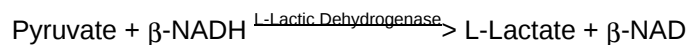


Product Information

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**)

Enzymatic Assay of L-LACTIC DEHYDROGENASE¹ (EC 1.1.1.27)

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

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

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

Enzymatic Assay of L-LACTIC DEHYDROGENASE¹
(EC 1.1.1.27)

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.

Sigma warrants that the above procedure information is currently utilized at Sigma and that Sigma products conform to the information in Sigma publications. Purchaser must determine the suitability of the information and products for its particular use. Upon purchase of Sigma products, see reverse side of invoice or packing slip for additional terms and conditions of sale.