Amplite™ Fluorimetric Glutathione Peroxidase Assay Kit

Blue Fluorescence

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 11560 (200 tests)	Keep in freezer and protect from light	Fluorescence microplate readers

Introduction

Glutathione peroxidase (GPx) is an enzyme family with peroxidase activity to protect the organism from oxidative damage. GPx plays an important role in reducing organic hydroperoxides such as lipid hydroperoxides to their corresponding alchols, or reducing free hydrogen peroxide to water. It therefore guards against oxidative damage to the cell membranes and other oxidant-sensitive sites in the cell. It has been noticed that altered GPx levels correlate with lesions caused by many comment and complex diseases. GPx 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 GPx levels in biological samples. This assay is based on the oxidation of glutathione (GSH) to oxidized glutathione (GSSG) catalyzed by GPx. The generated GSSG is recycled to its reduced state GSH by glutathione reductase (GR) and NADPH:

$$\text{R-O-O-H} + 2\text{GSH} \xrightarrow{\text{GPx}} \text{R-O-H} + \text{GSSG} + \text{H}_2\text{O}$$

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

The product $NADP^+$ 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 at Ex/Em= 420/480 nm, which is directly proportional to the GPx 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 GPx assay, we were able to detect as low as1.25 mU/mL GPx in a 155 µL reaction volume.

Kit Components

Components	**Amount**
Component A: Glutathione Peroxidase Standard	1 vial (0.5 U/vial)
Component B: Assay Buffer	1 bottle (10 mL)
Component C: Enzyme Mix	2 bottles (lyophilized powder)
Component D: GSH	1 vial (3mg/vial)
Component E: GPx Substrate	1vial (11 µL/vial)
Component F: Quest Fluor™ NADP Probe	1 bottle (5 mL)
Component G: NADP Assay Solution	1 bottle (5 mL)
Component H: Enhancer Solution	1 bottle (3.5 mL)

Assay Protocol for One 96-Well Plate

Brief Summary

Prepare GPx assay mixture (50 µL)→ Add GPx standards or test samples (50 µL)→ Incubate at RT for 30 min→ Add 20 µL Quest Fluor™ NADP Probe→ Add 20 µL NADP Assay Solution→ Incubate at RT for 10-20 min→ Add 15 µL Enhancer Solution→ Incubate at RT for 30-60 min→ Record Fluorescence at Ex/Em= 420/480nm

Note 1. To achieve the best results, it's strongly recommended to use the black plates.
Note 2. Thaw one vial of each kit component at room temperature before starting the experiment.

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1. Prepare Glutathione Peroxidase (GPx) standard stock solution:

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

Note: The unused GPx *standard stock solution should be divided into single use aliquots and stored at -20°C.*

2. Prepare serial dilutions of GPx standard:

2.1 Add 4 µL of GPx standard stock solution (10 U/mL, from Step 1) into 996 µL 1×PBS buffer to generate standard solution at the concentration of 40 mU/mL.

Note: Diluted GPx *standard solution is unstable, and should be used within 4 hours.*

2.2 Take 200 µL of 40 mU/mL GPx standard solution to perform 1:2 serial dilutions to get approximately 20, 10, 5, 2.5, 1.25, 0.625 and 0 mU/mL serial dilutions of GPx standard.

2.3 Add serial dilutions of GPx standard and GPx containing test samples into a solid black 96-well microplate as described in Tables 1 and 2.

Table 1 Layout of GPx standards and test samples in a solid black 96-well microplate

BL	BL	TS	TS	….	….						
GP 1	GP 1	….	….	….	….						
GP 2	GP 2										
GP 3	GP 3										
GP 4	GP 4										
GP 5	GP 5										
GP 6	GP 6										
GP 7	GP 7										

Note: GP= GPx Standards, BL=Blank Control, TS=Test Samples.

Table 2 Reagent composition for each well

GPx Standard	**Blank Control**	**Test Sample**
Serial Dilutions*: 50 µL	1×PBS Buffer : 50 µL	50 µL

**Note: Add the serially diluted GPx standards from approximately 0.6 mU/mL to 40 mU/mL into wells from GP1 to GP7 in duplicate.*

3. Prepare GSH stock solution (100X):

Add 100 µL of ddH2O into the vial of GSH (**Component D**) to make 100X GSH stock solution.

4. Prepare GPx substrate stock solution (100X):

Make 100 µL of ddH_2O into the vial of substrate (**Component E**) to make 100X substrate stock solution.

5. Prepare GPx assay mixture:

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

5.2 Add 50 µL GSH stock solution (**Component D**, from Step 3), 50 µL substrate stock solution (**Component E**, from Step 4) into the bottle of **Component B+C** (from Step 5.1), and mix well to make GPx assay mixture (**Component B+C+D+E).**

Note 1: This GPx assay mixture is enough for one 96-well plate. It is not stable, please use it promptly.

Note 2: It is not recommend storing unused GPx assay mixture. One might divide the unused ***Components B+C*** *mixture (from Step 5.1) into single use aliquots and stored at -20°C although the sensitivity might decrease.*

Note3; Divide unused 100X GSH stock solution (from step 3), and 100X GPx substrate stock solution (from step 4) into single use aliquots and stored at -20°C.

6. Run GPx assay:

6.1 Add 50 µL of GPx assay mixture (from Step 5.2) to each well of GPx standard, blank control, and test samples (see Step 2.3) to make the total volume of 100 µL/well.

Note: For a 384-well plate, add 25 µL of sample and 25 µL of GPx assay mixture into each well.

6.2 Incubate the reaction at room temperature for 30 minutes, protected from light.

7. Run NADP assay:

7.1 Add 20 µL Quest Fluor™ NADP Probe (Component F) into each well of GPx standard, blank control, and test samples, mix well.

7.2 Add 20 µL NADP Assay Solution (Component G) into each well, mix well.
Note: 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.

7.3 Incubate the reaction at room temperature for 10-20 minutes, protected from light.

7.4 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.
Note: For a 384-well plate, add 7.5 µL Enhancer.

7.5 Monitor the fluorescence increase with a fluorescence plate reader at Ex/Em = 420/480 nm.

Data Analysis

The fluorescence reading in blank wells (with PBS buffer and GPx, NADP reaction mixtures only) is used as a control, and is subtracted from the values of those wells with the GPx standards and test samples. A GPx standard curve is shown in Figure 1. Calculate the GPx concentrations of the samples according to the GPx standard curve.

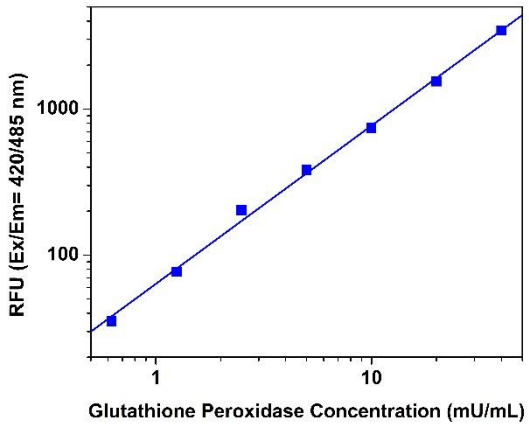

Figure 1. Glutathione Peroxidase (GPx) dose response was measured with the Fluorimetric Glutathione Peroxidase Assay Kit (Cat#11560) on a solid black 96-well plate using a Gemini microplate reader (Molecular Devices). As low as 1.25 mU/mL GPx can be detected with 30-60 minutes incubation (n=3).

Note: The fluorescence background increases with time, thus it is important to subtract the fluorescence intensity value of the blank wells for each data point.

References

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4. Beal MF. (1997) Oxidative damage in neurodegenerative diseases. The Neuroscientist.
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