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ProductInformation

SIGMA QUALITY CONTROL TEST PROCEDURE

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

PRINCIPLE:

Pyruvate + β-NADH $\xrightarrow{\text{L-Lactic Dehydrogenase}}$ L-Lactate + β-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, Anhydrous, Monobasic, 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)
(Dissolve the contents of a 1 mg vial of β-Nicotinamide Adenine Dinucleotide, Reduced Form, Disodium Salt, Sigma Stock No. 340-101, in the appropriate volume of cold Reagent A **or** prepare 10 ml in cold Reagent A using β-Nicotinamide Adenine Dinucleotide, Reduced Form, Disodium Salt, Sigma Prod. No. N-8129. **PREPARE FRESH.)**

C. 34 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**)

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REAGENTS: (continued)

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.)

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 milliliters) 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, 1.1 mM pyruvate, 0.03% (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., 2nd ed.; Academic Press: New York, NY, Volume II, 574-579

NOTES:

1. This assay procedure is to be used to assay L-Lactic Dehydrogenase, Sigma Prod. Nos.: L-0133, L-0377, L-1006, L-2625, L-7525, L-2889, L-7755, L-9126, L-9382, and L-9889.

2. This assay is based on the cited reference.

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