Enzymatic Assay of GLUTATHIONE REDUCTASE
(EC 1.6.4.2)
Coenzyme A–Glutathione Reductase Activity

**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:**  \( T = 25^\circ C, \text{ pH } = 5.5, A_{340nm}, \text{ Light path } = 1 \text{ cm} \)

**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 β-Nicotinamide Adenine Dinucleotide Phosphate, Reduced Form Solution (β-NADPH)
   (Dissolve the contents of one 5 mg vial of β-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 β-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:

<table>
<thead>
<tr>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent A (Buffer)</td>
<td>2.00</td>
</tr>
<tr>
<td>Reagent B (CoA-S-S-G)</td>
<td>0.50</td>
</tr>
<tr>
<td>Reagent C (β-NADPH)</td>
<td>0.10</td>
</tr>
<tr>
<td>Deionized Water</td>
<td>0.30</td>
</tr>
</tbody>
</table>

Mix by inversion and equilibrate to 25°C. Monitor the A\textsubscript{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\textsubscript{340nm} for approximately 5 minutes. Obtain the r A\textsubscript{340nm}/minute using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

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

3 = Total volume (in milliliters) of assay  
\(df\) = Dilution factor  
6.22 = Millimolar extinction coefficient of β-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 β-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.

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