Enzymatic Assay of GLUTATHIONE REDUCTASE (EC 1.6.4.2)

PRINCIPLE:

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

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

CONDITIONS: T = 25°C, pH = 7.6, A_{340nm}, Light Path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 100 mM Potassium Phosphate Buffer with 3.4 mM Ethylenediaminetetraacetic Acid (EDTA), pH 7.6 at 25°C
(Prepare 200 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. P-5379 and Ethylenediaminetetraacetic Acid, Dipotassium Salt, Sigma Stock No. ED2P. Adjust to pH 7.6 at 25°C with 1 M KOH.)

B. 30 mM Glutathione Substrate Solution (GSSG)
(Prepare 5 ml in deionized water using Glutathione, Oxidized Form, Disodium Salt, Sigma Prod. No. G-4626.)

C. 0.8 mM ß-Nicotinamide Adenine Dinucleotide Phosphate, Reduced Form Solution (ß-NADPH)
(Prepare 5 ml in cold Reagent A using ß-Nicotinamide Adenine Dinucleotide Phosphate, Tetrasodium Salt, Sigma Prod. No. N-1630.)

D. 1.0% (w/v) Bovine Serum Albumin (BSA)
(Prepare 100 ml in Reagent A using Albumin, Bovine, Sigma Prod. No. A-4503. This solution should be kept cold.)

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REAGENTS: (continued)

E. Glutathione Reductase Enzyme Solution
(Immediately before use, prepare a solution containing 0.30 - 0.60 unit/ml of Glutathione Reductase in cold Reagent D.)

PROCEDURE:

Pipette (in milliliters) the following reagents into suitable cuvettes:

	Test	Blank
Deionized Water	0.65	0.65
Reagent A (Buffer)	1.50	1.50
Reagent B (GSSG)	0.10	0.10
Reagent C (ß-NADPH)	0.35	0.35
Reagent D (BSA)	0.30	0.40

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

Reagent E (Enzyme Solution)	0.10	------

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

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(\Delta A_{340nm}/\text{min Test} - \Delta A_{340nm}/\text{min Blank})(3)(df)}{(6.22)(0.1)}$$

3 = Total volume (in milliliters) of assay
df = Dilution factor
6.22 = Millimolar extinction coefficient of ß-NADPH at 340 nm
0.1 = Volume (in milliliters) of enzyme used

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

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CALCULATIONS: (continued)

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

UNIT DEFINITION:

One unit will reduce 1.0 μmole of oxidized glutathione per minute at pH 7.6 at 25°C.

FINAL ASSAY CONCENTRATION:

In a 3.00 ml reaction mix, the final concentrations are 75 mM potassium phosphate, 2.6 mM ethylenediaminetetraacetic acid, 1 mM glutathione, 0.09 mM ß-nicotinamide adenine dinucleotide phosphate, reduced form, 0.13% (w/v) bovine serum albumin, and 0.03 - 0.06 unit of glutathione reductase.

REFERENCE:

Mavis, R.D. and Stellwagen, E. (1968) *Journal of Biological Chemistry* **243**, 809-814

NOTES:

1. This assay is based on the cited reference.

2. 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.