Glutathione Peroxidase Assay Kit (Colorimetric)

LS-K197-100 (100 Tests) • **Store at -20°C**

LSBio
LifeSpan BioSciences, Inc.

Introduction

GLUTATHIONE PEROXIDASE represents an enzyme family with peroxidase activity whose main biological role is to protect the organism from oxidative damage. It helps prevent lipid peroxidation of cellular membranes by removing free peroxide in the cell. GPX catalyzes the following reaction with glutathione reductase

$$2GSH + ROOH \xrightarrow{GPX} GSSG + ROH + H_2O, \quad GSSG + NADPH \xrightarrow{GR} 2GSH + NADP^+$$

Simple, direct and high-throughput assays for GPX activity find wide applications. LSBio's improved assay directly measures NADPH consumption in the enzyme coupled reactions. The measured decrease in optical density at 340 nm is directly proportional to the enzyme activity in the sample.

Key Features

- Sensitive and accurate. Use 10 µL sample. Linear detection range 40 to 800 U/L GPX activity.

Applications

- Direct Assays: GPX activity in biological samples.
- Drug Discovery/Pharmacology: effects of drugs on GPX activity.

Components

Component	K197-100
	100 Tests
Assay Buffer	25 mL
GR Enzyme	250 µL
Glutathione	Dried
NADPH	Dried
Calibrator	Dried
Peroxide Solution	50 µL

Materials Not Supplied

Pipetting devices, centrifuge tubes, clear flat-bottom uncoated 96-well plates, plate reader capable of reading optical density at 340 nm every minute, homogenizer, etc.

Storage

The kit is shipped on ice. Store all kit components at -20 °C.

Glutathione Peroxidase Assay Kit (Colorimetric)

LS-K197-100 (100 Tests) • **Store at -20°C**

LSBio
LifeSpan BioSciences, Inc.

Sample Preparation

All samples should be clear and free of any turbidity or particles. Liquid samples (e.g. non-hemolyzed serum, plasma) can be assayed directly.

Homogenize tissue (10 mg) and cells (106) in 200 µL cold PBS and then centrifuge 10 min at 14,000 rpm to pellet any debris. Use the clear supernatant for the assay. If not assayed immediately, freeze supernatant at -80°C (stable for 1 month).

Assay Procedure

1. Reagent Preparation. Equilibrate all components to room temperature. Briefly centrifuge all tubes before opening.

   Reconstitute the NADPH tube with 400 µL dH_2O (final 35 mM).

   Reconstitute the Glutathione tube with 540 µL dH_2O (final 100 mM). Reconstitute the Calibrator with 100 µL dH_2O (final 100 mM). Vortex tubes to mix. Keep these reconstituted reagent tubes on ice. Unused reagents are stable for three weeks when stored frozen at -20°C.

2. Standards and Samples. Mix 12 µL of the Calibrator with 188 µL dH_2O (equivalent to 6 mM NADPH). Dilute this calibrator stock as shown in the Table below.

No	6 mM Calibrator + H_2O	Vol (µL)	[Equiv. NADPH] (mM)
1	100 µL + 0 µL	100	6.0
2	60 µL + 40 µL	100	3.6
3	30 µL + 70 µL	100	1.8
4	0 µL + 100 µL	100	0

   Transfer 10 µL standards into wells of a clear flat-bottom 96-well plate. Add 190 µL Assay Buffer to all standard wells. Transfer 10 µL of each sample into separate wells of the 96-well plate. In addition, for each assay run, include a background control that only contains 10 µL Assay Buffer.

   Note: For unknown samples, perform several dilutions to ensure that GPX activity is within the linear range of 40 to 800 U/L.

3. Assay. Prepare enough Working Reagent for all Sample and Control wells by mixing, for each well, 90 µL Assay Buffer, 5 µL Glutathione, 3 µL 35 mM NADPH and 2 µL GR enzyme. Add 90 µL Working Reagent quickly to the Sample/Control wells. Tap plate to mix.

   Transfer 5 µL Peroxide Solution into an EMPTY 1.5-mL Eppendorf tube. Add 1495 µL dH_2O and mix well by vortexing for at least 30 seconds. Immediately dilute this solution 1:10 in dH_2O to generate the 1X Substrate Solution. Use the diluted Solutions within one hour.

   With a multi-channel pipette, add 100 µL 1X Substrate Solution to all Sample and Control wells. Tap plate quickly to mix well contents thoroughly. Immediately read OD_{340nm} (time zero, OD_0) and again at 4 min (OD_4).

Glutathione Peroxidase Assay Kit (Colorimetric)

LS-K197-100 (100 Tests) • **Store at -20°C**

LSBio
LifeSpan BioSciences, Inc.

Calculations

Use OD values at 4 min for NADPH standards. Subtract blank value (#4) from the standard values. Plot the ΔOD against standard concentrations and determine the slope of the standard curve. Calculate the $\Delta OD_S = (OD_0 - OD_4)$ for the samples and $\Delta OD_B = (OD_0 - OD_4)$ for the background control. Calculate the GPX activity of Sample,

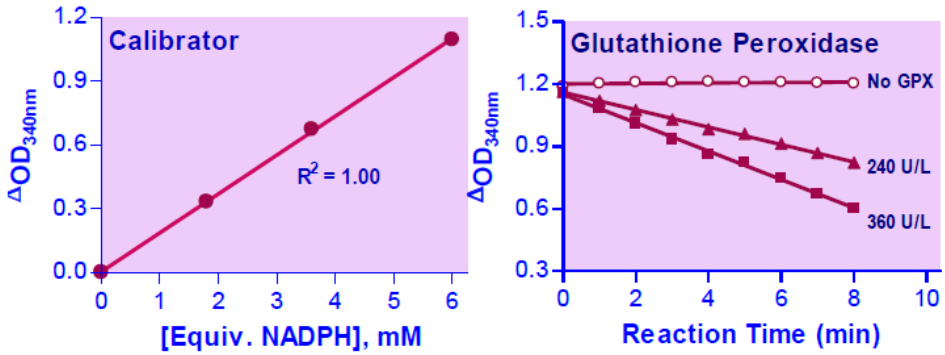
$$\text{GPX Activity (U/L)} = \frac{\Delta OD_S - \Delta OD_B}{\text{Slope (mM}^{-1}\text{) x 4 (min)}} \times 1000 \times n$$

The factor 1000 converts mmoles to µmoles. n is the sample dilution factor.

Note: if calculated GPX activity is higher than 800 U/L, or initial OD340nm is >1.5 in sample wells, dilute sample in dH_2O and repeat assay. Multiply the results by the dilution factor.

Unit definition: one unit is the amount of GPX that produces 1 µmole of GS-SG per min at pH 7.6 and room temperature.

Sample Data



Version: V.08.09.2018