Enzymatic Assay of \textit{d}-LACTIC DEHYDROGENASE (EC 1.1.1.28)

**PRINCIPLE:**

\[
\text{Pyruvate} + \beta\text{-NADH} \xrightarrow{\text{d-Lactic Dehydrogenase}} \text{d-Lactate} + \beta\text{-NAD}
\]

Abbreviations used:

\begin{align*}
\beta\text{-NADH} &= \beta\text{-Nicotinamide Adenine Dinucleotide, Reduced Form} \\
\beta\text{-NAD} &= \beta\text{-Nicotinamide Adenine Dinucleotide, Oxidized Form}
\end{align*}

**CONDITIONS:** \( T = 25^\circ C, \ \text{pH} = 7.0, \ A_{340nm}, \ \text{Light path} = 1 \ \text{cm} \)

**METHOD:** Continuous Spectrophotometric Rate Determination

**REAGENTS:**

\begin{enumerate}
\item[A.] 100 mM Potassium Phosphate Buffer, pH 7.0 at 25°C  
(Prepare 200 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. P-5379. Adjust to pH 7.0 at 25°C with 1 M KOH.)
\item[B.] 11 mM \( \beta\text{-Nicotinamide Adenine Dinucleotide, Reduced Form Solution (\( \beta\text{-NADH} \))} 
(Prepare 1 ml in cold deionized water using \( \beta\text{-Nicotinamide Adenine Dinucleotide, Reduced Form, Disodium Salt, Sigma Prod. No. N-8129. PREPARE FRESH.} \)
\item[C.] 20 mM Sodium Pyruvate Solution (Pyruvate)  
(Prepare 1.0 ml in cold deionized water using Pyruvic Acid, Sodium Salt, Sigma Prod. No. P-2256.)
\item[D.] 1.0\% (w/v) Bovine Serum Albumin Solution (BSA)  
(Prepare 50 ml in Reagent A using Albumin, Bovine, Sigma Prod. No. A-4503 or equivalent.)
\item[E.] \textit{d}-Lactic Dehydrogenase Enzyme Solution  
(Immediately before use, prepare a solution containing 0.3 - 0.60 unit/ml of \textit{d}-Lactic Dehydrogenase in cold Reagent D. PREPARE FRESH.)
\end{enumerate}
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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>
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</thead>
<tbody>
<tr>
<td>Reagent A (Buffer)</td>
<td>2.50</td>
<td>2.50</td>
</tr>
<tr>
<td>Reagent B (β-NADH)</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>Reagent C (Pyruvate)</td>
<td>0.10</td>
<td>0.10</td>
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Mix by inversion and equilibrate to 25°C. Monitor the $A_{340nm}$ until constant, using a suitably thermostatted spectrophotometer. Then add:  

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<tbody>
<tr>
<td>Reagent D (BSA)</td>
<td>------</td>
<td>0.05</td>
</tr>
<tr>
<td>Reagent E (Enzyme Solution)</td>
<td>0.05</td>
<td>------</td>
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</tbody>
</table>

Immediately mix by inversion and record the decrease in $A_{340nm}$ for approximately 5 minutes. Obtain the $\Delta A_{340nm}$/minute using the maximum linear rate for both the Test and Blank.  

CALCULATIONS:  

$$\text{Units/ml enzyme} = \frac{(\Delta A_{340nm}/\text{min Test} - \Delta A_{340nm}/\text{min Blank})(2.7)(df)}{(6.22)(0.05)}$$  

2.7 = Total volume (in milliliters) of the assay  

df = Dilution  

6.22 = Millimolar extinction coefficient of β-NADH at 340 nm  

0.05 = Volume (in milliliters) of assay  

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

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

UNIT DEFINITION:  

One unit will reduce 1.0 µmole of pyruvate to d-lactate per minute at pH 7.0 at 25°C.  

FINAL ASSAY CONCENTRATION:  

In a 2.75 ml reaction mix, the final concentrations are 94 mM potassium phosphate, 0.20 mM β-nicotinamide adenine dinucleotide, reduced form, 0.74 mM pyruvate, 0.015 - 0.03 unit d-lactic dehydrogenase.
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NOTES:

1. This assay is based on the cited reference.

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.