Enzymatic Assay of LIPOAMIDE DEHYDROGENASE
(EC 1.8.1.4)

PRINCIPLE:

$$\text{ß-NADH} + \text{DL-Lipoamide} \xrightarrow{\text{Lipoamide Dehydrogenase}} \text{ß-NAD} + \text{Dihydrolipoamide}$$

Abbreviations used:
ß-NADH = ß-Nicotinamide Adenine Dinucleotide, Reduced Form
ß-NAD = ß-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_{340nm}, 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 ß-Nicotinamide Adenine Dinucleotide, Reduced Form Solution (ß-NADH)
(Prepare 2 ml in deionized water using ß-Nicotinamide Adenine Dinucleotide, Reduced Form, Disodium Salt, Sigma Prod.No. N-8129 or dissolve the contents of one 10 mg vial of ß-Nicotinamide Adenine Dinucleotide, Reduced Form, Disodium Salt, Sigma Stock No. 340-110, in the appropriate volume of deionized water. **PREPARE FRESH**.)

REAGENTS: (continued)

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

	Test	Blank
Reagent A (Buffer)	2.50	2.60
Reagent B (DL-Thio)	0.10	0.10
Reagent C (ß-NADH)	0.10	0.10
Reagent D (ß-NAD)	0.10	0.10
Reagent E (EDTA)	0.10	0.10

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

Reagent F (Enzyme Solution)	0.10	------

Immediately mix by inversion and record the decrease in A_{340nm} for approximately 5-10 minutes. Obtain the ΔA_{340nm}/minute using the maximum linear rate for both the Test and Blank.

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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 = Volume (in milliliters) of assay
df = Dilution factor
6.22 = Millimolar extinction coefficient of ß-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 µmole 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 ß-nicotinamide adenine dinucleotide, reduced form, 0.67 mM ß-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.