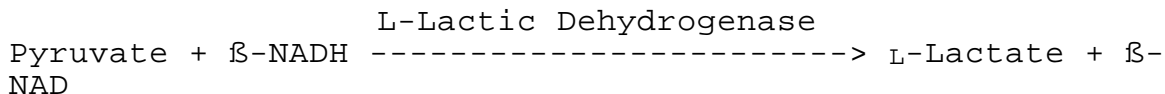


**Enzymatic Assay of L-LACTIC DEHYDROGENASE
(EC 1.1.1.27)**

PRINCIPLE:



Abbreviations used:

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

β -NAD = β -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 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, 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.)

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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 (β-NADH)	0.06	0.06

Mix by inversion and equilibrate to 30°C. Monitor the $A_{340\text{nm}}$
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
 $A_{340\text{nm}}$ for approximately 5 minutes. Obtain the $r A_{340\text{nm}}/\text{minute}$
using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

($r A_{340\text{nm}}/\text{min Test} - r A_{340\text{nm}}/\text{min Blank}$)(3.08)(df)

Units/ml enzyme =

(6.22)(0.05)

3.08 = Total volume (in milliliters) of assay

df = Dilution factor

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

0.05 = Volume (in milliliter) of assay

units/ml enzyme

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

units/ml enzyme

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

**Enzymatic Assay of L-LACTIC DEHYDROGENASE
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UNIT DEFINITION:

One unit will reduce 1.0 μ mole 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 β -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.