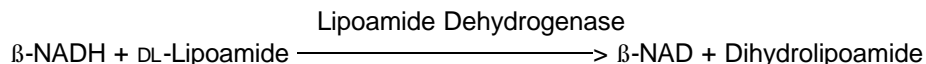


## Enzymatic Assay of LIPOAMIDE DEHYDROGENASE (EC 1.8.1.4)

### PRINCIPLE:



Abbreviations used:

$\beta$ -NADH =  $\beta$ -Nicotinamide Adenine Dinucleotide, Reduced Form

$\beta$ -NAD =  $\beta$ -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  $\beta$ -Nicotinamide Adenine Dinucleotide, Reduced Form Solution ( $\beta$ -NADH)  
(Prepare 2 ml in deionized water using  $\beta$ -Nicotinamide Adenine Dinucleotide, Reduced Form, Disodium Salt, Sigma Prod.No. N-8129 or dissolve the contents of one 10 mg vial of  $\beta$ -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  $\beta$ -Nicotinamide Adenine Dinucleotide, Oxidized Form Solution ( $\beta$ -NAD)  
(Dissolve the contents of one 5.0 mg vial of  $\beta$ -Nicotinamide Adenine Dinucleotide, Sigma Stock No. 260-150, in the appropriate volume of deionized water or prepare 2.0 ml in deionized water using  $\beta$ -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 ( $\beta$ -NADH) | 0.10        | 0.10         |
| Reagent D ( $\beta$ -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 | ----- |
|-----------------------------|------|-------|

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  $\beta$ -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  $\mu$ 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  $\beta$ -nicotinamide adenine dinucleotide, reduced form, 0.67 mM  $\beta$ -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.**