Glutathione Peroxidase (GSH-PX) Assay Kit

Catalog No.: abx294069

Size: 100 tests

Detection Range: 12.65 U – 387 U

Sensitivity: 12.65 U

Storage: Store all components at 4°C. Store the DTNB Solution and Salt Reagent in the dark.

Application: For detection and quantification of GSH-PX activity in serum, plasma, tissue, cell lysates, and cell culture supernatants.

Introduction

Glutathione peroxidase (GPX1) is an enzyme that reduces lipid hydroperoxides back to carboxylic acids and hydrogen peroxide to water using glutathione as a hydrogen donor. This produces dimeric glutathione (connected via a disulphide bond) that can be regenerated to two separate glutathione molecules by NADPH. In humans, GPX1 contains a selenocysteine amino acid residue crucial to the reduction process, which is coded for using a UGA stop codon. Deficiencies in glutathione peroxidases are associated with vitiligo, and double knockout mice had increased rates of hearing loss and cataract development.

Abbexa's GSH-PX Assay Kit is a quick, convenient, and sensitive method for measuring and calculating GSH-PX activity. The absorbance should be measured at 412 nm. The reaction of reduced glutathione and hydrogen peroxide can occur without the enzyme, so the calculated activity must be normalized against non-enzymatic activity. The intensity of the color is proportional to the activity of the GSH-PX enzyme(s), which can then be calculated.

Kit components

1. Stock Solution: 2 ml
2. Acid Reagent: 4 × 60 ml
3. Phosphate: 2 vials
4. DTNB Solution: 30 ml
5. Salt Reagent: 4 vials
6. GSH Standard: 2 × 3.07 mg
7. GSH Standard Diluent: 6 ml

Materials Required But Not Provided

1. Spectrophotometer (412 nm)
2. Cuvettes (1 cm optical path)
3. Double distilled water
4. Normal saline (0.9% NaCl)
5. PBS (0.01 M, pH 7.4)
6. Pipette and pipette tips
7. Vials/tubes
8. Sonicating water bath
9. Centrifuge
10. Vortex mixer

Protocol

A. Preparation of samples and reagents

1. Samples

Isolate the test samples soon after collecting and analyze immediately or aliquot and store at -20°C or -80°C for long term storage. Avoid multiple freeze-thaw cycles.

The following sample preparation methods are intended as a guide and may be adjusted as required depending on the specific samples used.

- **Serum/ Plasma :** Samples should be detected directly. Unused serum/ plasma samples can be aliquot and stored at -80°C for up to 1 month.
- **Tissue Homogenates:** Weigh the tissue homogenate and wash in cold PBS (0.01 M, pH 7.4). For each 1 g of homogenate, add 9 ml normal saline (0.9 % NaCl) or PBS (0.01 M, pH 7.4). Homogenize at 4°C by hand, using a mechanical homogenizer, or by ultrasonication. Centrifuge the homogenate at 10,000 x g at 4°C for 10 minutes. Collect the supernatant and assay immediately. The protein content in the supernatant should be determined separately **(abx097193)**.
- **Cell (adherent or suspension):** Collect cells into a centrifuge tube (recommended 1 × 10^6 cells) and wash with PBS (0.01 M, pH 7.4). Add 300 µl normal saline (0.9 % NaCl) or PBS (0.01 M, pH 7.4) per 1 × 10^6 cells, then homogenize by ultrasonication at 4°C. Centrifuge at 10,000 × g at 4°C for 10 minutes. Take the supernatant into a new centrifuge tube and store on ice for immediate assay. The protein content in the supernatant should be determined separately **(abx097193)**.

Samples should not contain detergents such as SDS Tween-20, NP-40 and Triton X-100, or reducing agents such as DTT.

We recommend carrying out a preliminary experiment to determine the optimal dilution factor of samples before carrying out the formal experiment. Dilute samples into different concentrations with Double distilled water, then carry out the assay procedure and calculate the inhibition ratio of the samples. This kit is suitable for measuring an inhibition ratio in the range 20% - 60%. The optimal dilution factor is an inhibition ratio in the range of 45% - 55%, where the inhibition ratio can be calculated as:

$$\text{Inhibition Ratio (\%)} = \frac{\text{OD}_{\text{Non-Enzyme Tube}} - \text{OD}_{\text{Enzyme Tube}}}{\text{OD}_{\text{Non-Enzyme Tube}}} \times 100$$

If the inhibition ratio is > 60%, the sample should be diluted further. If the inhibition ratio is < 20%, the sample concentration should be increased.

Note:

- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.
- Lysis buffers may interfere with the kit. It is therefore recommended to use mechanical lysis methods for cell lysates and tissue homogenates.

Samples may be diluted using Normal Saline (0.9% NaCl) or PBS (0.01 M, pH 7.4) according to the following values. These values are intended as a guide and the final dilution factor should be determined by the end user.

The recommended dilution factor for different samples is as follows (for reference only):

Sample	Dilution factor
Human serum	1-3
Mouse plasma	4-8
Rat serum	5-8
10% Mouse liver tissue homogenate	20-40

2. Reagents

- **Stock working solution:** Dilute the Stock Solution 1:99 with Double distilled water. Incubate at 37°C for 5 minutes prior to use. Prepare immediately before carrying out the assay.
- **Phosphate working solution:** Dissolve a vial of Phosphate with 60 ml double distilled water and mix thoroughly. Prepare immediately before carrying out the assay. If crystals appear, take the supernatant for assay.
- **Salt Reagent working solution:** Dissolve a vial of Salt Reagent with 10 ml double distilled water and mix thoroughly. The prepared solution can be stored at 2-8°C in the dark for up to 1 month.
- **GSH diluent working solution:** Dilute the GSH Standard Diluent 1:9 with Double distilled water. If the GSH Standard Diluent is frozen then dissolve at 65 °C. Prepare immediately before carrying out the assay.
- **1 mmol/L GSH Standard solution:** Dissolve a vial of GSH Standard with 10 ml GSH diluent working solution. Prepare immediately before carrying out the assay and mix fully. Aliquot and store at -20°C for up to one month.
- **20 µmol/L GSH Standard solution**: Dilute the 1 mmol/L GSH Standard solution 1:49 with GSH Standard Diluent working solution. Prepare immediately before carrying out the assay and mix fully.

B. Assay Procedure

1. Enzymatic Reaction:

1.1. Set the Non-Enzyme tubes and Enzyme tubes. Each sample to be tested will require at least one Non-Enzyme tube and one Enzyme tube.

1.2. Add 200 µl of 1 mmol/L GSH Standard solution to each tube.

1.3. Add sample to each Enzyme tube:

Serum and plasma samples: Add 100 µl of sample to each Enzyme tube.

Tissue homogenates, cell lysates and cell culture supernatants samples: Add 200 µl of sample to each Enzyme tube.

1.4. Heat all tubes and the Stock working solution in a water bath at 37°C for 5 minutes.

1.5. Add 100 µl of Stock working solution to each Non-Enzyme tube and each Enzyme tube.

1.6. Heat all tubes at 37°C in a water bath for 5 minutes.

1.7. Add 2 ml of Acid Reagent to each tube.

1.8. Add sample to each Non-Enzyme tube.

Serum and plasma samples: Add 100 µl of sample to each Enzyme tube.

Tissue homogenates, cell lysates and cell culture supernatants samples: Add 200 µl of sample to each Enzyme tube.

1.9. For each tube, mix fully and centrifuge at 3100 × g. Then take 1 ml of supernatant for the chromogenic reaction. If the supernatant contains sediment, transfer to a fresh tube and re-centrifuge.

2. Chromogenic Reaction:

2.1. Set the Blank, Standard, Non-Enzyme, and Enzyme tubes and label accordingly.
2.2. Add 1 ml of supernatant from the Non-Enzyme tubes to the new Non-Enzyme tube.
2.3. Add 1 ml of supernatant from the Enzyme tubes to the new Enzyme tube.
2.4. Add 1 ml of GSH Standard Diluent to the Blank Tube.
2.5. Add 1 ml of 20 μmol/L GSH Standard solution to the Standard Tube.
2.6. Add 1 ml of Phosphate working solution to each tube.
2.7. Add 0.25 ml of DTNB working solution to each tube.
2.8. Add 0.05 ml of Salt Reagent working solution to each tube.
2.9. Mix fully and allow to stand at room temperature for 15 minutes.
2.10. Set the spectrophotometer to zero using Double distilled water. Measure the OD values at 412 nm with a 1 cm optical path cuvette..

C. Calculation of Results

1. Serum, plasma and cell culture supernatant samples:

One unit of GSH-PX activity is defined as the quantity of GSH-PX in 0.1 ml of sample that catalyzes the consumption of 1 μmol/L GSH after deducting the effect of non-enzyme reaction at 37°C over 5 minutes.

$$\text{GSHPX activity (U)} = \frac{\text{OD}_{\text{Non-Enzyme Tube}} - \text{OD}_{\text{Enzyme Tube}}}{\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}} \times f_1 \times f \times c$$

2. Tissues and cell lysate samples:

One unit of GSH-PX activity is defined as the quantity of GSH-PX in 1 mg of protein that catalyzes the consumption of 1 μmol/L GSH after deducting the effect of non-enzyme reaction at 37°C over 5 minutes.

$$\text{GSHPX activity (U/mg protein)} = \frac{\text{OD}_{\text{Non-Enzyme Tube}} - \text{OD}_{\text{Enzyme Tube}}}{\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}} \times \frac{c \times f_2 \times f}{VC_{\text{P}}}$$

Cell culture supernatant samples:

One unit of GSH-PX activity is defined as the quantity of GSH-PX in 0.1 ml of cell culture supernatant that catalyzes the consumption of 1 μmol/L GSH after deducting the effect of non-enzyme reaction at 37°C over 5 minutes.

$$\text{GSHPX activity (U)} = \frac{\text{OD}_{\text{Non-Enzyme Tube}} - \text{OD}_{\text{Enzyme Tube}}}{\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}} \times \frac{c \times f_2 \times f}{2}$$

where:

GSHPX	activity of glutathione peroxidase
c	concentration of the standard (20 µmol/L)
f	dilution factor of sample prior to assay
f_1	dilution factor of serum/plasma samples in the enzymatic reaction ($f_1 = 6$)
f_2	dilution factor of tissue, cell lysate or cell culture supernatant samples in the enzymatic reaction ($f_2 = 5$)
V	volume of sample
C_P	concentration of protein in sample (mg protein/ml)
2	conversion factor (definition of U for cell culture supernatants uses 0.1 ml of sample; the actual volume used in the experiment is 0.2 ml)

Technical Support

For troubleshooting and technical assistance, please contact us at support@abbexa.com.