Glutathione Peroxidase (GPx) Assay
Cat. No. 8238, 100 tests

Introduction

Glutathione peroxidase (GPx) is an antioxidant enzyme located in cytoplasma and mitochondria. It catalyzes the reduction of hydrogen peroxide (H_2O_2) and a variety of organic hydroperoxides (R-O-O-H) to water and the corresponding stable alcohols (R-OH) using glutathione as the reducing reagent, thus inhibiting the formation of free radicals and protecting the cells from oxidative damage. The ScienCell™ GPx Assay provides an indirect method to quantify the activity of total glutathione peroxidase. In this assay, oxidized glutathione, produced upon reduction of peroxide substrate by glutathione peroxidase, is then recycled back to glutathione using glutathione reductase and NADPH. The oxidation of NADPH to $NADP^+$ is monitored by a decrease in the absorbance at 340 nm. Since all other reagents are provided in excess, the GPx activity is rate-limiting. The rate of decrease in A_{340nm} is directly proportional to the GPx activity. In this assay, cumene hydroperoxide is used as the peroxide substrate, so the total GPx (selenium and non-selenium containing) activity in a variety of samples can be measured.

Kit Components

Cat. No.	# of vials	Reagent	Amount	Storage
8238a	1	GPx Assay Buffer, 10×	8 ml	2-8˚C
8238b	1	GPx Sample Buffer	12 ml	2-8˚C
8238c	5	β-NADPH	Powder	2-8˚C
8238d	5	Glutathione	Powder	2-8˚C
8238e	1	Glutathione reductase	50 µl	-20˚C
8238f	1	Cumene Hydroperoxide (10 mM)	8 ml	-20˚C
8238g	1	GPx Standard (125 mU/ml)	1.25 ml	-20˚C

Quality Control

Serially diluted GPx solutions with concentrations ranging from 62.5 to 3.9 mU/ml are measured with the ScienCell™ Glutathione Peroxidase Assay. The decrease in A_{340nm} is monitored as a function of time (Figure 1), and the resulting standard curve of ΔA_{340nm}/min vs. GPx concentration are plotted (Figure 2). A positive linear relationship between ΔA_{340nm}/min & GPx concentration can be observed.

Procedures

A. Preparation of reagents

1. Reconstitute each vial of β-NADPH with 2 ml DI H_2O. Store the solution at 2-8˚C and use it within 3 hours. Do not freeze. Each vial is sufficient for 20 tests.
2. Reconstitute each vial of glutathione with 2 ml DI H_2O. Store the solution at 2-8˚C and use it within 3 hours. Do not freeze. Each vial is sufficient for 20 tests.

B. Preparation of GPx standards

1. Obtain 6 test tubes, add 250 µl of GPx Sample Buffer into each tube and label them #1 through #6.

2. Add 250 µl of the 125 mU/ml GPx solution into tube #1 and mix well to get the 62.5 mU/ml GPx standard.
3. Transfer 250 µl of the 62.5 mU/ml GPx standard from tube #1 to tube #2 and mix well to get the 31.3 mU/ml GPx standard.
4. Repeat step 3 for tubes #3-5 to serially dilute the GPx standards. Do not add any GPx to tube #6, which serves as the blank.
5. Obtain a 48-well test plate, prepare 3 replicates (A, B, C) of each GPx standard by aliquoting 80 µl/well of each GPx standard into triplicate wells of the 48-well test plate, according to the following plate format:

	#1	**#2**	**#3**	**#4**	**#5**	**#6**
A	62.5 mU/ml	31.3 mU/ml	15.6 mU/ml	7.8 mU/ml	3.9 mU/ml	Blank
B	62.5 mU/ml	31.3 mU/ml	15.6 mU/ml	7.8 mU/ml	3.9 mU/ml	Blank
C	62.5 mU/ml	31.3 mU/ml	15.6 mU/ml	7.8 mU/ml	3.9 mU/ml	Blank

C. Sample preparation

1. Collect cells by centrifuge. For adherent cells, do not use detergents containing high levels of endogenous peroxidase, such as TWEEN® 20 and TRITON® X-100, rather use a rubber policeman. Wash the cell pellet once with PBS. Count the number of cells.
2. Resuspend cells in pre chilled GPx Sample Buffer at 1×10^6-1×10^7 cells/ml. Leave the cells on ice for 15 minutes with gentle agitation.
3. Centrifuge at 14,000 × g in pre-cooled centrifuge for 3 minutes, transfer the supernatant to a fresh tube and discard the pellet. Prepare at least 80 µl cell lysate for each sample, used immediately or stored at -80 ºC.

D. Assay procedure

1. Prepare enough reaction mixture according to the number of tests to be performed. For each test, mix 360 µl of H_2O, 80 µl of 10× GPx Assay Buffer, 100 µl of reconstituted β-NADPH, 100 µl of reconstituted glutathione and 0.5 µl of glutathione reductase.
2. Add 640 µl of the reaction mixture to each well of the 48-well plate containing 80 µl of GPx standard or test sample.
3. Initiate the reaction by adding 80 µl of cumene hydroperoxide solution to each well as quickly as possible. Carefully shake the plate to mix well. The total volume in each well should be 0.8 ml.
4. Follow the decrease in A_{340nm} using a plate reader. Read the absorbance once every minute for at least 4 minutes.

E. Calculations

1. Plot the absorbance at 340 nm as a function of time to obtain the slope (ΔA_{340nm}/min) of the linear portion of the curves (e.g. Figure 1).
2. Plot a GPx standard curve of ΔA_{340nm}/min vs. GPx concentration (e.g. Figure 2).
3. Determine the GPx activity of the samples by comparing to the GPx standard curve.

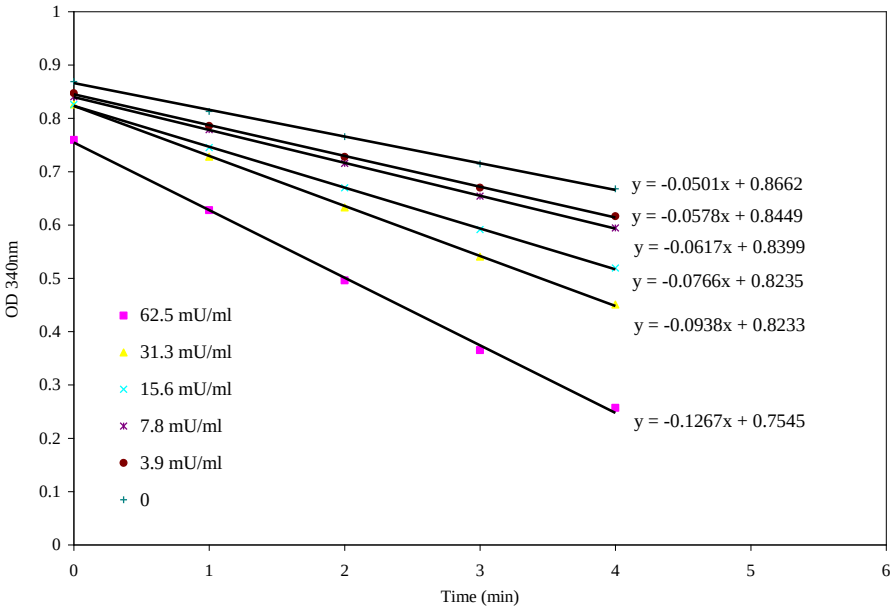

Figure 1. Absorbance at 340 nm as a function of time (interval: 1 min; Number of reading: 5).

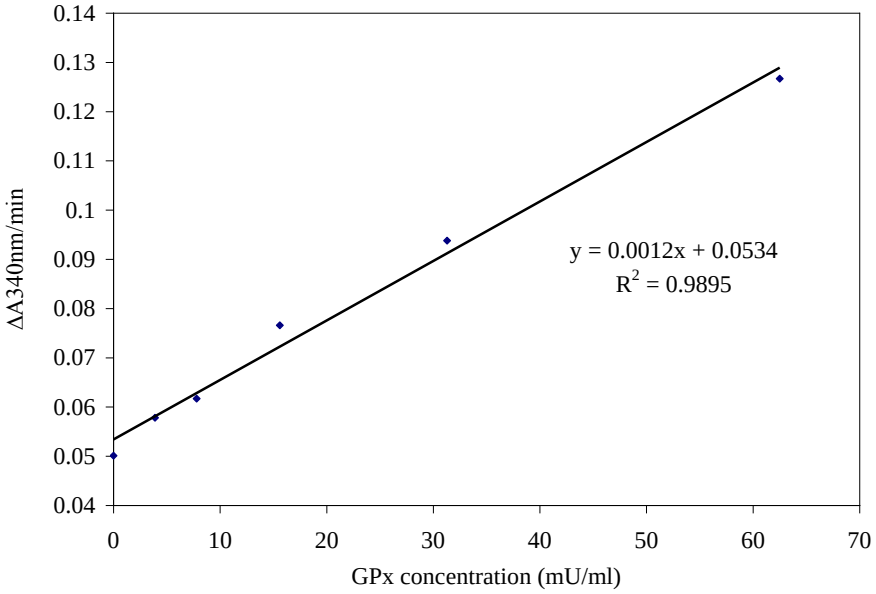

Figure 2. GPx standard curve of ΔA_{340nm}/min vs. GPx concentration.