Enzymatic Assay of GLUTATHIONE PEROXIDASE
(EC 1.11.1.9)

PRINCIPLE:

$$2\ GSH + H_2O_2 \xrightarrow{\text{Glutathione Peroxidase}} GSSG + 2\ H_2O$$

$$GSSG + \text{ß-NADPH} \xrightarrow{\text{Glutathione Reductase}} \text{ß-NADP} + 2\ GSH$$

Abbreviations used:
GSH = Glutathione, Reduced Form
GSSG = Glutathione, Oxidized Form
ß-NADPH = ß-Nicotinamide Adenine Dinucleotide Phosphate, Reduced Form
ß-NADP = ß-Nicotinamide Adenine Dinucleotide Phosphate, Oxidized Form

CONDITIONS: T = 25°C, pH = 7.0[1], A_{340nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 50 mM Sodium Phosphate Buffer with 0.40 mM Ethylenediaminetetraacetic Acid (EDTA), pH 7.0[1] at 25°C
(Prepare 100 ml in deionized water using Sodium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. S-0751 and Ethylenediaminetetraacetic Acid, Tetrasodium Salt, Sigma Stock No. ED4SS. Adjust to pH 7.0 at 25°C with 1 M NaOH.)

B. 1.0 mM Sodium Azide Solution (Buffer w/Azide)
(Prepare 50 ml in Reagent A using Sodium Azide, Sigma Prod. No. S-2002.[2])

C. ß-Nicotinamide Adenine Dinucleotide Phosphate, Reduced Form (ß-NADPH)
(Use 1.0 mg vial of ß-Nicotinamide Adenine Dinucleotide Phosphate, Reduced Form, Tetrasodium Salt, Sigma Stock No. 201-201.)

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REAGENTS: (continued)

D. Glutathione Reductase Enzyme Solution (GR)
(Immediately before use, prepare a solution containing 100 units/ml of Glutathione Reductase, Sigma Prod. No. G-3664, in cold deionized water.)

E. 200 mM Glutathione, Reduced (GSH)
(Prepare 5 ml in deionized water using Glutathione, Free Acid, Reduced Form, Sigma Prod. No. G-4251.)

F. 10.0 mM Sodium Phosphate Buffer with
1.0 mM Dithiothreitol, pH 7.0 at 25EC (Buffer w/DTT)
(Prepare 100 ml in deionized water using Sodium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. S-0751 and DL-Dithiothreitol, Sigma Prod. No. D-0632. Adjust to pH 7.0 at 25°C with 1 M NaOH.)

G. Glutathione Peroxidase Enzyme Solution
(Immediately before use, prepare a solution containing 1.5 - 3.0 units/ml of Glutathione Peroxidase in cold Reagent F.)

H. 0.042% (w/w) Hydrogen Peroxide (H_2O_2)
(Prepare 5 ml in deionized water using Hydrogen Peroxide, 30% (w/w) Solution, Sigma Prod. No. H-1009[3]. **PREPARE FRESH.**)

PROCEDURE:

Prepare a reaction cocktail by pipetting (in milliliters) the following reagents into Reagent C (ß-NADPH vial):

Reagent B (Buffer w/Azide)	9.20
Reagent D (GR)	0.10
Reagent E (GSH)	0.05

Mix by inversion and adjust to pH 7.0 at 25°C with 1 M HCl or 1 M NaOH, if necessary. Pipette (in milliliters) the following reagents into suitable cuvettes:

	Test	Blank
Reaction Cocktail	3.00	3.00
Reagent F (Buffer w/DTT)	------	0.05
Reagent G (Glutathione Peroxidase)	0.05	------

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PROCEDURE: (continued)

Mix by inversion and equilibrate to 25°C. Monitor the A_{340nm} until constant, using a suitably thermostatted spectrophotometer. Then add:

	Test	Blank
Reagent H (H_2O_2)	0.05	0.05

Immediately mix by inversion and record the decrease in A_{340nm} for approximately 5 minutes. Obtain the ΔA_{340nm}/minute using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(\Delta A_{340nm}/\text{min Test} - \Delta A_{340nm}/\text{min Blank})(2)(3.1)(df)}{(6.22)\ (0.05)}$$

2 = 2 µmoles of GSH produced per µmole of ß-NADPH oxidized
3.1 = Total volume (in milliliters) of assay
df = Dilution factor
6.22 = Millimolar extinction coefficient of ß-NADPH at 340 nm
0.05 = Volume (in milliliters) of enzyme used

$$\text{Units/mg solid} = \frac{\text{units/ml enzyme}}{\text{mg solid/ml enzyme}}$$

$$\text{Units/mg protein} = \frac{\text{units/ml enzyme}}{\text{mg protein/ml enzyme}}$$

UNIT DEFINITION:

One unit will catalyze the oxidation by H_2O_2 of 1.0 µmole of reduced glutathione to oxidized glutathione per minute at pH 7.0 at 25°C.

FINAL ASSAY CONCENTRATION:

In a 3.05 ml reaction mix, final concentrations are 48 mM sodium phosphate, 0.38 mM ethylenediaminetetraacetic acid, 0.12 mM ß-nicotinamide adenine dinucleotide phosphate, reduced form, 0.95 mM sodium azide, 3.2 units of glutathione reductase, 1 mM glutathione, 0.02 mM DL-dithiothreitol, 0.0007% (w/w) hydrogen peroxide and 0.075 - 0.15 unit of glutathione peroxidase.

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REFERENCE:

Wendel, A. (1980) *Enzymatic Basis of Detoxication*, Volume 1, p. 333, Academic Press, NY

NOTES:

1. The enzyme activity at the pH optimum of 8.8 is almost 10 times that at pH 7.0. However, non-enzymatic oxidation of GSH increases at alkaline pH, complicating the assay considerably.

2. Sodium Azide is included to inhibit the competitive catalase activity of impurities such as hemoglobin. If hemoglobin is not present, then sodium azide is not required.

3. Hydrogen peroxidase, 30% (w/w) solution, has a limited shelf-life. The A_{240nm} of a 0.042% (w/w) hydrogen peroxide solution should be 0.540.

4. Glutathione Reductase Unit Definition: One unit will reduce 1.0 µmole of oxidized glutathione per minute at pH 7.6 at 25°C.

5. This assay is based on the cited reference.

6. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.

This procedure is for informational purposes. For a current copy of Sigma's quality control procedure contact our Technical Service Department.