

Product Information

SIGMA QUALITY CONTROL TEST PROCEDURE

Enzymatic Assay of L-LACTIC DEHYDROGENASE¹ (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 = 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:

	<u>Test</u>	<u>Blank</u>
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 $A_{340\text{nm}}$ 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 $A_{340\text{nm}}$ for approximately 5 minutes. Obtain the $\Delta A_{340\text{nm}}$ /minute using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

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

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 μ mole 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.

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