SIGMA QUALITY CONTROL TEST PROCEDURE

ProductInformation

Enzymatic Assay of L-LACTIC DEHYDROGENASE[1] (EC 1.1.1.27)

PRINCIPLE:

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

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

CONDITIONS: T = 37°C, pH = 7.5, A_{340nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 100 mM Sodium Phosphate Buffer, pH 7.5 at 37°C
(Prepare 200 ml in deionized water using Sodium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. S-0751. Adjust to pH 7.5 at 37°C with 1 M NaOH.)

B. 0.13 mM β-Nicotinamide Adenine Dinucleotide, Reduced Form Solution (β-NADH)
(Prepare 10 ml in cold Reagent A using β-Nicotinamide Adenine Dinucleotide, Reduced Form, Disodium Salt, Sigma Prod. No. N-8129 or dissolve the contents of 1 mg vial of β-Nicotinamide Adenine Dinucleotide, Reduced Form, Disodium Salt, Sigma Stock No. 340-101, in the appropriate volume of Reagent A. **PREPARE FRESH**.)

C. 69 mM Sodium Pyruvate Solution (Pyruvate)
(Prepare 1.0 ml in cold Reagent A 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. **PREPARE FRESH**.)

E. L-Lactic Dehydrogenase Enzyme Solution
(Immediately before use, prepare a solution containing 0.25 - 0.75 unit/ml of L-Lactic Dehydrogenase in cold Reagent D.)

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PROCEDURE:

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

	Test	Blank
Reagent B (β-NADH)	2.80	2.80
Reagent C (Pyruvate)	0.10	0.10

Mix by inversion and equilibrate to 37°C. Monitor the A_{340nm} until constant, using a suitably thermostatted spectrophotometer. Then add:

Reagent D (BSA)	------	0.10
Reagent E (Enzyme Solution)	0.10	------

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:

$$\text{Units/ml enzyme} = \frac{(\Delta A_{340nm}\text{/min Test} - \Delta A_{340nm}\text{/min Blank})(3)(df)}{(6.22)(0.1)}$$

3 = Total volume (in milliliters) of assay
df = Dilution factor
6.22 = Millimolar extinction coefficient of β-NADH at 340 nm
0.1 = Volume (in milliliter) of enzyme used

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

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

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UNIT DEFINITION:

One unit will reduce 1.0 μmole of pyruvate to L-lactate per minute at pH 7.5 at 37°C.

FINAL ASSAY CONCENTRATION:

In a 3.00 ml reaction mix, the final concentrations are 100 mM sodium phosphate, 0.12 mM β-nicotinamide adenine dinucleotide, reduced form, 2.3 mM pyruvate, 0.033% (w/v) bovine serum albumin and 0.025 - 0.075 unit L-lactic dehydrogenase.

REFERENCES:

Bergmeyer, H.U. and Bernt, E. (1974) in *Methods of Enzymatic Analysis*, (Bergmeyer, H.U. ed.) Volume 2, 574-578, Academic Press, New York, NY

NOTES:

1. This assay is suitable for the following L-Lactic Dehydrogenases, Sigma Prod. Nos.: L-2375, L-2500, L-5132, L-1378, L-1254, L-3379, L-0755, L-3632, L-3757, L-3882, L-9757, L-4387, L-0883, L-2518, L-6504, L-6383, L-5008, L-9887, L-0508, L-6508, and L5762.

2. This assay is based on the cited reference.

3. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.