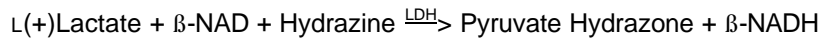


Determination of the Concentration and Molecular Weight of L(+)-LACTIC ACID

PRINCIPLE:



Abbreviations used:

β -NAD = β -Nicotinamide Adenine Dinucleotide, Oxidized Form

β -NADH = β -Nicotinamide Adenine Dinucleotide, Reduced Form

LDH = L-Lactic Dehydrogenase

CONDITIONS: T = 25°C, pH = 9.5, $A_{340\text{nm}}$, 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
(Prepare 50 ml in deionized water using Hydrazine Sulfate, Sigma Prod. No. H-3376, and Glycine, Free Base, Sigma Prod. No. G-7126 and Ethylenediaminetetraacetic Acid, Sigma Stock No. ED3SS. Adjust to pH 9.5 at 25°C with 10 M NaOH.)
- B. 0.3 mM L(+)-Lactic Acid Solution
(Immediately before use, prepare 100 ml deionized water.)
- C. 50 mM β -Nicotinamide Adenine Dinucleotide (β -NAD)
(Dissolve the contents of one 50 mg vial of β -Nicotinamide Adenine Dinucleotide, Sigma Stock No. 260-150, in the appropriate volume of deionized water or prepare 2 ml in deionized water using β -Nicotinamide Adenine Dinucleotide, Sigma Prod. No. N-7004. **PREPARE FRESH.**)
- D. L-Lactic Dehydrogenase
(Immediately before use, prepare a solution containing approximately 5000 units/ml in cold deionized water using L-Lactic Dehydrogenase, Sigma Prod. No. L-2500.)

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PROCEDURE:

Pipette (in milliliters) the following reagents into suitable cuvettes:

		<u>Test</u>	<u>Blank</u>
Deionized Water		0.35	1.35
Reagent A (Buffer)	1.40	1.40	
Reagent B (Lactate)		1.00	-----
Reagent C (β-NAD)	0.15	0.15	

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:

		<u>Test</u>	<u>Blank</u>
Reagent D (L-Lactic Dehydrogenase)		0.10	0.10

Immediately mix by inversion and record the increase in absorbance at $A_{340\text{nm}}$ until the reaction is complete¹. Record the final absorbance at $A_{340\text{nm}}$ (A_f) of both the Test and Blank.

CALCULATIONS:

$$\Delta A = A_f - A_i$$

A_i = Initial Absorbance

A_f = Final Absorbance

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

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

3 = Volume (in milliliters) of assay

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

$$\text{Apparent Molecular Weight} = \frac{(\text{mg sample weighed})(1000)}{\mu\text{moles Lactic Acid/weighed sample}}$$

1000 = Conversion factor from mg to μg

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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 β -nicotinamide adenine dinucleotide,
500 units L-lactic dehydrogenase, and varying amounts of L(+)-lactic acid.

REFERENCE:

Gutmann, I. and Wahlefeld, A.W. (1974) in *Methods of Enzymatic Analysis*, (Bergmeyer, H.U., Ed,) Academic Press Inc., New York, NY, Vol. 3, 1464-1468

NOTES:

1. Reaction time is approximately 30 minutes.
2. L-Lactic Dehydrogenase Unit Definition: One unit will reduce 1.0 μ mole of pyruvate to 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.