Glutathione Peroxidase (GSH-PX) Assay Kit

Catalog No.: abx294002

Size: 96 tests

Detection Range: 34.34 U – 1036.64 U

Sensitivity: 34.34 U

Storage: Store all components at 4°C for up to 6 months.

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

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 intensity of the color is proportional to the activity of the GSH-PX enzyme(s), which can then be calculated.

Kit components

1. 96-well microplate
2. Stock solution: 0.5 ml
3. Acid reagent: 50 ml
4. Phosphate solution: 12 ml
5. DTNB solution: 7 ml
6. GSH standard: 3.07 mg
7. GSH standard diluent: 2 × 1.5 ml
8. Plate sealer: 2

Materials Required But Not Provided

1. Microplate reader (412 nm)
2. Distilled water
3. Normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4)
4. Homogenization medium (10 mM Tris-HCl, pH 7.4, containing 10 mM NaCl, 10 mM sucrose, 0.1 mM EDTA)
5. Pipette and pipette tips
6. Vials/tubes
7. Sonicating water bath
8. Centrifuge
9. 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:** Samples should be collected into a serum separator tube. Coagulate the serum by leaving the tube undisturbed in a vertical position overnight at 4°C or at room temperature for up to 1 hr. Centrifuge at approximately 2000 × g for 15 mins at 4°C. If a precipitate appears, centrifuge again. Take the supernatant, keep on ice and assay immediately, or aliquot and store at -80°C for up to 1 month.
- **Plasma:** Collect plasma using heparin as the anticoagulant. Centrifuge for 10 mins at 1000-2000 x g at 4°C, within 30 mins of collection. If precipitate appears, centrifuge again. Avoid hemolytic samples. Take the supernatant (avoid taking the middle layer containing white blood cells and platelets), keep on ice and assay immediately, or aliquot and store at -80°C for up to 1 month.
- **Tissue Homogenates:** Weigh the tissue homogenate. For each 1 g of homogenate, add 9 ml homogenization medium. Homogenize by hand, using a mechanical homogenizer, or by ultrasonication. Centrifuge the homogenate at 10,000 x g at 4°C for 10 min. Collect the supernatant and assay immediately. The protein concentration in the supernatant should be determined separately.
- **Cell lysates:** Collect cells into a centrifuge tube and wash with PBS. Centrifuge at 1000 × g for 10 min and discard the supernatant. Add 300-500 µl homogenization medium per 1 × 10^6 cells, then sonicate in an ice water bath. Centrifuge at 1500 × g at 4 °C for 10 min. Take the supernatant into a new centrifuge tube (while kept on ice) and analyze immediately. The protein concentration in the supernatant should be determined separately.

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 distilled water, then carry out the assay procedure and calculate the inhibition ratio of the samples. The optimal dilution factor is an inhibition ratio in the range of 25% - 45%, 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 > 50%, the sample should be diluted further. If the inhibition ratio is < 10%, 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.

2. Reagents

- **Stock working solution:** Dilute the Stock solution 1:99 with distilled water. Prepare immediately before carrying out the assay.
- **GSH diluent working solution:** Dilute the GSH Standard diluent 1:9 with distilled water. Prepare immediately before carrying out the assay.
- **1 mmol/L GSH standard solution:** Dissolve a vial of GSH standard with GSH diluent working solution to a final volume of 10 ml. Prepare immediately before carrying out the assay and mix fully.
- **100 µmol/L GSH standard solution**: Dilute the 1 mmol/L GSH standard solution 1:9 with GSH standard diluent working solution. Prepare immediately before carrying out the assay and mix fully.

B. Assay Procedure

1. **Standard curve preparation:** Label 7 tubes with 80, 60, 50, 40, 20, 10 and 0 µmol/L. Dilute the 100 µmol/L GSH standard solution with GSH diluent working solution to concentrations of 80, 60, 50, 40, 20 and 10 µmol/L. The GSH diluent working solution itself serves as the 0 µmol/L (blank) standard.

2. Enzymatic Reaction:

2.1. 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.
2.2. Add 20 µl of 1 mmol/L GSH standard solution to each tube.
2.3. Add 20 µl of sample to each Enzyme tube.
2.4. Heat all tubes and the Stock working solution in a water bath at 37°C for 5 minutes.
2.5. Add 10 µl of Stock working solution to each Non-Enzyme tube and each Enzyme tube.
2.6. Heat all tubes at 37°C in a water bath for 5 minutes.
2.7. Add 200 µl of Acid reagent to each tube.
2.8. Add 20 µl of sample to each Non-Enzyme tube.
2.9. For each tube, mix fully and centrifuge at 3100 × g. Then take 100 µl of supernatant for the chromogenic reaction.

3. Chromogenic Reaction:

3.1. Set the Standard, Non-Enzyme and Enzyme wells on the well-plate.
3.2. Add 100 µl of prepared standards to the Standard wells.
3.3. Add 100 µl of supernatant from the Non-Enzyme tubes to the Non-Enzyme wells.
3.4. Add 100 µl of supernatant from the Enzyme tubes to the Enzyme wells.
3.5. Add 100 µl of Phosphate solution to each well.
3.6. Add 50 µl of DTNB solution to each well.
3.7. Tap the plate gently to mix. Allow to stand at room temperature for 5 minutes.
3.8. Measure the OD values at 412 nm with a microplate reader.

C. Calculation of Results

The standard curve can be plotted as the absolute OD_{412} of each standard solution (*y*) vs. the respective concentration of the standard solution (*x*). A linear fit is recommended for the standard curve (*y* = a*x* + b). The GSH-PX concentration of the samples can be interpolated from the standard curve.

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.

$$\mathrm{GSHPX\ (U)} = \frac{\Delta A_{412} - b}{a} \times f_1 f_2 \times \frac{0.1}{V}$$

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.

$$\mathrm{GSHPX\ (U/mg\ protein)} = \frac{\Delta A_{412} - b}{a} \times f_1 f_2 \times \frac{1}{V C_{\mathrm{P}}}$$

where:

GSHPX	activity of glutathione peroxidase
ΔA_{412}	OD value of the sample ($\mathrm{OD_{Non-Enzyme\ Tube}} - \mathrm{OD_{Enzyme\ Tube}}$)
a	gradient of the standard curve (linear fit)
b	y-intercept of the standard curve (linear fit)
f_1	dilution factor of the reaction system in the enzymatic reaction $\left(\frac{0.23+V}{0.03+V}\right)$
f_2	dilution factor of the sample before carrying out the assay
V	volume of sample
C_{P}	concentration of protein in sample (mg protein/ml)