Enzymatic Assay of LEUCINE DEHYDROGENASE  
(EC 1.4.1.9)

PRINCIPLE:

\[ \text{L-Leucine} + \beta-\text{NAD} \xrightarrow{\text{Leucine Dehydrogenase}} \alpha-\text{Ketoisocaproate} + \beta-\text{NADH} \]

Abbreviations used:

\( \beta\text{-NAD} = \beta\text{-Nicotinamide Adenine Dinucleotide, Oxidized Form} \)

\( \beta\text{-NADH} = \beta\text{-Nicotinamide Adenine Dinucleotide, Reduced Form} \)

CONDITIONS:  \( T = 37^\circ\text{C}, \  \text{pH} = 10.5, \ A_{340\text{nm}}, \  \text{Light path} = 1 \ \text{cm} \)

METHOD:  Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 200 mM Glycine with 200 mM Potassium Chloride Buffer, pH 10.5 at 37°C  

B. 20 mM \text{L-Leucine Solution (Leu)}  
(Prepare 20 ml in Reagent A using L-Leucine, Prod. No. L-8000.)

C. 12.5 mM \beta-Nicotinamide Adenine Dinucleotide Solution (\beta-NAD)  
(Prepare 2 ml in deionized water using \beta-Nicotinamide Adenine Dinucleotide, Prod. No. N-7004 or dissolve the contents of one 20 mg vial of \beta-Nicotinamide Adenine Dinucleotide, Stock No. 260-120, in the appropriate volume of deionized water. \text{PREPARE FRESH.})

D. 25 mM Potassium Phosphate Solution, pH 7.2 at 37°C  
(Enzyme Diluent)  
(Prepare 100 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous Prod. No. P-5379. Adjust to pH 7.2 at 37°C with 1 M KOH.)
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REAGENTS: (continued)

E. Leucine Dehydrogenase Enzyme Solution  
(Immediately before use, prepare a solution containing 
0.1 - 0.5 unit/ml of Leucine Dehydrogenase in cold 
Reagent D.)

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 B (Leu)</td>
<td>3.00</td>
<td>3.00</td>
</tr>
<tr>
<td>Reagent C (β-NAD)</td>
<td>0.30</td>
<td>0.30</td>
</tr>
</tbody>
</table>

Mix by inversion and equilibrate to 37°C. Monitor the 
A$_{340\text{nm}}$ until constant, using a suitably thermostatted 
spectrophotometer. Then add:

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent D (Enzyme Diluent)</td>
<td>------</td>
<td>0.05</td>
</tr>
<tr>
<td>Reagent E (Enzyme Solution)</td>
<td>0.05</td>
<td>------</td>
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</tbody>
</table>

Immediately mix by inversion and record the increase in 
A$_{340\text{nm}}$ for approximately 5 minutes. Obtain the r A$_{340\text{nm}}$/minute 
using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

\[
\text{Units/mg enzyme} = \frac{r \ A_{340\text{nm}}/\text{min Test} - r \ A_{340\text{nm}}/\text{min Blank}}{(6.22) \ (\text{mg enzyme/ml RM})}
\]

6.22 = Millimolar extinction coefficient of β-NADH at 340 

RM = Reaction Mix

UNIT DEFINITION:

One unit will convert 1.0 µmole of L-leucine to a-ketoiso-
caproate per minute at pH 10.5 at 37°C.
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FINAL ASSAY CONCENTRATION:

In a 3.35 ml reaction mix, the final concentrations are 179 mM glycine, 179 mM potassium chloride, 18 mM L-leucine, 1.1 mM β-NAD, 0.37 mM potassium phosphate and 0.005 - 0.025 unit leucine dehydrogenase.

REFERENCES:


NOTES:

1. All product and stock numbers, unless otherwise indicated, are Sigma product and stock numbers.

This procedure is for informational purposes. For a current copy of Sigma's quality control procedure contact our Technical Service Department.