Enzymatic Assay of D-LACTIC DEHYDROGENASE (EC 1.1.1.28)

PRINCIPLE:

Pyruvate + β-NADH $\xrightarrow{\text{D-Lactic Dehydrogenase}}$ D-Lactate + β-NAD

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

CONDITIONS: T = 25°C, pH = 7.0, A_{340nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

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.)

B. 11 mM β-Nicotinamide Adenine Dinucleotide, Reduced Form Solution (β-NADH)
(Prepare 1 ml in cold deionized water using β-Nicotinamide Adenine Dinucleotide, Reduced Form, Disodium Salt, Sigma Prod. No. N-8129. **PREPARE FRESH.)**

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.)

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.)

E. D-Lactic Dehydrogenase Enzyme Solution
(Immediately before use, prepare a solution containing 0.3 - 0.60 unit/ml of D-Lactic Dehydrogenase in cold Reagent D. **PREPARE FRESH**.)

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PROCEDURE:

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

	Test	Blank
Reagent A (Buffer)	2.50	2.50
Reagent B (β-NADH)	0.05	0.05
Reagent C (Pyruvate)	0.10	0.10

Mix by inversion and equilibrate to 25°C. Monitor the A_{340nm} until constant, using a suitably thermostatted spectrophotometer. Then add:

Reagent D (BSA)	------	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 ΔA_{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.