# Amplite® Fluorimetric Glutathione Peroxidase Assay Kit *Blue Fluorescence*

Catalog number: 11560
Unit size: 200 Tests

| Component | Storage | Amount (Cat No. 11560) |
|---|---|---|
| Component A: Glutathione Peroxidase Standard | Freeze (< -15 °C), Minimize light exposure | 1 vial (0.5 U/vial) |
| Component B: Assay Buffer | Freeze (< -15 °C) | 1 bottle (10 mL) |
| Component C: Enzyme Mix | Freeze (< -15 °C), Minimize light exposure | 2 bottles (lyophilized powder) |
| Component D: GSH | Freeze (< -15 °C), Minimize light exposure | 1 vial (3 mg/vial) |
| Component E: GPx Substrate | Freeze (< -15 °C), Minimize light exposure | 1vial (11 µL/vial) |
| Component F: Quest Fluor™ NADP Probe | Freeze (< -15 °C), Minimize light exposure | 1 bottle (5 mL) |
| Component G: NADP Assay Solution | Freeze (< -15 °C), Minimize light exposure | 1 bottle (5 mL) |
| Component H: Enhancer Solution | Freeze (< -15 °C), Minimize light exposure | 1 bottle (3.5 mL) |

## OVERVIEW

Glutathione peroxidase is an enzyme family with peroxidase activity to protect the organism from oxidative damage. Glutathione peroxidase plays an important role in reducing organic hydroperoxides such as lipid hydroperoxides to their corresponding alcohols, or reducing free hydrogen peroxide to water. Glutathione peroxidase guards against oxidative damage to cell membranes and other oxidant-sensitive sites in cells. The altered glutathione peroxidase levels correlate with lesions caused by many common and complex diseases. Glutathione peroxidase level is measured in biological samples as a potential indicator for the potential treatment of cancer, diabetes, neurodegenerative and cardiovascular diseases. AAT Bioquest's Fluorimetric Glutathione Peroxidase Assay Kit offers a sensitive fluorimetric assay for measuring glutathione peroxidase levels in biological samples. This assay is based on the oxidation of glutathione (GSH) to oxidized glutathione (GSSG) catalyzed by glutathione peroxidase. The generated GSSG is recycled to its reduced state GSH by glutathione reductase (GR) and NADPH to generate $NADP^+$ that can be specifically monitored using Quest Fluor™ NADP Probe, our newly developed proprietary NADP sensor. The NADP sensor reacts only with NADP to generate a fluorescent product. The fluorescence signal can be measured with a fluorescence microplate reader and is directly proportional to the glutathione peroxidase activity. Compared to other commercial kits that measure the decrease in absorbance of NADPH at 340 nm, our Quest Fluor™ NADP Probe can be used for quantify NADP level directly. With this fluorimetric glutathione peroxidase assay, as low as 1.25 mU/mL glutathione peroxidase can be detected in a 155 µL reaction volume.

## AT A GLANCE

### Protocol Summary

1. Prepare GPx standards or test samples (50 µL)
2. Add GPx working solution (50 µL)
3. Incubate at room temperature for 30 min
4. Add 20 µL Quest Fluor™ NADP Probe
5. Add 20 µL NADP Assay Solution
6. Incubate at room temperature for 10 - 20 min
7. Add 15 µL Enhancer Solution
8. Incubate at room temperature for 30 - 60 min
9. Record Fluorescence at Ex/Em= 420/480nm (Cutoff = 430 nm)

### Important Note

To achieve the best results, it's strongly recommended to use the black plates. Thaw one vial of each kit component at room temperature before starting the experiment.

## KEY PARAMETERS

**Fluorescence microplate reader**

| | |
|---|---|
| Cutoff | 430 nm |
| Emission | 480 nm |
| Excitation | 420 nm |
| Recommended plate | Solid black |

## PREPARATION OF STOCK SOLUTIONS

*Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles*

### Glutathione Peroxidase (GPx) standard solution (10 U/mL)

Add 50 µL of $ddH_2O$ or 1× PBS buffer into the vial of GPx standard (Component A) to make 10 U/mL standard solution.

### GSH stock solution (100X)

Add 100 µL of $ddH_2O$ into the vial of GSH (Component D) to make 100X GSH stock solution.

### GPx Substrate stock solution (100X)

Add 100 µL of $ddH_2O$ into the vial of substrate (Component E) to make 100X GPx Substrate stock solution.

## PREPARATION OF STANDARD SOLUTIONS

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/11560

### GPx standard

Add 10 µL of GPx standard solution (10 U/mL) into 990 µL 1x PBS buffer + 0.1% BSA to generate 100 mU/mL GPx standard solution (GP7). Take 100 mU/mL GPx standard solution (GP7) and perform 1:1.5 serial dilutions in PBS + 0.1% BSA to get serial dilutions of GPx standard (GP6 - GP1). Note: Diluted GPx standard solution is unstable, and should be used within 4 hours.

## PREPARATION OF WORKING SOLUTION

1. Add 5 mL of Assay Buffer (Component B) into a bottle of Enzyme Mix (Component C).

2. Add 50 µL GSH stock solution (Component D), 50 µL GPx

Substrate stock solution (Component E) into the bottle of Component B+C, and mix well to make GPx working solution (Component B+C+D+E). *Note:* This GPx working solution is enough for one 96-well plate. It is not stable; please use it promptly. It is not recommend storing unused GPx working solution.

## SAMPLE EXPERIMENTAL PROTOCOL

**Table 1.** Layout of GPx standards and test samples in a solid black 96-well microplate. GP= GPx Standards (GP1 - GP7, 8.78 to 100 mU/mL), BL=Blank Control, TS=Test Samples.

| BL | BL | TS | TS |
|---|---|---|---|
| GP1 | GP1 | ... | ... |
| GP2 | GP2 | ... | ... |
| GP3 | GP3 | | |
| GP4 | GP4 | | |
| GP5 | GP5 | | |
| GP6 | GP6 | | |
| GP7 | GP7 | | |

**Table 2.** Reagent composition for each well.

| Well | Volume | Reagent |
|---|---|---|
| GP1 - GP7 | 50 µL | Serial Dilution (8.78 to 100 mU/mL) |
| BL | 50 µL | 1X PBS Buffer + 0.1% BSA |
| TS | 50 µL | Test Sample |

**Run GPx assay:**

1. Prepare GPx standards (GP), blank controls (BL), and test samples (TS) according to the layout provided in Tables 1 and 2. For a 384- well plate, use 25 µL of reagent per well instead of 50 µL.
2. Add 50 µL of GPx working solution to each well of GPx standard, blank control, and test samples to make the total assay volume of 100 µL/well. For a 384-well plate, add 25 µL GPx working solution into each well instead, for total of 50 µL/well.
3. Incubate the reaction at room temperature for 30 minutes, protected from light.

**Run NADP assay:**

1. Add 20 µL Quest Fluor™ NADP Probe (Component F) into each well of GPx standard, blank control, and test samples, mix well.
2. Add 20 µL NADP Assay Solution (Component G) into each well, mix well. For a 384-well plate, add 25 µL of sample and 10 µL of Quest Fluor™ NADP Probe (Component F) and 10 µL NADP Assay Solution (Component G) into each well.
3. Incubate the reaction at room temperature for 10 - 20 minutes, protected from light.
4. Add 15 µL Enhancer (Component H) to each well to make the total assay volume of 155 µL/well, and incubate at room temperature for 30 - 60 minutes, protected from light. For a 384-well plate, add 7.5 µL Enhancer.
5. Monitor the fluorescence increase with a fluorescence plate reader at Ex/Em = 420/480 nm (Cutoff = 430nm).

## EXAMPLE DATA ANALYSIS AND FIGURES

Placeholder for image details

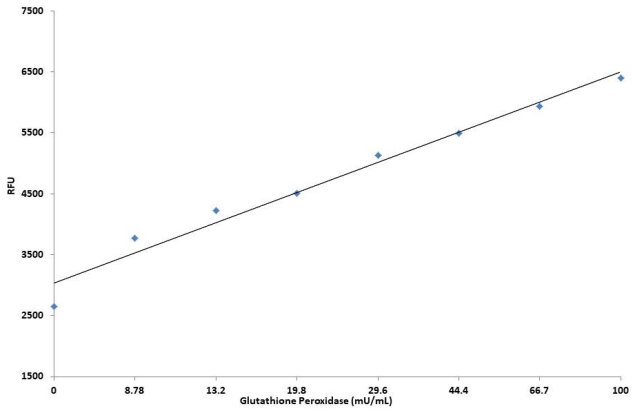


**Figure 1.** Glutathione Peroxidase (GPx) dose response was measured with Amplite® Fluorimetric Glutathione Peroxidase Assay Kit (Cat#11560) on a solid black 96-well plate using a Gemini microplate reader (Molecular Devices).

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