DATA SHEET

EnzyChrom™ Glutathione Peroxidase Assay Kit (EGPX-100)

Quantitative Colorimetric Glutathione Peroxidase Determination

DESCRIPTION

GLUTATHIONE PEROXIDASE (GPX, EC 1.11.1.9) 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 (GR),

$$2\,GSH + H_2O_2 \xrightarrow{GPX} GS\text{-}SG + 2\,H_2O, \quad GS\text{-}SG + NADPH \xrightarrow{GR} 2\,GSH + NADP^+$$

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

KEY FEATURES

Sensitive and accurate. Use 10 µL sample. Linear detection range 12 to 300 U/L GPX activity.

APPLICATIONS

Direct Assays: GPX activity in biological samples.

Drug Discovery/Pharmacology: effects of drugs on GPX activity.

KIT CONTENTS (100 TESTS IN 96-WELL PLATES)

Assay Buffer: 25 mL **GR Enzyme**: 1 mL **Glutathione**: 240 µL
NADPH: 40 µL **H2O2 Solution**: 1 mL **Calibrator**: 100 µL
Positive Control: 9 µL Glutathione Peroxidase (GPX)

Storage conditions. The kit is shipped on ice. store all components at -20 ℃. Shelf life of three months after receipt.

Precautions: reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

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 1 x 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℃ (stable for 1 month).

ASSAY PROCEDURE

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

Add 360 µL dH_2O to the NADPH tube (final 35 mM). Add 500 µL Assay Buffer to the "Positive Control" tube. Vortex tubes to mix. Keep these reconstituted reagent tubes on ice. Unused reagents are stable for three weeks when stored frozen at -20℃.

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. Transfer 10 µL standards into wells of a clear flat-bottom 96-well plate. Add 190 µL Assay Buffer to all standard wells.

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

Transfer 10 µL sample and 10 µL reconstituted GPX Positive Control 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: (1). For unknown samples, perform several dilutions to ensure that GPX activity is within the linear range of 12 to 300 U/L. (2) The provided GPX serves as a positive control to ensure assay is working and should not be used to calculate the Sample GPX activity.

3. *Assay*. Prepare enough Working Reagent for Sample and Control wells by mixing, for each well, 85 µL Assay Buffer, 2 µL Glutathione, 2 µL 35 mM NADPH and 8 µL GR enzyme. Add 90 µL Working Reagent quickly to the Sample/Control wells. Tap plate to mix.

Dilute 8 µL 3% H_2O_2 with 1992 µL dH_2O (final 3.5 mM). Prepare enough 0.35 mM H_2O_2 Reagent by mixing, for each Sample/Control well, 12 µL 3.5 mM with 108 µL dH_2O. Use this Reagent within one hour.

With a multi-channel pipettor, add 100 µL 0.35 mM H_2O_2 Reagent 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).

CALCULATION

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,

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

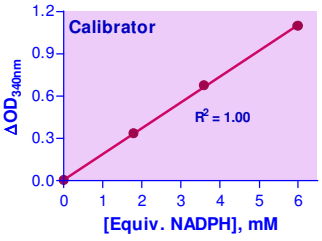
The factor 1000 converts mmoles to µmoles. *n* is the sample dilution factor.

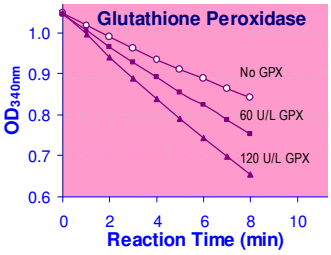
Note: if calculated GPX activity is higher than 300 U/L, or initial OD340nm is >1.5 in sample wells, dilute sample in dH_2O and repeat assay. Multiply the results by the dilution factor.

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.

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipetting devices, centrifuge tubes, clear flat-bottom uncoated 96-well plates, plate reader capable of reading optical density at 340nm every minute, homogenizer (e.g. Sigma # Z359971) etc.





LITERATURE

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3. Pascual, P. et al. (1992). Direct assay of glutathione peroxidase activity using high-performance capillary electrophoresis. J Chromatogr. 581:49-56.

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