Enzymatic Assay of L-LACTIC DEHYDROGENASE\(^{1}\)
(\text{EC \, 1.1.1.27})

**PRINCIPLE:**

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

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

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

**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 \(\beta\text{-Nicotinamide Adenine Dinucleotide, Reduced Form Solution (}\beta\text{-NADH}\)
(Prepare 10 ml in cold Reagent A using \(\beta\text{-Nicotinamide Adenine Dinucleotide, Reduced Form, Disodium Salt, Sigma Prod. No. N-8129 or dissolve the contents of 1 mg vial of }\beta\text{-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>
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<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>
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</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:

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<table>
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<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 $\Delta A_{340\text{nm}}$/minute using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

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

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:

Volume 2, 574-578, Academic Press, New York, NY

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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