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

# Glutathione Peroxidase (GSH-Px) Activity Assay Kit (NADPH Method)

Catalog No.: BC00012

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

☎ Tel: 0086-27-87002838

Website: www.enkilife.com


**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 (NADPH Method) |
| **Detection Method** | Colorimetric |
| **Sample Type** | Tissue, Cells |
| **Assay Type** | Enzyme activity |
| **Detection Instrument** | Microplate reader (340 nm) |

## Product Introduction

Glutathione Peroxidase (GSH-Px) Activity Assay Kit (NADPH Method) is a commonly used metabolism assay kit for detecting the activity of Glutathione Peroxidase (GPx). 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.

## Product Features

- The organic peroxide reagent (t-Bu-OOH) provided in this kit does not react with GSH 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 Peroxidase.
- Since t-Bu-OOH cannot be catalyzed by selenium-free Glutathione Peroxidase, this kit can relatively specifically quantify the most common selenium-containing Glutathione Peroxidase.

## Principle

GPx catalyzes the reaction of reduced glutathione (GSH) with peroxide reagent (R-OOH) to convert it into oxidized glutathione (GSSG). Glutathione reductase (GR) can use NADPH to catalyze GSSG to produce GSH. In the above reaction, Glutathione Peroxidase

is the rate-limiting step of the entire reaction system, so the decrease in NADPH is linearly related to the activity of Glutathione Peroxidase. Therefore, by detecting the change in A340, the amount of NADPH decrease, the activity level of GPx can be calculated.

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

$$NADPH + H^+ + GSSG \xrightarrow{GR} NADP^+ + 2GSH$$

## Components

| Components | Size(100T) | Storage |
|---|---|---|
| Sample Homogenate | 100ml | -20℃ |
| Glutathione Peroxidase Detection Buffer | 50ml | -20℃ |
| Glutathione Reductase | 100μl | -20℃ |
| NADPH | 11.5mg | Store at -20℃, after dissolving NADPH, aliquot and store at -70ºC, can be stored at 4ºC for one day, NADPH will degrade by more than 10% after one week at -20ºC |
| Reduced Glutathione (GSH) | 10mg | Store at -20℃, GSH should be prepared as a solution, aliquot and store at -20ºC |
| 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.

4. Sample dilution: Before the formal test, it is necessary to select 2-3 samples with large differences and dilute them into different concentrations for preliminary experiments. 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 appropriately increased.

5. Protein concentration determination: 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.

- Preparation of the assay kit

1. Preparation of 62.5mM NADPH solution

Add 220μl of Milli-Q grade pure water to the 11.5mg NADPH provided in this kit, dissolve and mix to obtain a 62.5mM NADPH solution. Except for the part to be used immediately, the remaining NADPH solution should be appropriately aliquoted and stored at -70° C.

2. Preparation of 75mM GSH solution

Add 433μl of Milli-Q grade pure water to the 10mg GSH provided in this kit, dissolve and mix to obtain a 75mM GSH solution. Except for the part to be used immediately, the remaining GSH solution should be appropriately aliquoted and stored at -20° C.

3. Preparation of GPx detection working solution

Prepare an appropriate amount of GPx detection working solution according to the number of samples to be measured (including controls), according to the volume of 40μl of GPx detection working solution required for each test. The prepared GPx detection working solution should only be used on the same day and should be stored on ice as much as possible. Please refer to the table below for specific preparation methods.

| Samples No. that can be measured (including controls) | 1 sample | 10 samples | 20 samples |
|---|---|---|---|
| Glutathione Peroxidase Detection Buffer | 35μl | 350μl | 700μl |
| 62.5mM NADPH | 2μl | 20μl | 40μl |
| 75mM GSH | 2μl | 20μl | 40μl |
| Glutathione Reductase | 1μl | 10μl | 20μl |
| GPx Detection Working Solution | 40μl | 400μl | 800μl |

4. Preparation of 30mM peroxide reagent solution

Take 21.5μl of peroxide reagent (t-Bu-OOH) and add 5 ml of Milli-Q grade pure water. Mix well to prepare 30mM peroxide reagent solution. The prepared 30mM peroxide reagent solution can only be used on the same day and should be stored on ice as much as possible.

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

## Operation process

1. Refer to the table below, use a 96-well plate, add the detection buffer, the sample to be tested and the GPx detection working solution in sequence, mix well, add 40μl of GPx detection working solution, and incubate at room temperature for 15 minutes to consume the GSSG in the sample and eliminate interference with subsequent detection.

| | Blank | Sample |
|---|---|---|
| Glutathione Peroxidase Detection Buffer | 50μl | 0-50μl |
| Test Sample | —— | 0-50μl |
| GPx Detection Working Solution | 40μl | 40μl |
| Total Volume | 90μl | 90μl |

2. Add 10μl of 30 mM peroxide reagent solution to each well and mix well.

3. Immediately measure A340 using an appropriate microplate reader or micro-volume UV spectrophotometer, and record the 0-minute reading. If the instrument can set the temperature, set it to 25° C. Otherwise, adjust the room temperature to 25° C through the air conditioner, and start measuring A340 after the instrument is expected to reach 25° C.
4. Measure A340 continuously for 5 minutes or automatically every 1 minute. If the instrument does not have the corresponding function, you can manually record the A340 value every 1 minute for at least 5 minutes to obtain 6 points of data.
Note 1: The time of continuous measurement can be adjusted according to the activity of GPx in the sample, but it is necessary to ensure that 6 points of data are obtained. For samples with higher GPx activity, it is recommended to measure for 5 minutes or 10 minutes, and the corresponding measurement interval is set to 1 minute and 2 minutes; for samples with very low GPx activity, the measurement time can be extended to 10, 15 or 20 minutes, and the corresponding measurement interval is set to 2, 3 or 4 minutes. It can also be measured continuously for 20 minutes, once every 1 minute, and finally the data before the linear time point is taken for analysis.
Note 2: If the first reading of the sample, such as the A340 reading at 0 minutes, is lower than 1, it means that the GPx activity in the sample is too high, or the GSSG content in the sample itself is too high, and the sample needs to be appropriately diluted or the sample dosage reduced.
5. The measured △A340/min should preferably be controlled within the range of 0.01-0.2. If the measured △A340/min value is too large, the sample can be appropriately diluted or the sample dosage can be reduced. If the △A340/min value is too small, the sample needs to be concentrated as much as possible and the sample dosage should be appropriately increased when processing the sample. See Table 1, Figure 1, Table 2, and

Figure 2.

Table 1. Data Analysis of High-Protein Fish Tissue Sample Testing

| Detection data | 0min | 1min | 2min | 3min | 4min | 5min |
|---|---|---|---|---|---|---|
| A340(blank) | 1.6789 | 1.6585 | 1.6384 | 1.6187 | 1.5990 | 1.5794 |
| A340(sample) | 1.0408 | 0.9172 | 0.7775 | 0.6482 | 0.5274 | 0.4163 |
| △A340(blank) | 0.0000 | 0.0204 | 0.0405 | 0.0602 | 0.0799 | 0.0995 |
| △A340(sample) | 0.0000 | 0.1236 | 0.2633 | 0.3926 | 0.5134 | 0.6245 |
| △A340 | 0.0000 | 0.1032 | 0.2228 | 0.3324 | 0.4335 | 0.5250 |
| △A340/min | 0.0000 | 0.1032 | 0.1114 | 0.1108 | 0.108375 | 0.105 |

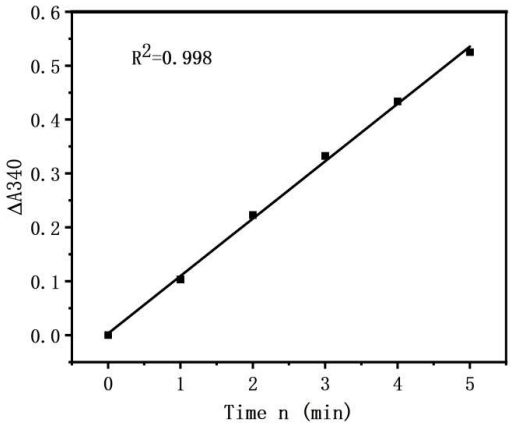


Figure 1. Detection Effect of High-Protein Fish Tissue Samples Measured for 5 Minutes

Table 2.Data Analysis of Low-Protein Fish Tissue Sample Testing

| Detection data | 0min | 3min | 6min | 9min | 12min | 15min |
|---|---|---|---|---|---|---|
| A340(blank) | 1.7105 | 1.6790 | 1.6549 | 1.6327 | 1.6107 | 1.5902 |
| A340(sample) | 1.1529 | 0.9996 | 0.8519 | 0.7161 | 0.5904 | 0.4738 |
| △A340(blank) | 0 | 0.0315 | 0.0556 | 0.0778 | 0.0998 | 0.1203 |
| △A340(sample) | 0 | 0.1533 | 0.301 | 0.4368 | 0.5625 | 0.6791 |
| △A340 | 0.0000 | 0.1218 | 0.2454 | 0.3590 | 0.4627 | 0.5588 |
| △A340/min | 0.0000 | 0.0406 | 0.0409 | 0.0399 | 0.0386 | 0.0373 |

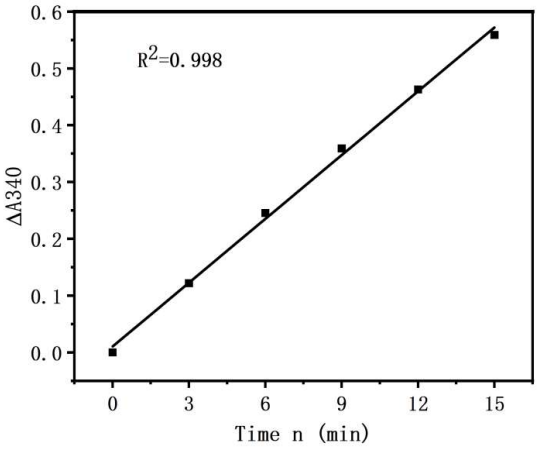


Figure 2. Detection Effect of Low-Protein Fish Tissue Samples Measured for 15 Minutes

Note： ΔA340(blank)=A340(blank)(Time 0) - A340(blank)(Time n)

ΔA340(sample)=A340(sample)(Time 0) - A340(sample)(Time n)

ΔA340= ΔA340(sample) - A340(blank)

ΔA340/min= ΔA340/n

## Result calculation

1. Definition of glutathione peroxidase activity unit

1 unit of enzyme activity (1 unit) can catalyze the conversion of 1 μmol of NADPH into NADP+ in 1 minute at 25ºC, pH 8.0, in the presence of GSH, glutathione reductase, and t-Bu-OOH. 1 U = 1000 mU.

2. For Glutathione Peroxidase Solution

1mU/ml＝1nmol NADPH/min/ml＝(ΔA340/min)/($\varepsilon^{\mu M}$×L(cm))

3. This is equivalent to:

**[Glutathione peroxidase activity in the test system]** = (ΔA340/min)/($\varepsilon^{\mu M}$×L(cm)) = [(ΔA340 (sample)-ΔA340 (blank))/min]/($\varepsilon^{\mu M}$×L(cm))

**[Glutathione peroxidase activity in the sample]** = [Glutathione peroxidase activity in the test system] × [dilution factor]/[protein concentration in the sample] = [(ΔA340/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 test system] is mU/ml, and the unit of [protein concentration in the sample] is mg/ml, so the final unit of [glutathione peroxidase activity in the sample] is: U/mg protein or mU/mg protein;

$\varepsilon^{\mu M}$ is the molar extinction coefficient: the molar extinction coefficient of NADPH in A340 is 0.00622$\mu M^{-1}cm^{-1}$;

L(cm) is the path length when measuring absorbance: the height of 100μl sample in a

general 96-well is about 0.276cm. 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, and this reaction system is 0.1ml;

$V_{sample}$(ml) is the volume of the sample in the reaction system, expressed in ml.

4. Calculation example:

The protein concentration of the sample was determined to be 1.2 mg/ml. After diluting it 2 times with the sample diluent, 20μl of the diluted sample was taken for measurement according to Table 1. The measurement time was set to 15 minutes. If A340 (sample) = 1.53, A340 (blank) = 1.75 at 0 minutes, A340 (sample) = 0.81, A340 (blank) = 1.35 at 15 minutes, then △A340 (sample) = 1.53-0.81 = 0.72, △A340 (blank) = 1.75-1.35 = 0.40, then:

**[Glutathione peroxidase activity in the detection system]** = [ (0.72-0.40)/15]/(0.00622×0.276) = 12.43mU/ml

**[Glutathione peroxidase activity in the sample]** = 12.43mU/ml×(2×0.1/0.02) /(1.2mg/ml) = 104mU/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. The first reading after adding the peroxide reagent solution, such as the A340 reading at 0 minutes, should not be lower than 1. If the reading is lower than 1, it means that the GPx activity in the sample is too high, or the GSSG content in the sample itself is too high. The sample needs to be appropriately diluted or the sample dosage needs to be reduced, otherwise the reaction will quickly enter the plateau phase and normal detection data cannot be obtained. The sample can be measured immediately or frozen at -70°C for later measurement.
4. The reaction temperature must be strictly controlled at 25°C, otherwise it will cause more errors.
5. NADPH is not very stable, so please strictly follow the subsequent instructions to prevent inactivation.
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.