Enzymatic Assay of GLUTATHIONE-S-TRANSFERASE
(EC 2.5.1.18)
1-Chloro-2,4-Dinitrobenzene as Substrate

PRINCIPLE:

\[ \text{G-SH} + \text{CDNB} \xrightarrow{\text{Glutathione-S-Transferase}} \text{G-SDNB Conjugate} + \text{HCl} \]

Abbreviations:
G-SH = Glutathione, Reduced Form
CDNB = 1-Chloro-2,4-Dinitrobenzene
G-SDNB Conjugate = Glutathione-2,4-Dinitrobenzene

CONDITIONS: T = 25°C, pH = 6.5, \( A_{340\text{nm}} \), Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 100 mM Potassium Phosphate Buffer with 1.0 mM Ethylenediaminetetraacetic Acid, pH 6.5 at 25°C
(Prepare 100 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. P-5379, and Ethylenediaminetetraacetic Acid, Tetrasodium Salt: Hydrate, Sigma Stock No. ED4S. Adjust to pH 6.5 at 25°C with 1 M KOH.)

B. 75 mM Glutathione, Reduced Solution (G-SH)
(Prepare 10 ml in Reagent A using Glutathione, Free Acid, Reduced Form, Sigma Prod. No. G-4251. PREPARE FRESH.)

C. 30 mM 1-Chloro-2,4-Dinitrobenzene Solution (CDNB)
(Prepare 1 ml in Reagent D using 1-Chloro-2,4-Dinitrobenzene, Sigma Prod. No. C-6396. PREPARE FRESH.)

D. 95% Ethanol (Nondenatured)

E. Glutathione-S-Transferase Enzyme Solution
(Immediately before use, prepare a solution containing 0.075 - 0.15 unit/ml of Glutathione-S-Transferase in cold Reagent A.)
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PROCEDURE:

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

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent A (Buffer)</td>
<td>2.70</td>
<td>2.80</td>
</tr>
<tr>
<td>Reagent B (G-SH)</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>Reagent C (CDNB)</td>
<td>0.10</td>
<td>0.10</td>
</tr>
</tbody>
</table>

Equilibrate to 25°C. Monitor the A$_{340}$nm until constant, using a suitably thermostatted spectrophotometer. Then add:

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

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

CALCULATIONS:

\[
\text{Units/ml enzyme} = \frac{(r \ A_{340\text{nm}/\text{min Test}} - r \ A_{340\text{nm}/\text{min Blank}})(3.0)(\text{df})}{(9.6)(0.10)}
\]

- 3.0 = Total volume (in milliliters) of assay
- df = Dilution factor
- 9.6 = Millimolar extinction coefficient of Glutathione-1-Chloro-2,4-Dinitrobenzene conjugate at 340 nm
- 0.10 = Volume (in milliliter) of enzyme used

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

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

UNIT DEFINITION:

One unit will conjugate 1.0 µmole of 1-chloro-2,4-dinitrobenzene with reduced glutathione per minute at pH 6.5 at 25°C.
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FINAL ASSAY CONCENTRATION:

In a 3.00 ml reaction mix, the final concentrations are 97 mM potassium phosphate, 0.97 mM ethylenediaminetetraacetic acid, 2.5 mM glutathione, reduced, 1.0 mM 1-chloro-2,4-dinitrobenzene, 3.2% (v/v) ethanol and 0.0075 - 0.015 unit glutathione-S-transferase.

REFERENCES:


NOTES:

1. This assay is based on the cited references.

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.