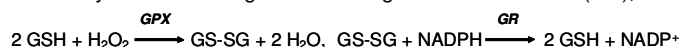


EnzyChrom™ Glutathione Peroxidase Assay Kit (EGPX-100)

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),



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

Assay Buffer: 25 mL **GR Enzyme:** 1 mL **Glutathione:** lyophilized

NADPH: lyophilized **H₂O₂ Solution:** 100 μL 3% H₂O₂

Positive Control: 9 μL Glutathione Peroxidase (GPX)

Storage conditions. The kit is shipped on ice. Store all components at -20 °C. 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°C (stable for 1 month).

ASSAY PROCEDURE

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

Add 240 μL dH₂O to the Glutathione tube (final 225 mM) and 400 μL dH₂O 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°C.

2. **NADPH Standards and Samples.** Mix 45 μL of the reconstituted 35 mM NADPH with 217 μL dH₂O (final 6 mM). Dilute standards 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 NADPH + H ₂ O	Vol (μL)	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 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 Reconstituted 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% H₂O₂ with 1992 μL dH₂O (final 3.5 mM). Prepare enough 0.35 mM H₂O₂ Reagent by mixing, for each Sample/Control well, 12 μL 3.5 mM with 108 μL dH₂O. Use this Reagent within one hour.

With a multi-channel pipettor, add 100 μL 0.35 mM H₂O₂ Reagent to all Sample and Control wells. Tap plate quickly to mix well contents thoroughly.

Immediately read OD_{340nm} (time zero, OD₀) and again at 4 min (OD₄).

Note: if calculated GPX activity is higher than 300 U/L, or initial OD_{340nm} is >1.5 in sample wells, dilute sample in dH₂O and repeat assay. Multiply the results by the dilution factor.

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 $\Delta\text{OD}_s = (\text{OD}_0 - \text{OD}_4)$ for the samples and $\Delta\text{OD}_B = (\text{OD}_0 - \text{OD}_4)$ for the background control. Calculate the GPX activity of Sample,

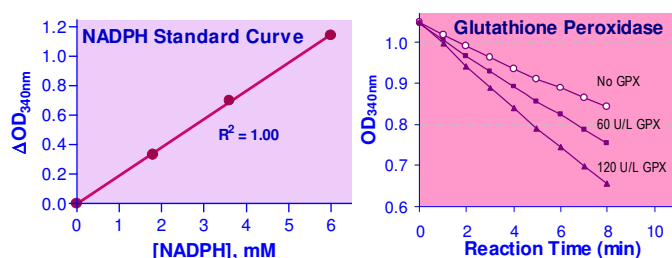
$$\text{GPX Activity (U/L)} = \frac{\Delta\text{OD}_s - \Delta\text{OD}_B}{\text{Slope (mM}^{-1}) \times 4 \text{ (min)}} \times 1000 \times n$$

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

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

- Paglia, D.E. and Valentine, W.N. (1967). Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J Lab Clin Med.* 70:158-169.
- Jacobson, B. et al. (1988). Adaptation of glutathione peroxidase assay to the Technicon RA-1000. *Clin Chem.* 34:2164-2165.
- Pascual, P. et al. (1992). Direct assay of glutathione peroxidase activity using high-performance capillary electrophoresis. *J Chromatogr.* 581:49-56.