Determination of the Concentration and Molecular Weight of L(+)LACTIC ACID

PRINCIPLE:

\[ \text{L}(+)^{\text{L}}\text{Lactate} + \beta\text{-NAD} + \text{Hydrazine} \xrightarrow{\text{LDH}} \text{Pyruvate Hydrazone} + \beta\text{-NADH} \]

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

CONDITIONS: \( T = 25^\circ C \), \( pH = 9.5 \), \( A_{340nm} \), Light path = 1 cm

METHOD: Spectrophotometric Rate Determination

REAGENTS:

A. 600 mM Hydrazine Buffer with 1 M Glycine and 5.6 mM Ethylenediaminetetraacetic Acid (EDTA), pH 9.5 at 25°C

B. 0.3 mM \text{L}(+)^{\text{L}}\text{Lactic Acid} Solution
(Immediately before use, prepare 100 ml dionized water.)

C. 50 mM \( \beta\text{-Nicotinamide Adenine Dinucleotide (} \beta\text{-NAD)} \)
(Dissolve the contents of one 50 mg vial of \( \beta\text{-Nicotinamide Adenine Dinucleotide, Sigma Stock No. 260-150, in the appropriate volume of deionized water or prepare 2 ml in deionized water using } \beta\text{-Nicotinamide Adenine Dinucleotide, Sigma Prod. No. N-7004. PREPARE FRESH.)}

D. \text{L-Lactic Dehydrogenase}
(Immediately before use, prepare a solution containing approximately 5000 units/ml in cold deionized water using \text{L-Lactic Dehydrogenase, Sigma Prod. No. L-2500.})
Determination of the Concentration and Molecular Weight of L(+)-Lactic Acid

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>Deionized Water</td>
<td>0.35</td>
<td>1.35</td>
</tr>
<tr>
<td>Reagent A (Buffer)</td>
<td>1.40</td>
<td>1.40</td>
</tr>
<tr>
<td>Reagent B (Lactate)</td>
<td>1.00</td>
<td>-----</td>
</tr>
<tr>
<td>Reagent C (β-NAD)</td>
<td>0.15</td>
<td>0.15</td>
</tr>
</tbody>
</table>

Mix by inversion and equilibrate to 25°C. Monitor the $A_{340\text{nm}}$ until constant, using a suitably thermostatted spectrophotometer. Record the initial absorbance at $A_{340\text{nm}}$ ($A_i$) of both the Test and Blank. Then add:

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent D (L-Lactic Dehydrogenase)</td>
<td>0.10</td>
<td>0.10</td>
</tr>
</tbody>
</table>

Immediately mix by inversion and record the increase in absorbance at $A_{340\text{nm}}$ until the reaction is complete. Record the final absorbance at $A_{340\text{nm}}$ ($A_f$) of both the Test and Blank.

CALCULATIONS:

$r_A = A_f - A_i$

$A_i =$ Initial Absorbance

$A_f =$ Final Absorbance

$r_A$ $A_{340\text{nm}}$ Test - $r_A$ $A_{340\text{nm}}$ Blank)$(3)(df)$

Micromoles Lactic Acid/weighed sample = $\frac{(r_A)_{340\text{nm}}\text{ Test} - (r_A)_{340\text{nm}}\text{ Blank})\times(3)}{(df)}$ $\times 6.22$

$df =$ Dilution factor of L(+)-Lactic Acid

$3 =$ Volume (in milliliters) of assay

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

Apparent Molecular Weight = $\frac{(\text{mg sample weighed})\times(1000)}{\mu\text{moles Lactic Acid/weighed sample}}$
1000 = Conversion factor from mg to μg
Determination of the Concentration and Molecular Weight of 
\( \text{L}(\text{+})\text{LACTIC ACID} \)

**FINAL ASSAY CONCENTRATIONS:**

In a 3.00 ml reaction mix, the final concentrations are 
280 mM hydrazine, 467 mM glycine, 2.6 mM ethylenediaminetetraacetic acid, 2.5 mM \( \beta \)-nicotinamide adenine dinucleotide, 
500 units \( \text{L} \)-lactic dehydrogenase, and varying amounts of \( \text{L}(\text{+}) \)-lactic acid.

**REFERENCE:**


**NOTES:**

1. Reaction time is approximately 30 minutes.

2. \( \text{L} \)-Lactic Dehydrogenase Unit Definition: One unit will reduce 1.0 \( \mu \)mole of pyruvate to \( \text{L} \)-lactate per minute at pH 7.5 at 37°C.

3. This assay is based on the cited reference.

4. 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.