

Glutathione Peroxidase Assay Kit

(Catalog #K762-100; 100 reactions; Store kit at -20°C)

- 82 µl Assay Buffer
- 4 µl 40 mM NADPH solution
- 2 µl GR solution
- 2 µl GSH solution

I. Introduction:

Glutathione Peroxidase (GPx, EC 1.11.1.9) is an enzyme family with peroxidase activity, and plays important role in protecting of organisms from oxidative damage. It converts reduced glutathione (GSH) to oxidized glutathione (GSSG), to reduce lipid hydroperoxides to their corresponding alcohols, or reduce free hydrogen peroxide to water. Several isozymes have been found in different cellular locations and with different substrate specificity. Low levels of GPx have been correlated with free radical related disorders. BioVision's Glutathione Peroxidase Assay Kit measures glutathione peroxidase (GPx) activity through a coupled reaction with glutathione reductase (GR). In the assay, GPx reduce Cumene Hydroperoxide, and oxidize GSH to GSSG. The generated GSSG is reduced to GSH with consumption of NADPH by GR. The decrease of NADPH is proportionally to GPx activity in the reactions. The decrease of NADPH can be easily measured by absorbance at 340 nm. The assay can be used to measure all of the glutathione dependent peroxidases in plasma, erythrocyte lysates, tissue homogenates, and cell lysates with detection sensitivity ~ 0.5 mU/ml of GPx in samples.

II. Kit Contents:

Components	K762-100	Cap Code	Part No.
GPx Assay Buffer	25 ml	WM	K762-100-1
NADPH	Lyophilized	Blue	K762-100-2
Glutathione Reductase	2 µl	Green	K762-100-3
Glutathione	Lyophilized	Brown	K762-100-4
Cumene Hydroperoxide	2 µl	Yellow	K762-100-5
GPx Positive Control	Lyophilized	Red	K762-100-6

III. Storage and Handling:

Store the kit at -20°C, protect from light. Warm the assay buffer to room temperature before use. Briefly centrifuge vials before opening. Read the entire protocol before performing the assay.

IV. Reagent Reconstitution and General Consideration:

NADPH: Dissolve with 0.5 ml dH₂O to get 40mM NADPH solution. It is sufficient for 100 assays.

GR: Dissolve one vial with 0.22 ml Assay Buffer; sufficient for 100 assays.

GSH: Dissolve one vial with 0.22 ml Assay Buffer; sufficient for 100 assays.

Cumene Hydroperoxide: Dissolve one vial with 1.25 ml Assay Buffer; sufficient for 100 assays.

GPx Positive Control: Dissolve with 100µl Assay Buffer, add 5-10 µl per well as the positive control; bring the volume to 100µl with Assay Buffer.

All the solutions are stable for at least 1 week at 4°C and 1 month at -20°C.

Ensure that the assay buffer is at room temperature before use. Keep samples, GR mix solution and GPx Positive Control on ice during the assay.

V. Glutathione Reductase Activity Assay:

1. Sample Preparations:

Homogenize 0.1 gram tissues, 10⁶ cells, or 0.2 ml erythrocytes on ice in 0.5 ml cold assay buffer; Centrifuge at 10,000 x g for 15 min at 4°C; Collect the supernatant for assay and store on ice, serum can be tested directly. Keep samples at -80°C for storage. Add 2-100 µl of the samples into a 96-well plate; bring the volume to 100 µl with Assay Buffer. We suggest testing several doses of your sample to make sure the readings are within the standard curve range.

2. NADPH Standard Curve:

Dilute 25µl of the 40 mM NADPH solution into 975 µl dH₂O to generate 1 mM NADPH standard. Add 0, 20, 40, 60, 80, 100 µl of the 1 mM NADPH Standard into 96-well plate in duplicate to generate 0, 20, 40, 60, 80, 100 nmol/well standard. Bring the final volume to 200µl with Assay Buffer. Measure O.D. 340 nm to plot the NADPH Standard Curve.

3. Reaction Mix:

Mix enough reagents for the number of assays to be performed. For each well, prepare 90 µl Reaction Mix:

Add 90 µl of the Reaction Mix to each test samples, mix well, and incubate for 15 minutes to deplete all GSSG in your sample^A. Add 10 µl Cumene Hydroperoxide Solution to start GPx reaction. Mix well. Measure O.D. 340 nm at T1 to read A1^B, measure O.D.340 nm again at T2 after incubating the reaction at 25°C for 5 min (or longer if the GPx activity is low) to read A2^C, protect from light. $\Delta A_{340\text{ nm}} = A1 - A2$. Sometimes background is high in samples, we recommend setting background control with 10 µl assay buffer instead of Cumene Hydroperoxide Solution when start GPx reaction.

Notes:

A. Measure the O.D.340 nm before adding Cumene Hydroperoxide. Add more NADPH if the O.D. at 340 nm is lower than 1.0 to ensure there is enough NADPH in the reaction system. 1 µl of 40 mM NADPH will give ~0.5 O.D. at 340 nm.

B. If A1 reading is too low (<0.7), it means either too much GPx or too much GSSG presence in the sample. You may need to dilute the samples, or remove GSSG from your sample using methods, such as dialyzing the sample or using spin filters (BioVision Cat.# 1997-25) to remove GSSG.

C. It is essential to read A1 and A2 in the reaction linear range. It will be more accurate if you read the reaction kinetics. Then choose A1, A2, in the reaction linear range.

4. Calculation:

Plot NADPH standard Curve. Apply the $\Delta A_{340\text{nm}}$ to the NADPH standard curve to get NADPH amount B and B⁰, B for Sample and B⁰ for Sample background control (samples without Cumene Hydroperoxide).

$$\text{GPx Activity} = \frac{(B - B^0)}{(T_2 - T_1) \times V} \times \text{Sample dilution} = \text{nmol/min/ml} = \text{mU/mL}$$

Where: B is the NADPH amount that was decreased between T1 and T2 (in nmol).

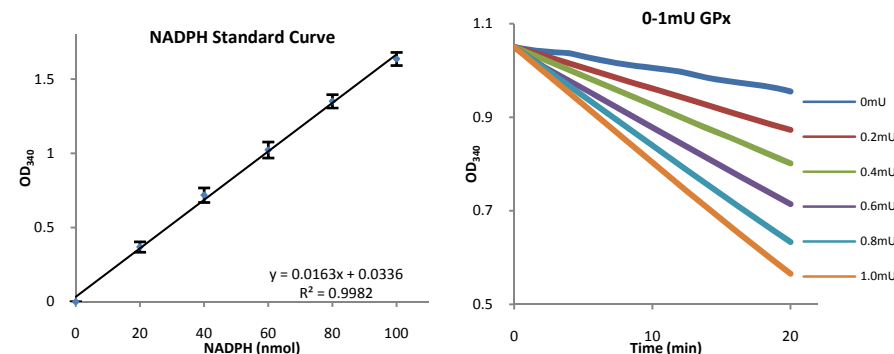
B⁰ is the background change (without Cumene Hydroperoxide) between T1 and T2.

T1 is the time of first reading (A1) (in min).

T2 is the time of second reading (A2) (in min).

V is the pretreated sample volume added into the reaction well (in ml).

One unit is defined as the amount of enzyme that will cause the oxidation of 1.0 µmol of NADPH to NADP⁺ under the assay kit condition per minute at 25°C.



VI. Related Products:

- Glutathione Reductase Assay Kit
- Colorimetric Glutathione Detection Kit
- GST Assay Kit
- Acid Phosphatase Assay Kit
- Phosphate Assay Kit
- Pyruvate Assay Kit
- Glutamate Assay Kit
- Ethanol Assay Kit

- Catalase Assay Kit
- Glutathione Kit (GSH, GSSG and Total)
- Triglyceride and Fatty Acid Assay Kit
- ADP/ATP Ratio Assay Kit
- NAD(P)/NAD(P)H Quantification Kit
- Lactate Assay Kit/ II
- Glucose Assay Kit
- Uric Acid Assay Kit