Glutathione Peroxidase Assay Kit

Catalog Number KA0882

100 assays

Version: 03

Intended for research use only

www.abnova.com

Table of Contents

Introduction

Background

Glutathione Peroxidase (GPx, EC 1.11.1.9) is an enzyme family with peroxidase activity, and plays important role in protection of organisms from oxidative damage. GPx converts reduced glutathione (GSH) to oxidized glutathione (GSSG), to reduce lipid hydroperoxides to their corresponding alcohols or 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. 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 (easily measured at 340 nm) is proportionally to GPx activity. The assay can be used to measure all of the glutathione dependent peroxidases in plasma, erythrocyte lysates, tissue homogenates, and cell lysates with a detection sensitivity ~ 0.5 mU/ml of GPx in samples.

General Information

Materials Supplied

List of component

Component	Amount
GPx Assay Buffer	25 ml
NADPH (lyophilized)	1 vial
Glutathione Reductase	1 vial
Glutathione (GSH; lyophilized)	1 vial
Cumene Hydroperoxide	1 vial
GPx Positive Control (lyophilized)	1 vial

Storage Instruction

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.

Assay Protocol

Reagent Preparation

- NADPH: Reconstitute with 0.5 ml dH_2O to get 40mM NADPH solution.
- GR: Dilute with 0.22 ml Assay Buffer.
- GSH: Reconstitute with 0.22 ml Assay Buffer.
- Cumene Hydroperoxide: Dilute with 1.25 ml Assay Buffer. Mix well.
- GPx Positive Control: Reconstitute with 100µl 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.

Assay Procedure

1. Sample Preparations

   Homogenize 0.1 gram tissues, 10^6 cells, or 0.2 ml erythrocytes on ice in 0.2 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 2-50 µl of the samples into a 96-well plate; bring the volume to 50 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 25 µl of the 40 mM NADPH solution into 975 µl dH_2O 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 100 µl with Assay Buffer. Measure O.D. 340 nm to plot the NADPH Standard Curve.
3. Positive Control (optional) and Reagent Blank

   For Positive Control use 5 - 10 µl of the GPx Positive Control into the desired well(s) and adjust to 50 µl with Assay Buffer. Add 50 µl of Assay Buffer into a well (s) as a Reagent Control (RC).
4. Reaction Mix

   For each well, prepare 40 µl Reaction Mix:

   33 µ Assay Buffer

   3 µl 40 mM NADPH solution

   2 µl GR solution

   2 µl GSH solution

   Add 40 µl of the Reaction Mix to each test samples, Positive control (s) and RC (s) mix well, and incubate for 15 minutes to deplete all GSSG in your sample. Add 10 µl Cumene Hydroperoxide Solution to start

GPx reaction. Mix well. Measure O.D. 340 nm at T1 to read A1, 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, protect from light. $\Delta A_{340\ nm}$ = [(Sample_A1-Sample_A2) – (RC_A1 – RC_A2)]

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 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.

Data Analysis

Calculation of Results

Plot NADPH standard Curve. Apply the ΔA_{340nm} to the NADPH standard curve to get NADPH amount B.

$$\text{GPx Activity} = \frac{B}{(T2 - T1) \times V} \times \text{Sample dilution} = \text{nmol/min/mL} = \text{mU/mL}$$

Where:

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

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).

Unit Definition: 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.