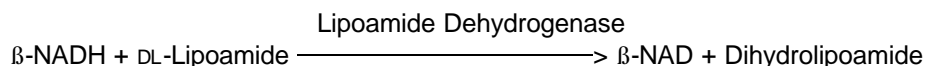


Enzymatic Assay of LIPOAMIDE DEHYDROGENASE (EC 1.8.1.4)

PRINCIPLE:



Abbreviations used:

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

β -NAD = β -Nicotinamide Adenine Dinucleotide, Oxidized Form

DL-Lipoamide = DL-6,8-Thioctic Acid Amide

Dihydrolipoamide = DL-6,8-Dihydrothioctic Acid Amide

CONDITIONS: T = 25°C, pH = 6.5, $A_{340\text{nm}}$, Light Path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

- A. 50 mM Sodium Phosphate Buffer, pH 6.5 at 25°C
(Prepare 200 ml in deionized water using Sodium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. S-0751. Adjust to pH 6.5 at 25°C with 1 M NaOH.)
- B. 28 mM DL-Thioctic Acid Amide Solution (DL-Thio)
(Prepare by dissolving 20 mg of DL-6,8-Thioctic Acid Amide, Sigma Prod. No. T-5875, in 2 ml Ethanol (Nondenatured). Dilute this solution with 1.5 ml Reagent A. **PREPARE FRESH.**)
- C. 7.0 mM β -Nicotinamide Adenine Dinucleotide, Reduced Form Solution (β -NADH)
(Prepare 2 ml in deionized water using β -Nicotinamide Adenine Dinucleotide, Reduced Form, Disodium Salt, Sigma Prod.No. N-8129 or dissolve the contents of one 10 mg vial of β -Nicotinamide Adenine Dinucleotide, Reduced Form, Disodium Salt, Sigma Stock No. 340-110, in the appropriate volume of deionized water. **PREPARE FRESH.**)

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REAGENTS: (continued)

- D. 20 mM β -Nicotinamide Adenine Dinucleotide, Oxidized Form Solution (β -NAD)
(Dissolve the contents of one 5.0 mg vial of β -Nicotinamide Adenine Dinucleotide, Sigma Stock No. 260-150, in the appropriate volume of deionized water or prepare 2.0 ml in deionized water using β -Nicotinamide Adenine Dinucleotide, Sigma Prod. No. N-7004. **PREPARE FRESH.**)

- E. 300 mM Ethylenediaminetetraacetic Acid with 2.0% (w/v) Albumin Solution, pH 7.0 at 25°C (EDTA)
(Prepare 50 ml in deionized water using Ethylenediaminetetraacetic Acid, Tetrasodium Salt, Hydrate, Sigma Stock No. ED4S, and Albumin Bovine, Sigma Prod. No. A-4503. Adjust to pH 7.0 at 25°C with 5 M HCl.)

- F. Lipoamide Dehydrogenase Enzyme Solution
(Immediately before use, prepare a solution containing 0.3 - 0.6 unit/ml of Lipoamide Dehydrogenase in cold Reagent A.)

PROCEDURE:

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

	<u>Test</u>	<u>Blank</u>
Reagent A (Buffer)	2.50	2.60
Reagent B (DL-Thio)	0.10	0.10
Reagent C (β -NADH)	0.10	0.10
Reagent D (β -NAD)	0.10	0.10
Reagent E (EDTA)	0.10	0.10

Mix by inversion and equilibrate to 25°C. Monitor the $A_{340\text{nm}}$ until constant, using a suitably thermostatted spectrophotometer. Then add:

Reagent F (Enzyme Solution)	0.10	-----
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Immediately mix by inversion and record the decrease in $A_{340\text{nm}}$ for approximately 5-10 minutes. Obtain the $\Delta A_{340\text{nm}}/\text{minute}$ using the maximum linear rate for both the Test and Blank.

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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 = 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

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

UNIT DEFINITION:

One unit will reduce 1.0 μ mole of DL-lipoamide to DL-dihydrolipoamide per minute at pH 6.5 at 25°C.

FINAL ASSAY CONCENTRATION:

In a 3.00 ml reaction mix, the final concentrations are 44 mM sodium phosphate, 0.93 mM DL-thioctic acid amide, 1.9% (v/v) ethanol, 0.2 mM β -nicotinamide adenine dinucleotide, reduced form, 0.67 mM β -nicotinamide adenine dinucleotide, 10 mM ethylenediaminetetraacetic acid, 0.07% (w/v) bovine serum albumin and 0.03 - 0.06 unit lipoamide 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.