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

PRINCIPLE:

L(+)Lactate + ß-NAD + Hydrazine $\xrightarrow{LDH}$ Pyruvate Hydrazone + ß-NADH

Abbreviations used:
ß-NAD = ß-Nicotinamide Adenine Dinucleotide, Oxidized Form
ß-NADH = ß-Nicotinamide Adenine Dinucleotide, Reduced Form
LDH = L-Lactic Dehydrogenase

CONDITIONS: T = 25°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
(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 dionized water.)

C. 50 mM ß-Nicotinamide Adenine Dinucleotide (ß-NAD)
(Dissolve the contents of one 50 mg vial of ß-Nicotinamide Adenine Dinucleotide, Sigma Stock No. 260-150, in the appropriate volume of deionized water or prepare 2 ml in deionized water using ß-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:

		Test	Blank
Deionized Water		0.35	1.35
Reagent A (Buffer)	1.40	1.40	
Reagent B (Lactate)		1.00	------
Reagent C (ß-NAD)	0.15	0.15	

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

	Test	Blank
Reagent D (L-Lactic Dehydrogenase)	0.10	0.10

Immediately mix by inversion and record the increase in absorbance at A_{340nm} until the reaction is complete[1]. Record the final absorbance at A_{340nm} (A_f) of both the Test and Blank.

CALCULATIONS:

$\Delta A = A_f - A_i$
A_i = Initial Absorbance
A_f = Final Absorbance

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

df = Dilution factor of L(+)Lactic Acid
3 = Volume (in milliliters) of assay
6.22 = Millimolar extinction coefficient of ß-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 ß-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 µmole 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.