Enzymatic Assay of GLUTATHIONE REDUCTASE
(EC 1.6.4.2)
Coenzyme A-Glutathione Reductase Activity[1]

PRINCIPLE:

$$\text{CoA-S-S-G} + \text{NADPH} \xrightarrow{\text{Glutathione Reductase}} \text{CoA-SH} + \text{GSH} + \text{NADP}$$

Abbreviations used:
CoA-S-S-G = Coenzyme A Glutathione Disulfide
NADPH = Nicotinamide Adenine Nucleotide Phosphate, Reduced Form
CoA-SH = Coenzyme A, Reduced Form
GSH = Glutathione, Reduced Form
NADP = Nicotinamide Adenine Dinucleotide Phosphate, Oxidized Form

CONDITIONS: T = 25°C, pH = 5.5, A_{340nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 75 mM Potassium Phosphate Buffer with 0.15% (w/v) Bovine Serum Albumin, pH 5.5 at 25°C
(Prepare 100 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. P-5379, and Albumin, Bovine, Sigma Prod. No. A-4503. Adjust to pH 5.5 at 25°C with 2 N NaOH.)

B. 6.0 mM Coenzyme A Glutathione Disulfide Solution (CoA-S-S-G)
(Prepare 5 ml in deionized water using Coenzyme A Glutathione Disulfide, Sodium Salt, Sigma Prod. No. C-5018.)

C. 4.5 mM ß-Nicotinamide Adenine Dinucleotide Phosphate, Reduced Form Solution (ß-NADPH)
(Dissolve the contents of one 5 mg vial of ß-Nicotinamide Adenine Dinucleotide Phosphate, Reduced Form, Tetrasodium Salt, Sigma Stock No. 201-205, in the appropriate volume of deionized water or prepare 1 ml in deionized water using ß-Nicotinamide Adenine Dinucleotide Phosphate, Reduced Form, Tetrasodium Salt, Sigma Prod. No. N-1630.)

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REAGENTS: (continued)

D. Glutathione Reductase Enzyme Solution
(Immediately before use, prepare a solution containing 0.25 - 0.50 unit/ml of Glutathione Reductase in cold deionized water.)

PROCEDURE:

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

	Test	Blank
Reagent A (Buffer)	2.00	2.00
Reagent B (CoA-S-S-G)	0.50	0.50
Reagent C (ß-NADPH)	0.10	0.10
Deionized Water	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 D (Enzyme Solution)	0.10	------

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

CALCULATIONS:

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

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 milliliter) of enzyme used

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

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

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UNIT DEFINITION:

One unit will reduce 1.0 µmole of CoA-S-S-G per minute at pH 5.5 at 25°C.

FINAL ASSAY CONCENTRATIONS:

In a 3.00 ml reaction mix, the final concentrations are 50 mM potassium phosphate, 1.0 mM coenzyme A glutathione disulfide, 0.15 mM ß-nicotinamide adenine dinucleotide phosphate, reduced form, 0.01% (w/v) bovine serum albumin, and 0.025 - 0.050 unit glutathione reductase.

REFERENCE:

Carlberg I. and Mannervik, B. (1977) *Biochimica et Biophysica Acta* **484**, 268-274

NOTES:

1. This assay is not used to assay Glutathione Reductase, Crude from Wheat Germ, Sigma Prod. No. G-6004.

2. This assay is based on the cited reference.

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