Technical Bulletin

Glutathione Peroxidase Assay Kit

Catalog Number MAK437

Product Description

Glutathione peroxidase (GPX) represents an enzyme family with peroxidase activity whose main biological role is to protect the organism from oxidative damage. Glutathione peroxidase helps prevent lipid peroxidation of cellular membranes by removing free peroxide in the cell. GPX catalyzes the following reaction with glutathione reductase (GR) (see Figure 1).

Simple, direct and high-throughput assays for GPX activity find wide application. The Glutathione Peroxidase Assay Kit directly measures NADPH consumption in the enzyme coupled reactions. The decrease in optical density measured at 340 nm (OD_{340}) is directly proportional to the enzyme activity in the sample. The linear range of the assay method is 40 to 800 units per liter (U/L) of glutathione peroxidase activity.

The kit is suitable for the quantitative determination of glutathione peroxidase activity and evaluation of drug effects on GPX activity in biological samples.

Figure 1.

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

Components

The kit is sufficient for 100 colorimetric assays in 96-well plates.

- Assay Buffer 25 mL
  Catalog Number MAK437A
- GR Enzyme 250 µL
  Catalog Number MAK437B
- Glutathione 1 vial
  Catalog Number MAK437C
- NADPH 1 vial
  Catalog Number MAK437D
- Calibrator 1 vial
  Catalog Number MAK437E
- Peroxide Solution 50 µL
  Catalog Number MAK437F

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories
- Spectrophotometric multiwell plate reader
- Clear flat-bottom 96-well plates. Cell culture or tissue culture treated plates are **not** recommended.
- 1.5 mL microcentrifuge tubes
- Dounce tissue grinder set (Catalog Number D9063 or equivalent)
- Microcentrifuge capable of RCF ≥ 14,000 × g
- Phosphate Buffered Saline (PBS) (Catalogue Number P3813 or equivalent)

Precautions and Disclaimer

For Research Use Only. Not for use in diagnostic procedures. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

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

Preparation Instructions

Briefly centrifuge small vials prior to opening. Equilibrate all components to room temperature prior to use. Keep reconstituted NADPH, Glutathione and Calibrator on ice during the assay.

NADPH: Reconstitute vial with 400 µL of purified water (final concentration 35 mM). Vortex to mix. Reconstituted vial is stable for three weeks when stored frozen at -20 °C.

Glutathione: Reconstitute vial with 540 µL of purified water (final concentration 100 mM). Vortex to mix. Reconstituted vial is stable for three weeks when stored frozen at -20 °C.

Procedure

All samples and standards should be run in duplicate. All samples should be clear and free of any turbidity or particles.

Sample Preparation

Liquid Samples:

Liquid samples (such as non-hemolyzed serum, plasma) can be assayed directly.

Tissues and Cells:

Homogenize tissue (10 mg) or cells (10^6) in 200 µL of cold 1× PBS and then centrifuge for 10 minutes at 14,000 × g to pellet any debris. Use the clear supernatant for the assay. If not assayed immediately, freeze supernatant at -80 °C for up to 1 month.

All Samples

Transfer 10 µL of each Sample into separate wells of a 96-well plate. For unknown samples, it is recommended to perform several dilutions to ensure that the GPX activity is within the linear standard curve range of 40 to 800 U/L.

In addition, for each assay run include a Background Control well that contains 10 µL of Assay Buffer.

Standard Curve Preparation

1. The Calibrator (MAK437E) is equivalent to 6 mM NADPH. Dilute this stock solution as shown in Table 1.

Table 1.
Preparation of Standards

Well	6 mM Calibrator	Purified Water	Equivalent NADPH (mM)
1	100 µL	-	6.0
2	60 µL	40 µL	3.6
3	30 µL	70 µL	1.8
4	-	100 µL	0

2. Mix well and transfer 10 µL of each Standard into separate wells.
3. Add 190 µL of Assay Buffer to all Standard wells.

Working Reagents

Mix enough reagents for the number of assays to be performed. For each Sample and Background Control well, prepare 100 µL of Working Reagent. Prepare Working Reagents according to Table 2. Mix well.

Table 2.
Preparation of Working Reagent

Reagent	Working Reagent
Assay Buffer	90 µL
Glutathione	5 µL
35 mM NADPH	3 µL
GR Enzyme	2 µL

Measurement

1. **Quickly** add 90 µL of Working Reagent to each Sample and Background Control well. Tap plate to mix.
2. Transfer 5 µL of Peroxide Solution into a 1.5 mL microcentrifuge tube. Add 1495 µL of purified water and mix well by vortexing for at least 30 seconds.
3. Immediately dilute the Peroxide Solution from Step 2 1:10 in purified water to generate the 1× Substrate Solution. Use the 1× Substrate Solution within one hour of preparation.
4. With a multichannel pipettor, add 100 µL of 1× Substrate Solution to each Sample and Background Control well. Tap plate quickly to mix wells.
5. Immediately read optical density (OD) at 340 nm (time zero, OD_{T0}) of all Samples and Controls. Read the optical density of all Samples, Background Controls, and Standards after a reaction time of 4 minutes (OD_{T4}).

Results

1. Use OD values at 4 minutes for the NADPH Standards.
2. Subtract the blank (Standard #4) OD_{T4} value from all other Standard OD_{T4} values.
3. Plot the Corrected OD_{T4} value for each Standard against Standard concentrations. Determine the slope of the standard curve.
4. Calculate the change in optical density (ΔOD_S) for each Sample:

   ΔOD_S = Sample OD_{T0} – Sample OD_{T4}
5. Calculate the change in optical density (ΔOD_B) for the Background Control:

   ΔOD_B = Background Control OD_{T0} – Background Control OD_{T4}

Note for Steps 4 and 5: The Time 4-minute reading is subtracted from the Time Zero reading because the optical density at 340 nm decreases with reaction time.

6. Calculate the GPX activity of Sample:

GPX Activity (U/L) =

$$\frac{\Delta OD_S - \Delta OD_B}{\text{Slope } (mM^{-1}) \times 4 \text{ (min)}} \times 1000 \times DF$$

Where:

ΔOD_s = Change in OD readings at 340 nm of the Sample

ΔOD_B = Change in OD readings at 340 nm of the Background Control

1000 = Conversion factor from mmoles to μmoles.

DF = Sample Dilution factor (DF = 1 for undiluted Samples)

Note: If the calculated GPX activity of the Sample is higher than 800 U/L, or the initial OD reading at 340 nm is >1.5 in Sample wells, dilute the Sample in purified water and repeat the assay.

Unit definition: One unit is the amount of GPX that produces 1 μmole of oxidized glutathione (GSSG) per minute at pH 7.6 and room temperature.

Figure 2.
Typical Calibrator Standard Curve.

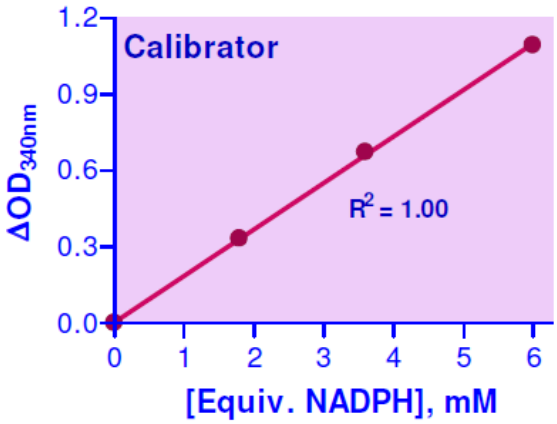
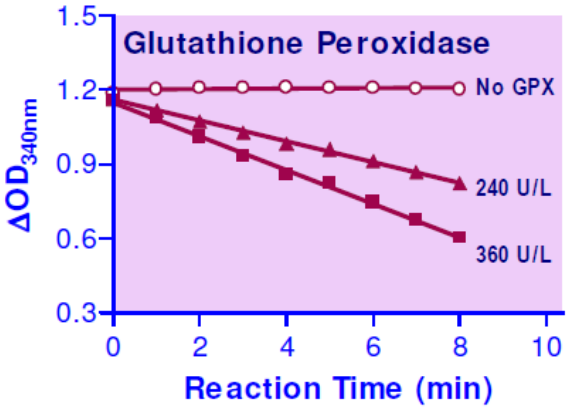


Figure 3.
Kinetics of Glutathione Peroxidase Reaction in a 96-Well Plate.



References

1. Medina-Leendertz, S., et al., Melatonin decreases oxidative stress in *Drosophila melanogaster* exposed to manganese. *Invest. Clin.*, **59(3)**, 230-241 (2018).
2. Hirahara, I., et al., Hypermetabolism of glutathione, glutamate and ornithine via redox imbalance in methylglyoxal-induced peritoneal injury rats. *J. Biochem.*, **167(2)**, 185-194 (2020).
3. Vieira da Silva, I., et al., Glutamine and cystine-enriched diets modulate aquaporins gene expression in the small intestine of piglets. *PloS One*, **16(1)**, e0245739 (2021).

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