Enzymatic Assay of LIPOAMIDE DEHYDROGENASE
(EC 1.8.1.4)

**PRINCIPLE:**

\[
\text{Lipoamide Dehydrogenase} \quad \beta\text{-NADH + DL-Lipoamide} \rightarrow \beta\text{-NAD + Dihydrolipoamide}
\]

Abbreviations used:
- $\beta\text{-NADH} = \beta\text{-Nicotinamide Adenine Dinucleotide, Reduced Form}$
- $\beta\text{-NAD} = \beta\text{-Nicotinamide Adenine Dinucleotide, Oxidized Form}$
- $\text{DL-Lipoamide} = \text{DL-6,8-Thioctic Acid Amide}$
- $\text{Dihydrolipoamide} = \text{DL-6,8-Dihydrothioctic Acid Amide}$

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

**METHOD:** Continuous Spectrophotometric Rate Determination

**REAGENTS:**

A. 50 mM Sodium Phosphate Buffer, $\pH$ 6.5 at $25^\circ C$
   (Prepare 200 ml in deionized water using Sodium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. S-0751. Adjust to $\pH$ 6.5 at $25^\circ C$ with 1 M NaOH.)

B. 28 mM $\text{DL-Thioctic Acid Amide Solution (DL-Thio)}$
   (Prepare by dissolving 20 mg of $\text{DL-6,8-Thioctic Acid Amide}$, 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 ($\beta\text{-NADH}$)
   (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 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:

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent A (Buffer)</td>
<td>2.50</td>
<td>2.60</td>
</tr>
<tr>
<td>Reagent B (DL-Thio)</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>Reagent C (β-NADH)</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>Reagent D (β-NAD)</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>Reagent E (EDTA)</td>
<td>0.10</td>
<td>0.10</td>
</tr>
</tbody>
</table>

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

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

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

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

- \( \Delta A_{340\text{nm}}/ \text{min Test} \) = Volume (in milliliters) of assay
- \( \Delta A_{340\text{nm}}/ \text{min Blank} \) = 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 \( \text{DL} \)-lipoamide to \( \text{DL} \)-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 \( \text{DL} \)-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.