SIGMA QUALITY CONTROL TEST PROCEDURE

Enzymatic Assay of GLUTATHIONE REDUCTASE
(EC 1.6.4.2)

PRINCIPLE:

\[
\beta\text{-NADPH} + \text{GSSG} \xrightarrow{\text{Glutathione Reductase}} \beta\text{-NADP} + 2 \text{GSH}
\]

Abbreviations used:
\(\beta\text{-NADPH}\) = \(\beta\)-Nicotinamide Adenine Dinucleotide Phosphate, Reduced Form
\(\beta\text{-NADP}\) = \(\beta\)-Nicotinamide Adenine Dinucleotide Phosphate, Oxidized Form
\text{GSSG} = \text{Glutathione, Oxidized Form}
\text{GSH} = \text{Glutathione, Reduced Form}

CONDITIONS: \(T = 25^\circ C\), 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 \(\beta\)-Nicotinamide Adenine Dinucleotide Phosphate, Reduced Form Solution (\(\beta\text{-NADPH}\))
   (Prepare 5 ml in cold Reagent A using \(\beta\)-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:

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deionized Water</td>
<td>0.65</td>
<td>0.65</td>
</tr>
<tr>
<td>Reagent A (Buffer)</td>
<td>1.50</td>
<td>1.50</td>
</tr>
<tr>
<td>Reagent B (GSSG)</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>Reagent C (β-NADPH)</td>
<td>0.35</td>
<td>0.35</td>
</tr>
<tr>
<td>Reagent D (BSA)</td>
<td>0.30</td>
<td>0.40</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent E (Enzyme Solution)</td>
<td>0.10</td>
<td>------</td>
</tr>
</tbody>
</table>

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 β-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 \( \mu \)mole 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 \( \beta \)-nicotinamide adenine dinucleotide phosphate, reduced form, 0.13% (w/v) bovine serum albumin, and 0.03 - 0.06 unit of glutathione reductase.

REFERENCE:


NOTES:

1. This assay is based on the cited reference.
2. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.

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