Enzymatic Assay of GLYOXALASE II
(EC 3.1.2.6)

PRINCIPLE:

\[ \text{S-Lactoylglutathione} + \text{H}_2\text{O} \xrightarrow{\text{Glyoxalase II}} \text{GSH} + \text{DL-Lactic Acid} \]

Abbreviations:
GSH = Reduced Glutathione

CONDITIONS:  \( T = 25^\circ C, \) \( \text{pH} = 7.4, A_{240nm}, \) Light path = 1 cm

METHOD:  Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 50 mM Tris HCl Buffer, pH 7.4 at 25°C
   (Prepare 100 ml in deionized water using Trizma Base,
   Adjust to pH 7.4 at 25°C using
   1 M HCl.)

B. 0.76% (w/v) S-Lactoylglutathione Solution (SLG)
   (Prepare 10 ml in deionized water using
   S-Lactoylglutathione, Free Acid.)

C. Glyoxalase II Enzyme Solution (Glyox II)
   (Immediately before use, prepare a solution containing
   0.2 - 0.4 unit/ml of Glyoxalase II in cold deionized
   water.)

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>Reagent A (Buffer)</td>
<td>2.90</td>
<td>2.90</td>
</tr>
<tr>
<td>Reagent B (SLG)</td>
<td>0.07</td>
<td>0.07</td>
</tr>
</tbody>
</table>
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PROCEDURE:

Mix by inversion and equilibrate to 25°C. The A_{240nm} should be 0.6 - 0.8. If not, add more Reagent B so that the A_{240nm} is within this range. Then add:

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

Immediately mix by inversion and record the decrease in A_{240nm} for approximately 3 minutes. Obtain the ΔA_{240nm}/minute using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

\[
\text{Units/ml enzyme} = \frac{(\Delta A_{240nm}/\text{min Test} - \Delta A_{240nm}/\text{min Blank})(3.07)(\text{df})}{(3.37)(0.1)}
\]

- 3.07 = Total volume (in milliliters) of assay
- df = Dilution factor
- 3.37 = Millimolar extinction coefficient of S-Lactoylglutathione at 240 nm
- 0.1 = Volume (in milliliter) of enzyme used

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

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

UNIT DEFINITION:

One unit will hydrolyze 1.0 µmole of S-lactoylglutathione per minute at pH 7.4 at 25°C.

FINAL ASSAY CONCENTRATIONS:

In a 3.07 ml reaction mix, the final concentrations are 48 mM Tris, 0.0176% (w/v) D-lactoylglutathione and 0.02 - 0.04 unit glyoxalase II.
REFERENCES:

Racker, E. (1951) Journal of Biological Chemistry 190, 685

NOTES:

1. This assay is based on the cited reference.