Signosis, Inc.
Innovative Plate Assay Solutions

Glutathione Peroxidase GPx Assay Kit

Catalog # EA-7023 **(For Research Use Only)**

Introduction

The Glutathione Peroxidase (GPx) Activity Assay determines the activity of GPx by measuring its ability to convert reduced glutathione (GSH) to oxidized glutathione (GSSG) in the presence of H_2O_2. The GSSG that is produced by GPx is quantified using the enzyme glutathione reductase (GR) which interacts with GSSG. GR reduces GSSG to GSH with NADPH as a cofactor, which converts the NADPH to its oxidized form, $NADP^+$. Because NADPH can be measured spectrophotometrically at an absorbance of 340 nm, the depletion of NADPH levels in samples can be used to determine GPx activity. Since GPx activity causes NADPH to be consumed, elevated GPx activity is observed as a decrease in absorbance at 340 nm.

Materials Required but Not Provided

- PBS
- 96-well clear microplate for absorbance reading
- Microplate reader capable of measuring absorbance at 340 nm

Materials Provided

- 1mM Peroxide Reagent (4°C)
- Serum Reagent (4°C)
- 10mM FAD (-20°C)
- 10mM GSH (-20°C)
- 10mM NADPH (-20°C)
- 1x GR Enzyme Stock (-80°C)

****Spin down small tubes before starting experiment. ****

Plasma Sample Preparation

1. Centrifuge citrated or EDTA-collected blood at 4°C (1,000 x *g* for 10 minutes) to separate plasma from erythrocytes. Alternatively, blood collected without anticoagulant can be centrifuged to collect serum.
2. Transfer the plasma layer to a new tube without disturbing the buffy layer.
3. The plasma may be assayed directly or stored away at -80°C.

Cell Sample Preparation

1. Wash the cells once with PBS before lysing the cells.
2. For a 96-well culture plate, add 40 µL of lysis buffer to each well and incubate at room temperature for 10 minutes.
3. Pipette the lysis buffer up and down to detach the cells and transfer the cell lysates into a new tube.
4. If necessary, homogenize the cell lysates with a sonicator.
5. The cell lysates may be assayed directly or stored at -80°C.
6. Use PBS to dilute the cell sample to the appropriate concentration for each assay, if necessary.

Tissue Sample Preparation

1. Weigh tissue sample and add 1 mL of lysis buffer per 100mg of tissue.
2. Homogenize the tissue samples with a tissue grinder.
3. If necessary, further homogenize the tissue samples with a sonicator.
4. Centrifuge the sample at 10,000 RPM for 5 minutes to pellet the tissue debris.
5. Collect the supernatant and measure the protein concentration of the supernatant. The tissue sample can be assayed directly or stored at -80°C.
6. Use PBS to dilute the tissue sample to the appropriate concentration for each assay, if necessary.

GPx Measurement

1. Sample preparation: A baseline GSSG measurement should be performed additionally for each sample. To obtain a baseline sample, take an aliquot from each sample and heat at 98°C to deactivate all enzymes in the sample. The heated sample contains only the endogenous GSSG from the sample, which will be used as the reference for the baseline GSSG level. The unheated sample will include additional GSSG produced by GPx activity and will have higher overall GSSG levels compared to the baseline.
2. Reaction mix preparation: calculate the amount of each reagent needed to make the reaction mix according to the table below.

Component	Reaction Mix (per well/sample)
1mM Peroxide Reagent	0.9 µL
Serum Reagent	0.9 µL
10mM FAD	0.09 µL
10mM GSH	0.9 µL
10mM NADPH	0.9 µL
1x GR Enzyme	0.09 µL
PBS	86.22 µL
Total	90 µL

3. Any unused enzyme stock can be stored at -80°C for future use.
4. Add 90 µL of reaction mix to each well of the plate.
5. Add 10 µL of sample to each well with reaction mix and mix thoroughly.
6. Cover the plate and incubate at room temperature for 10-20 minutes.
7. Measure the absorbance of the plate at 340 nm using a plate reader. Multiple readings of the plate can be done at 5 minute intervals to observe potential kinetic changes in the measurements.