Enzymatic Assay of L-LACTIC DEHYDROGENASE
(EC 1.1.1.27)

PRINCIPLE:

$$\text{Pyruvate} + \text{ß-NADH} \xrightarrow{\text{L-Lactic Dehydrogenase}} \text{L-Lactate} + \text{ß-NAD}$$

Abbreviations used:
ß-NADH = ß-Nicotinamide Adenine Dinucleotide, Reduced Form
ß-NAD = ß-Nicotinamide Adenine Dinucleotide, Oxidized form

CONDITIONS: T = 30°C, pH = 6.0, A_{340nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 100 mM Sodium Acetate Buffer, pH 6.0 at 30°C
(Prepare 100 ml in deionized water using Sodium Acetate, Trihydrate, Prod. No S-8625. Adjust to pH 6.0 at 30°C with 1 M Acetic Acid.)

B. 250 mM Pyruvate Solution (Pyruvate)
(Prepare 10 ml in deionized water using Pyruvic, Acid, Sodium Salt, Prod. No. P-2256.)

C. 13 mM ß-Nicotinamide Adenine Dinucleotide, Reduced Form Solution (ß-NADH)
(Prepare 1 ml in cold deionized water using ß-Nicotinamide Adenine Dinucleotide, Reduced Form, Disodium Salt, Prod. No. N-8129. **PREPARE FRESH.)**

D. 50 mM Potassium Phosphate Buffer, pH 7.5 at 30°C
(Enzyme Diluent)
(Prepare 100 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Prod. No. P-5379. Adjust to pH 7.5 at 30°C with 1 M KOH.)

REAGENTS: (continued)

E. L-Lactic Dehydrogenase Enzyme Solution
(Immediately before use, prepare a solution containing 0.5 - 1.0 unit/ml of L-Lactic Dehydrogenase in cold Reagent D.)

PROCEDURE:

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

	Test	Blank
Reagent A (Buffer)	2.85	2.85
Reagent B (Pyruvate)	0.12	0.12
Reagent C (ß-NADH)	0.06	0.06

Mix by inversion and equilibrate to 30°C. Monitor the A_{340nm} until constant, using a suitably thermostatted spectrophotometer. Then add:

Reagent D (Enzyme Diluent)	------	0.05
Reagent E (Enzyme Solution)	0.05	------

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

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(\text{r } A_{340nm}/\text{min Test} - \text{r } A_{340nm}/\text{min Blank})(3.08)(df)}{(6.22)(0.05)}$$

3.08 = Total volume (in milliliters) of assay
df = Dilution factor
6.22 = Millimolar extinction coefficient of ß-NADH at 340 nm
0.05 = Volume (in milliliter) of assay

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

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

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UNIT DEFINITION:

One unit will reduce 1.0 μmole of pyruvate to L-lactate per minute at pH 6.0 at 30°C.

FINAL ASSAY CONCENTRATION:

In a 3.08 reaction mix, the final concentrations are 93 mM sodium acetate, 9.7 mM pyruvate, 0.25 mM ß-nicotinamide adenine dinucleotide, reduced form, 0.81 mM potassium phosphate, and 0.025 - 0.05 unit L-lactic dehydrogenase.

NOTES:

1. 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.