can choose either of the subsequent steps a or b (step a is recommended):
a. You can use the Western and IP Cell Lysis Buffer (RC0009) produced by EnkiLife to lyse the cell sample according to the corresponding instructions. Add 100-200μl of lysis buffer for per 1 million cells for lysis. If the lysis effect is not good, the cell sample in the lysis buffer can be homogenized with a glass homogenizer at 4ºC or on an ice bath. Then centrifuge at 4ºC, 12,000 x g for 10 minutes. Take the supernatant for enzyme activity determination.

b. You can use the sample homogenate in this kit, add 100-200μl of sample homogenate for per 1 million cells, and homogenize with a glass homogenizer at 4ºC or on an ice bath. Then centrifuge at 4ºC, 12,000 x g for 10 minutes. Take the supernatant for enzyme activity determination.
2. Preparation of tissue samples: The animals were perfused with saline containing 0.16mg/ml heparin (0.9% NaCl containing 0.16mg/ml heparin) to remove blood and obtain tissue samples. According to the ratio of adding 200μl of sample homogenate per 20mg of tissue, the tissue was homogenized at 4ºC or in an ice bath using a tissue grinder or glass homogenizer. Centrifuge at 12,000 x g for 10 minutes at 4ºC. The supernatant was used for enzyme activity determination.
3. Preparation of red blood cell lysate: Collect blood in an anticoagulant tube and mix by inversion. Take at least 500μl of whole blood and centrifuge at 2500 x g for 5 minutes at 4ºC. Discard the supernatant, resuspend the pellet with ice-cold sample homogenate of about 10 times the volume of the red blood cell pellet, centrifuge again as before, and discard the supernatant. Add ice-cold Milli-Q grade pure water of about 4 times the volume of the red blood cell pellet to lyse the red blood cells. Centrifuge at 12,000 x g for 5 minutes and take the supernatant.
5. The above-mentioned samples can be measured for protein concentration using BCA Protein Assay Kit (BCA Method) (**BC00006**) produced by EnkiLife. Usually, samples containing 1-100μg of protein can be taken for glutathione peroxidase detection. Note: For tissue samples with higher GPx activity, samples containing 1-10μg of protein may meet the detection requirements, while for samples with lower GPx activity, such as certain cell samples, 10-100μg of protein may be required.If the activity of glutathione peroxidase in the sample is found to be too high, it can be diluted with glutathione peroxidase detection buffer. If the activity of glutathione peroxidase in the sample is too low, the amount of protein should be increased appropriately. If the prepared sample is measured on the

same day, it can be stored in an ice bath. If it is measured at a later time, it can be frozen at -70ºC.

- Preparation of the assay kit

1. Preparation of 10mM GSH solution.

Add 1.5 ml of Milli-Q grade pure water to the 4.5 mg of GSH provided in this kit, dissolve and mix to make a 75mM GSH solution. Except for the part to be used immediately, the rest of the GSH solution needs to be appropriately divided and stored at -20º C.

2. Preparation of DTNB stock solution.

Add 1.5 ml of DMSO provided in this kit to the 4.5 mg of DTNB provided in this kit, dissolve and mix to make a DTNB stock solution. Except for the part to be used immediately, the rest of the DTNB stock solution needs to be appropriately divided and stored at -20º C away from light.

3. Preparation of 15mM peroxide reagent solution.

Take 21.5 μl of peroxide reagent (t-Bu-OOH) and add 10 ml of Milli-Q grade pure water, mix well, and prepare a 15mM peroxide reagent solution. The prepared 15mM peroxide reagent solution is only used on the same day and should be stored on an ice bath as much as possible.

4. All reagents must be incubated to 25º C in a water bath or on a PCR instrument before use.

Operation process

1. Refer to the table below, add each solution in turn and mix well. It should be noted that the activity levels of glutathione peroxidase in different cells or tissue samples vary greatly, and the initial detection requires appropriate exploration of the sample dosage.

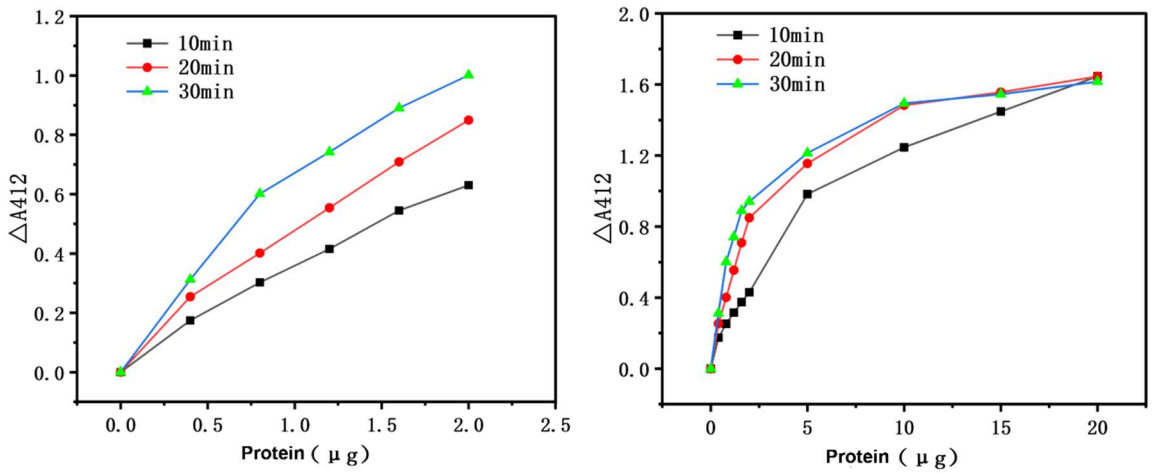
	Blank	Sample
Glutathione Peroxidase Detection Buffer	183μl	173-181μl
10mM GSH Solution	5μl	5μl
Test Sample	0μl	2~10μl
15mM Peroxide Reagent Solution	12μl	12μl
Total Volume	200μl	200μl

2. Incubate at 25℃ or room temperature for 10-30 minutes. Incubate for 10 minutes for the first test. If the results are not satisfactory, consider extending the incubation time to 20-30 minutes.

3. Add 6.6μl of DTNB solution to each well and mix well. Incubate at 25°C or room temperature for 10 minutes.

4. Measure A412 using an microplate reader or micro-spectrophotometer. If the absorbance of the sample is very close to that of the blank control, it means that the activity of glutathione peroxidase in the sample is too low. In this case, the sample volume should be increased appropriately and the incubation time of the sample with GSH and peroxide reagent in step (2) should be extended. If the difference between the sample and the blank control is too large, it means that the activity of glutathione peroxidase in the sample is too high. In this case, the sample should be diluted appropriately or the sample volume should be reduced. The actual measured effect diagram of mouse liver lysate samples with a protein content of 0-20μg is shown in Figure 1.

Figure 1. Detection effect of Glutathione Peroxidase (GSH-Px) Activity Assay Kit (DTNB Method) on mouse liver homogenate supernatant sample.



The horizontal axis is the protein amount of the mouse liver sample, and the vertical axis △A412 is the absorbance value of the blank control minus the sample after adding DTNB solution and incubating for 10 minutes. The incubation time of 10, 20, and 30 minutes is the incubation time of operation flow. (2). The left figure is the result of protein amount 0-2μg, and the right figure is the result of protein amount 0-20μg. The data in the figure are for reference only, and the actual test results may vary depending on the specific reaction conditions.

Result calculation

Note: After adding DTNB solution, the remaining GSH can be consumed in a very short time, so that the reaction catalyzed by glutathione peroxidase is terminated. Therefore, if you need to calculate the enzyme activity of glutathione peroxidase, please use the

reaction time before adding DTNB in operation flow (2) to perform the following calculation.
1. Definition of glutathione peroxidase activity unit: 1 unit of enzyme activity (1 unit) can oxidize 1μmol of GSH in 1 minute at 25°C, pH 7.5. 1 U = 1000 mU.
2. △A412/min = [A412(Blank) - A412(Sample)]/min
3. For glutathione peroxidase solution: 1mU/ml = 1nmol TNB/min/ml＝(△A412/min)/($\varepsilon^{\mu M}$×L(cm))
4.[Glutathione peroxidase activity in the detection system]=(△A412/min)/($\varepsilon^{\mu M}$×L(cm))
[Glutathione peroxidase activity in the sample]＝[Glutathione peroxidase activity in the detection system]×[dilution factor]/[protein concentration in the sample]=[(△A412/min)/($\varepsilon^{\mu M}$×L(cm))]×[dil×(V(ml)/V_{sample}(ml))]/[protein concentration in the sample]
Note: The unit of [glutathione peroxidase activity in the detection system] is mU/ml, [protein concentration in the sample] is mU/ml. The unit of [activity of glutathione peroxidase in sample] is mg/ml, so the final unit of [Glutathione peroxidase activity in sample] is: U/mg protein or mU/mg protein;
$\varepsilon^{\mu M}$ is the molar extinction coefficient: the molar extinction coefficient of TNB in A412 is 0.0136$\mu M^{-1}cm^{-1}$;
L(cm) is the path length when measuring absorbance: the height of 200μl sample in a general 96-well is about 0.552cm. If different reaction wells are used, please note that it is modified to the height of the solution in the well;
dil is the dilution multiple of the sample;
V(ml) is the reaction system, which is 0.2ml in this reaction system;
V_{sample}(ml) is the volume of the sample in the reaction system, expressed in ml.
5. Calculation example: The protein concentration of the sample is determined to be 5 mg/ml. The incubation time in operation flow (2) is 10 minutes. After diluting 10 times with sample diluent (i.e. dil=10), take 10μl of the diluted sample (i.e. V_{sample}(ml)=0.01) for measurement. If A412(Blank)＝1.549, A412(Sample)＝1.221.
[Glutathione peroxidase activity in the detection system]=[(1.549-1.221)/10]/(0.0136×0.552)= 4.369mU/ml
[Glutathione peroxidase activity in the sample]=4.369mU/ml×(10×0.2/0.01)/(5mg/ml)=174.76mU/mg=0.175U/mg(protein)

Notes

1. This kit involves redox reactions, so all oxidants or reducing agents will interfere with the determination of this kit. If reducing agents in the sample cannot be avoided, such as DTT,

mercaptoethanol, etc., the total concentration of these reducing agents should be at least less than 0.1mM. 0.15mM DTT can inhibit 40% of enzyme activity.
2. Commonly used detergents such as Triton X-100 and Tween-20 contain high levels of peroxides, which will affect the determination of this kit. If these detergents must be used, it is best to use detergents with higher purity and marked with lower peroxides.
3. After adding DTNB solution to the reaction system, the remaining GSH can be consumed in a very short time, so that the reaction catalyzed by GPx is terminated. Therefore,when calculating the enzyme activity,only the reaction time before the addition of DTNB is used for calculation.The sample can be measured immediately or frozen at -70^{o}C for later measurement.
4. The reaction temperature must be strictly controlled at 25^{o}C, otherwise it will cause more errors.
5. Compared with the NADPH method (Cat. No. BC00012), the DTNB used in this kit is more stable and easier to operate than NADPH, but the NADPH method has higher sensitivity. Researchers can choose according to the content of glutathione peroxidase in the sample and experimental conditions.
6. This product is for Research Use Only and shall not be used for clinical diagnosis or treatment, food or medicine, or stored in ordinary residences.