MANUAL

Glutathione Peroxidase (GPX) Activity Assay Kit (Colorimetric)

Catalog #: MA-GPX

ISO 13485:2016

Introduction

Glutathione peroxidase (GPX) is a tetramer composed of four identical subunits. It requires selenium as a cofactor and contains a selenocysteine amino acid residue in the active site of each monomer that participates in the actual mechanism of the enzyme. Glutathione peroxidase belongs to an enzyme family whose function is to detoxify peroxides in the cell. Because peroxides can decompose to form highly reactive radicals, the GPX enzymes play a critical role in protecting the cell from free radical damage, particularly lipid peroxidation.

Glutathione Peroxidase (GPX) Activity Assay Kit offers a user-friendly, reliable, and highly sensitive method for quantifying GPX activity in plasma, serum, cell lysates, and other biological fluid samples. In this assay, GPX reduces hydroperoxides, producing oxidized glutathione (GSSG), which is then recycled back to its reduced state by glutathione reductase (GR) and NADPH. The reduction of GSSG to GSH is accompanied by the oxidation of NADPH to NADP+, leading to a decrease in absorbance at 340nm. The rate of decrease in the absorbance is directly proportional to the GPX activity in the sample.

Storage

The entire kit may be stored at -20 °C or below for up to 6 months from the date of shipment. For prepared reagent storage, see table below.

Component	Size / Description	Storage After Preparation
Microplate	A 96-well (12 strips x 8 wells) plate	RT*
Assay Buffer	25 ml	2–8 °C for 4 weeks
Co-Substrate Mixture	2 Vials (Lyophilized)	Use immediately
NADPH	2 Vials (Lyophilized)	Use immediately
Glutathione Peroxidase (GPX) Positive Control	1 Vial (Lyophilized)	Aliquot and store at -20°C
Cumene Hydroperoxide	1 Vial (100 µL)	2–8 °C for 1 week

RT = room temperature

Additional Materials Required

1. Microplate reader capable of measuring absorbance at 340 nm at 25°C
2. Precision pipettes to deliver 2 µl to 1 ml volumes
3. Multi-channel pipettes to deliver 20 µl to 200 µl volumes
4. Tubes to prepare sample dilutions
5. 15 ml conical tubes
6. Incubator at 25°C
7. Deionized water

Sample Tips and General Considerations

NOTE: Optimal methods of sample preparation will need to be determined by each researcher empirically based on researched literature and knowledge of the samples.

- If not using fresh samples, freeze samples as soon as possible after collection.
- Avoid multiple freeze-thaw cycles. If possible, sub-aliquot samples prior to initial storage.
- It is strongly recommended to add a protease inhibitor cocktail to cell and tissue lysate samples.
- Avoid sonication of 1 ml or less as this can quickly heat and denature proteins.
- Most samples will not need to be concentrated. If concentration is required, a spin column concentrator with a chilled centrifuge is recommended.

1. Cell lysates can be prepared as follows:

For attached cells, remove supernatant from cell culture, wash cells twice with cold 1X PBS (for suspension cells, pellet the cells by spinning down the cells at 1,000 x g for 10 min) making sure to remove any remaining PBS before adding lysis buffer. Solubilize the cells at $2x10^7$ cells/ml in lysis buffer containing protease inhibitors. Pipette up and down to resuspend cells and rock the lysates gently at 2–8 °C for 30 minutes. Transfer extracts to microfuge tubes and centrifuge at 14,000 x g for 10 minutes.
It is recommended that sample protein concentrations should be determined using a total protein assay. Lysates should be used immediately or aliquot and stored at -70 °C. Thawed lysates should be kept on ice prior to use.
General tips for preparing lysate samples can be viewed on the online Resources page of the website: https://www.raybiotech.com/tips-on-sample-preparation/

2. Plasma samples:

Collect blood with an anticoagulant such as citrate, EDTA or oxalate and mix by inversion. Centrifuge the blood at 1000 x g at 4°C for 10 minutes. Collect plasma supernatant without disturbing the white buffy layer. Sample should be tested immediately or frozen at -80°C for storage. Plasma samples can be loaded neat. Use Assay Buffer to dilute if needed.

3. Serum samples:

Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 30 minutes. Centrifuge at 2500 x g for 20 minutes. Remove the yellow serum supernatant without disturbing the white buffy layer. Samples should be tested immediately or frozen at -80°C for storage. Serum samples can be loaded neat. Use Assay Buffer to dilute if needed.

NOTE:
If the calculated GPX activity of the sample is higher than 150 U/L, dilute the sample in Assay Buffer and repeat the assay.

Reagent Preparation

A. Working Solution

1. Co-Substrate Mixture: Briefly centrifuge the Co-Substrate Mixture vial. Then add 1.2mL Assay Buffer to each vial. Pipette up and down to mix gently.
2. NADPH: Briefly centrifuge the NADPH vial. Then add 600ul deionized water to the vial. Pipette up and down to mix gently.
3. **Working solution**: For half of a plate, mix 1.1ml reconstituted Co-Substrate Mixture, 550ul reconstituted NADPH, and 7.15ml of Assay Buffer to prepare the Working Solution. Mix well. Protect from light. Keep at 4 ^{o}C. The reconstituted reagent should be used within 4 hours.

B. Positive Control

Glutathione Peroxidase (GPX) Positive Control: Briefly spin down the vial, add 60 µL of Assay Buffer to the vial to make the diluted GPX positive control. Mix well.

C. Cumene Hydroperoxide Solution

Add 23.3 µL of Cumene Hydroperoxide into 10 mL deionized water to make Cumene Hydroperoxide Solution.

Assay Procedure

Positive control, samples, and Assay Buffer (used as a blank) should be assayed in duplicate or triplicate. A freshly prepared positive control should be used each time the assay is performed.

1. Prepare Working Solution (See Reagent Preparation, section A).
2. Prepare Positive Control (See Reagent Preparation, section B).
3. Prepare Cumene Hydroperoxide Solution (See Reagent Preparation, section C).
4. Add 20 µL of the diluted GPX positive control, Assay Buffer (served as a blank), or sample to each well of a 96-well microtiter plate.
5. Add 160 µL of Working Solution to each well. Mix thoroughly. Incubate for 5 min at 25 ^{o}C with gentle shaking.
6. After five minutes, initiate the reaction by adding 20 µL of the Cumene Hydroperoxide Solution into each well and mix thoroughly (it is recommended to use a multi-channel pipette).
7. Immediately after, read and record absorbance at 340nm every minute for 5 time points at 25 ^{o}C (A1, A2, A3, A4, A5 Reading).

Calculation of Results

Calculate the mean absorbance for each set of duplicate/triplicate samples, Positive Control, and Assay Buffer blank.

Calculate the average absorbance difference per minute ΔOD_{340nm}/min.

$$\Delta OD_{340nm}/min = \frac{(A1-A2) + (A2-A3) + (A3-A4) + (A4-A5)}{4}$$

Subtract the absorbance difference per minute of the Blank wells from absorbance difference per minute of the sample and positive control, this is the corrected absorbance difference per minute.

$$\Delta OD'_{340nm}/min = \Delta OD_{340nm}/min - \Delta OD^{Blank}_{340nm}/min$$

One international Unit (U/L) is defined as the amount of enzyme that catalyzes the transformation of one micromole of substrate per minute.

$$GPX\ activity\ (U/L) = \frac{\Delta OD'_{340nm}/min \times 200\ \mu l \times 1000}{3.66\ mM^{-1} \times 20\ \mu l} \times sample\ dilution$$

Where: $\Delta OD'_{340nm}$ */min* = Corrected absorbance change per minute

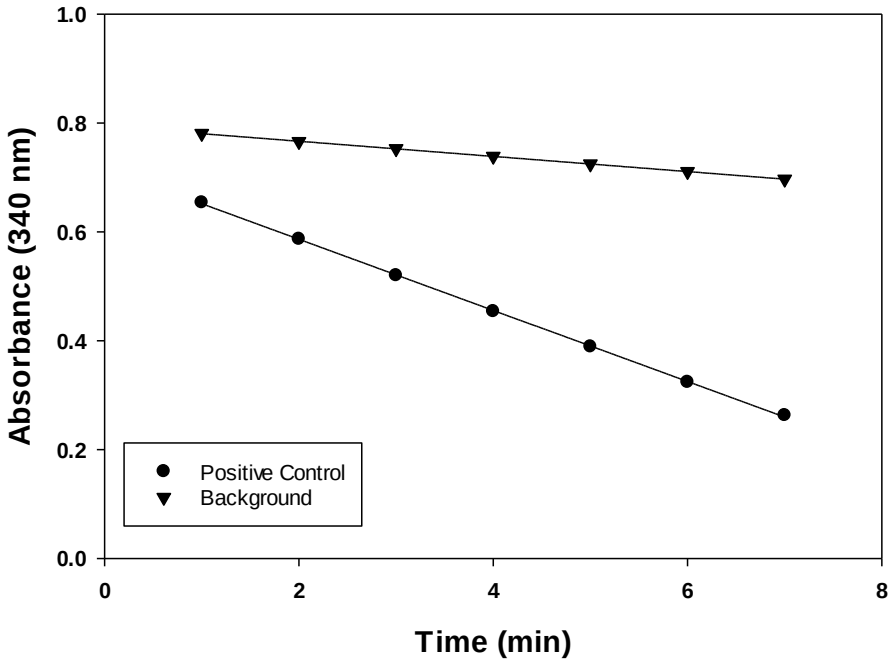
1000 = Conversion of U/ml to U/L

3.66 mM^{-1} is the adjusted extinction coefficient for NADH at 340nm with the current path length of the solution in a 96-well plate.

*GPX activity International System of Units (SI Units) (nkat/L) = GPX activity (U/L)*16.67*

Note: If the calculated GPX activity of the sample is higher than 150 U/L, dilute the sample in Assay Buffer and repeat the assay.

A. Typical Data



Example of OD values of Glutathione Peroxidase (GPX) Positive Control verse Time. This example is for demonstration purposes only. Results may vary.

B. Linear Range

0.5 U/L to 150 U/L GPX activity

C. Reproducibility

Intra-assay Precision (Precision within an assay):
To assess intra-assay precision, 16 wells per sample (total of 4 samples) were tested on a single plate. The intra-assay coefficient of variation was found to be 5.1%.

Inter-assay Precision (Precision between assays):
To assess inter-assay precision, 4 samples were tested in separate assays (n=4). The inter-assay coefficient of variation was found to be 9.1%.

This product is for research use only.