Enzymatic Assay of L-LACTIC DEHYDROGENASE1
(EC 1.1.1.27)

PRINCIPLE:

\[
\text{L-Lactic Dehydrogenase} \quad \text{Pyruvate} + \beta-\text{NADH} \quad \rightarrow \quad \text{L-Lactate} + \beta-\text{NAD}
\]

**Abbreviations used:**
- \(\beta\)-NADH = \(\beta\)-Nicotinamide Adenine Dinucleotide, Reduced Form
- \(\beta\)-NAD = \(\beta\)-Nicotinamide Adenine Dinucleotide, Oxidized Form

CONDITIONS: \(T = 37^\circ C\), pH = 7.5, \(A_{340nm}\), Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 100 mM Sodium Phosphate Buffer, pH 7.5 at 37°C
   (Prepare 200 ml in deionized water using Sodium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. S-0751. Adjust to pH 7.5 at 37°C with 1 M NaOH.)

B. 0.13 mM \(\beta\)-Nicotinamide Adenine Dinucleotide, Reduced Form Solution (\(\beta\)-NADH)
   (Prepare 10 ml in cold Reagent A using \(\beta\)-Nicotinamide Adenine Dinucleotide, Reduced Form, Disodium Salt, Sigma Prod. No. N-8129 or dissolve the contents of 1 mg vial of \(\beta\)-Nicotinamide Adenine Dinucleotide, Reduced Form, Disodium Salt, Sigma Stock No. 340-101, in the appropriate volume of Reagent A. **PREPARE FRESH**.)

C. 69 mM Sodium Pyruvate Solution (Pyruvate)
   (Prepare 1.0 ml in cold Reagent A using Pyruvic Acid, Sodium Salt, Sigma Prod. No. P-2256.)

D. 1.0% (w/v) Bovine Serum Albumin Solution (BSA)
   (Prepare 50 ml in Reagent A using Albumin, Bovine, Sigma Prod. No. A-4503. **PREPARE FRESH**.)

E. L-Lactic Dehydrogenase Enzyme Solution
   (Immediately before use, prepare a solution containing 0.25 - 0.75 unit/ml of L-Lactic Dehydrogenase in cold Reagent D.)
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**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 B (β-NADH)</td>
<td>2.80</td>
<td>2.80</td>
</tr>
<tr>
<td>Reagent C (Pyruvate)</td>
<td>0.10</td>
<td>0.10</td>
</tr>
</tbody>
</table>

Mix by inversion and equilibrate to 37°C. Monitor the \(A_{340\text{nm}}\) 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 D (BSA)</td>
<td>-----</td>
<td>0.10</td>
</tr>
<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 \(A_{340\text{nm}}\) for approximately 5 minutes. Obtain the \(\Delta A_{340\text{nm}}/\text{minute}\) using the maximum linear rate for both the Test and Blank.

**CALCULATIONS:**

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

3 = Total volume (in milliliters) of assay  
df = Dilution factor  
6.22 = Millimolar extinction coefficient of β-NADH at 340 nm  
0.1 = Volume (in milliliter) of enzyme used

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

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

One unit will reduce 1.0 μmole of pyruvate to L-lactate per minute at pH 7.5 at 37°C.

FINAL ASSAY CONCENTRATION:

In a 3.00 ml reaction mix, the final concentrations are 100 mM sodium phosphate, 0.12 mM β-nicotinamide adenine dinucleotide, reduced form, 2.3 mM pyruvate, 0.033% (w/v) bovine serum albumin and 0.025 - 0.075 unit L-lactic dehydrogenase.

REFERENCES:


NOTES:


2. This assay is based on the cited reference.

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

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