EZ-Glutathione Peroxidase (GPx) Assay Kit

Oxidative Stress Assay Kit

Cat. No. DG-GPX100

FOR RESEARCH USE ONLY.

NOT FOR USE IN DIAGNOSTIC PROCEDURES.

DoGenBio Co., Ltd.

▪ Product Description

Glutathione peroxidase (GPx) is an enzyme that plays an important role in protecting organisms from damage caused by oxidative stress. Glutathione (GSH) is converted to GSSG, its oxidized form, by GPx and peroxide (such as Cumene Hydroperoxide, and others).

GSSG is then reduced back to GSH by the enzyme glutathione reductase (GR), which consumes NADPH. Therefore, since the activity of GPx is proportional to NADPH consumption, it can be determined by measuring the amount of NADPH at 340 nm.

The EZ-Glutathione Peroxidase (GPx) Assay Kit can accurately measure GPx activity in samples such as plasma, erythrocyte lysates, tissue homogenates, and cell lysates.

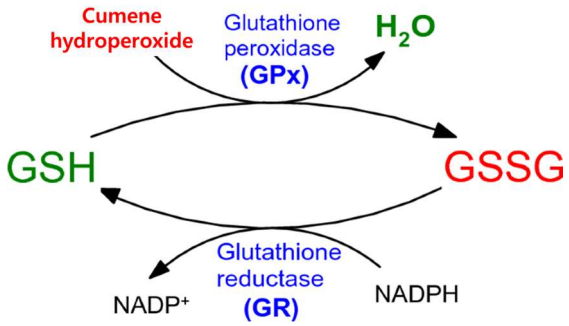

Fig. Activation mechanism of Glutathione peroxidase.

▪ Kit Contents and Storage Conditions

Component	100 assay	Storage
2X Cumene Hydroperoxide	5 ㎖	-20 ℃
Glutathione reductase (GR)	1 vial	
Glutathione peroxidase (GPx)	1 vial	
NADPH (30mM)	1 vial	
Glutathione (GSH)	1 vial	
GPx Assay Buffer (pH 7.0)	30 ㎖ X 2	

* This product should be used for research purposes only and should not be used for human or diagnostic purposes.

* Before use, ensure the product is brought to room temperature.

▪ Preparation of Reagent

Solution	Preparation	Storage and Stability
Glutathione reductase	Add 220 ㎕ assay buffer and mix well before using.	Dilute immediately before use. * After dilution, it can be stored for one month at -20°C or one week at 4°C.
Glutathione peroxidase	Add 110 ㎕ assay buffer and mix well before using.	
NADPH	Add 500 ㎕ D.W. and mix well before using.	
Glutathione	Add 220 ㎕ assay buffer and mix well before using.	
Cumene hydroperoxide	Mix 1:1 with the assay buffer and prepare before using.	

* This protocol is optimized for experiments/measurements using a 96-well plate.
* Ensure the Assay Buffer is fully dissolved before use.

▪ General Protocol

Standard preparation

Prepare 1 mM NADPH by mixing 25 ㎕ of a 40 mM NADPH standard solution with 975 ㎕ of D.W.
Mix 1 mM NADPH and assay buffer to match the total volume to 300 ㎕ as shown in the table below.

Standard No.	1mM NADPH Solution	Assay buffer	Total volume	NADPH Conc. (nmol/well)
1	0 ㎕	300 ㎕	300 ㎕	0
2	60 ㎕	240 ㎕	300 ㎕	20
3	120 ㎕	180 ㎕	300 ㎕	40
4	180 ㎕	120 ㎕	300 ㎕	60
5	240 ㎕	600 ㎕	300 ㎕	80
6	300 ㎕	0 ㎕	300 ㎕	100

* It is recommended to measure the standard alongside each experiment.

Dispense 100 ㎕ of each concentration into the wells in duplicate, then measure the absorbance at 340 nm.

Sample preparation

Note

- All sample concentrations should fall within the range of the Standard Curve. Prepare multiple sample concentrations for the experiment.
- It is recommended to use the samples immediately after preparation. If immediate analysis is not possible, store the samples in liquid nitrogen or a deep freezer (-80°C) and use them within one month.
- Conduct the experiment with the samples placed on ice.

1. Cell (adherent or suspension) samples and Tissue Samples

① Prepare the required number of cells for each experiment. (The following is a recommendation; please proceed according to your experimental conditions.)

- Cell (adherent or suspension): 2 X 10^6 cells
- Tissue Sample: 100 mg

② Wash the cells/tissue with cold PBS.

③ Add 200 ㎕ of cold Assay Buffer.

④ Homogenize quickly by pipetting several times on ice.

⑤ Centrifuge at 10,000 g for 15 minutes at 4°C to remove insoluble materials.

⑥ Transfer the supernatant to a new tube and use it for the experiment.

⑦ Use immediately or store in liquid nitrogen or a deep freezer (-80°C) and use within one month.

2. Erythrocytes

① Mix 200 ㎕ of the sample thoroughly with 200 µL of cold Assay Buffer.

② Centrifuge at 10,000 g for 15 minutes at 4°C to remove insoluble materials.

③ Transfer the supernatant to a new tube and either use it immediately or store it in liquid nitrogen or a deep freezer (-80°C) and use within one month.

3. Plasma and serum samples

① Plasma/serum samples can be diluted with Assay Buffer and measured directly without any pre-treatment.

② If the sample is not used immediately, store it in liquid nitrogen or a deep freezer (-80°C) and use it within one month.

Glutathione peroxidase Assay

① Dispense 2–10 ㎕ of the Glutathione peroxidase (GPx) solution (positive control) into the wells, and adjust the total volume to 50 ㎕ with Assay Buffer.

② For the Reagent Control (RC) wells, add 50 ㎕ of Assay Buffer.

③ Add 50 ㎕ of the prepared sample to each well according to the desired concentrations.

④ Reaction Mixture Preparation:

Prepare the reaction mix based on the volume needed for 1 assay, then calculate the total amount required for the experiment and prepare enough reaction mixture with one additional assay's volume (assay + 1).

Components	Reaction mixture
Assay Buffer	33 ㎕
30 mM NADPH solution	3 ㎕
GR solution	2 ㎕
GSH solution	2 ㎕
Total	**40 ㎕**

⑤ Add 40 ㎕ of the reaction mix solution to each well and incubate for 15 minutes to allow the reaction to occur, then remove the oxidized glutathione (GSSG).

- Ensure that the reaction is complete, as the presence of GSSG can interfere with accurate measurements

⑥ Measure the absorbance at 340 nm (A1).

- If the absorbance value of A1 is too low (e.g., below 0.7), dilute or dialyze the sample, or use a spin filter to remove GSSG from the sample before proceeding with the experiment.

⑦ Add 10 ㎕ of cumene hydroperoxide mixed with Assay Buffer in a 1:1 ratio to each well, mix well, and incubate at room temperature, protecting the wells from light. Allow the reaction to proceed for 5-20 minutes, then measure the absorbance at 340 nm (A2).

▪ Calculation

① Calculate the average absorbance values for each NADPH concentration.

② Plot a standard curve using the final NADPH concentration (X-axis) and the corresponding O.D. values (Y-axis) to obtain the linear equation.

③ Calculate the average absorbance for all samples.

④ Correct the absorbance values for the samples using the following formula:

$$\Delta A_{340nm} = \{(\text{Sample A1} - \text{Sameple A2}) - (\text{Reagent Conctrol A1} - \text{Reagent Conctrol A2})\}$$

⑤ Substitute the Δ340nm value into the NADPH linear equation to obtain B.

$$B = \frac{\Delta \text{A}_{340\text{nm}} - \text{intercet}}{\text{Slope}}$$

⑥ The GPx concentration of the sample is calculated as follows (nmol/min/mL = mU/mL).

$$GPx\ Activity = \frac{\text{B}}{(\text{T}_2 - \text{T}_1) \times \text{V}} \times \text{Sample dilution} = \text{nmol/min/mL} = \text{mU/mL}$$

T_1 = Time of the first reading (A1) (minutes).

T_2 = Time of second reading (A2) (minutes).

V = Pretreated sample volume added into the reaction well (mL).

- One unit of Glutathione peroxidase refers to the amount of enzyme that oxidizes NADPH to $NADP^+$ in 1 minute at 25°C.

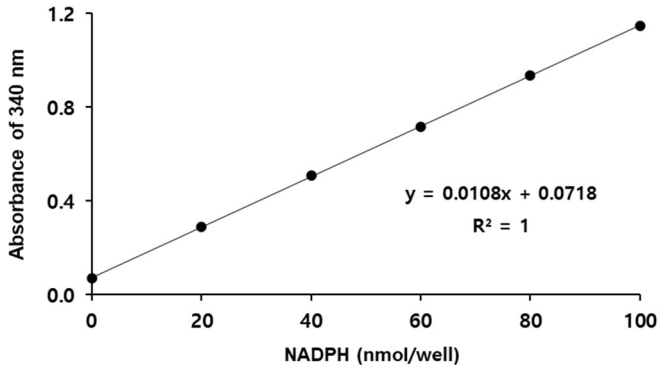

Fig. NADPH standard curve. Assay was performed following the kit protocol.

▪ Related Product

	Products	Catalog No.	Assay
Oxidative Stress Assay Kit	EZ-Superoxide Dismutase (SOD) Assay Kit (Colorimetric)	DG-SOD400	400 Assay
	EZ-Glutathione Assay Kit (Colorimetric)	DG-GLU200	200 Assay
	EZ-Catalase Assay Kit (Fluorometric/Colorimetric)	DG-CAT400	400 Assay
	EZ-Hydrogen peroxide/Peroxidase Assay Kit (Fluorometric/Colorimetric)	DG-PER500	500 Assay
	EZ-Lipid Peroxidation (TBARS) Assay Kit (Colorimetric)	DG-TBA200	200 Assay
	EZ-Total Antioxidant Capacity (TAC) Assay Kit (Colorimetric)	DG-TAC200	200 Assay
	EZ-DPPH Antioxidant Assay Kit (Colorimetric)	DG-DPH400	400 Assay
	EZ-ABTS Antioxidant Assay Kit (Colorimetric)	DG-ABT400	400 Assay
Metabolism Assay Kit	EZ-Lactate Assay Kit (Colorimetric)	DG-LAC100	100 Assay
	EZ-Acetylcholinesterase Assay Kit (Colorimetric)	DG-ACE100	100 Assay
	EZ-Ascorbic Acid Assay Kit (Colorimetric)	DG-ASC100	100 Assay
	EZ-ATP Assay Kit (Fluorometric/Colorimetric)	DG-ATP100	100 Assay
	EZ-Free Fatty Acid Assay Kit (Fluorometric/Colorimetric)	DG-FFA100	100 Assay
	EZ-Free Glycerol Assay Kit (Fluorometric/Colorimetric)	DG-FGC100	100 Assay
	EZ-Glucose Assay Kit (Fluorometric/Colorimetric)	DG-GCS100	100 Assay
	EZ-HDL, LDL/VDL Assay Kit (Fluorometric/Colorimetric)	DG-CHO100	100 Assay
	EZ-Total Cholesterol Assay Kit (Fluorometric/Colorimetric)	DG-TSC100	100 Assay
	EZ-Triglyceride Quantification Assay Kit (Fluorometric/Colorimetric)	DG-TGC100	100 Assay
	EZ-Nitric Oxide Assay Kit (Colorimetric)	DG-NO500	500 Assay
	EZ-Total Collagen Assay Kit (Colorimetric)	DG-COL100	100 Assay
	EZ-Ethanol Assay Kit (Colorimetric)	DG-ETH100	100 Assay