LEUCINE DEHYDROGENASE (LeuDH)

[EC 1. 4. 1. 9]

from *Bacillus stearothermophilus*

L-Leucine + NAD⁺ + H₂O ↔ α-Ketoisocaproate+ NH₄⁺ + NADH

**SPECIFICATION**

<table>
<thead>
<tr>
<th>State</th>
<th>Lyophilized</th>
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<tbody>
<tr>
<td>Specific activity</td>
<td>more than 40 U/mg protein</td>
</tr>
<tr>
<td>Contaminants NADH oxidase</td>
<td>&lt; 0.01 %</td>
</tr>
<tr>
<td>Contaminants Lactate dehydrogenase</td>
<td>&lt; 0.01 %</td>
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</tbody>
</table>

**PROPERTIES**

| Molecular weight | ca. 300,000 |
| Subunit molecular weight | ca. 49,000 |
| Optimum pH | 10.6 (Fig. 1) |
| pH stability | 6.0 - 11.5 (Fig. 2) |
| Thermal stability | No detectable decrease in activity up to 60 °C. (Fig. 3, 4) |
| Michaelis constants L-Leucine | 3.4 mM |
| Michaelis constants NAD⁺ | 0.3 mM |
| Substrate specificity L-Leucine | 100 % |
| Substrate specificity L-Valine | 86 % |
| Substrate specificity L-Isoleucine | 73 % |

**STORAGE**

Stable at -20 °C for at least one year

**APPLICATION**

The enzyme is useful for determination of L-leucine, L-valine or L-isoleucine.
ASSAY

Principle
The change in absorbance is measured at 340 nm according to the following reaction.

\[
\text{L-Leucine + NAD}^+ + \text{H}_2\text{O} \xrightarrow{\text{LeuDH}} \text{α-Ketoisocaproate} + \text{NH}_4^+ + \text{NADH}
\]

Unit Definition
One unit of activity is defined as the amount of LeuDH that forms 1 μmol of NADH per minute at 30 °C.

Solutions
I Buffer solution ; 250 mM Sodium phosphate, pH 10.5
II L-Leucine solution ; 60 mM (0.787 g L-leucine/80 mL distilled water, adjusted to pH 10.5 with 1 N-NaOH and filled up to 100 mL with distilled water)
III NAD⁺ solution ; 100mM (0.663 g NAD⁺/ 10mL with distilled water)

Preparation of Enzyme Solution
Dissolve the lyophilized enzyme with distilled water and dilute to 5 to 10 U/mL with 100 mM sodium phosphate buffer, pH 9.5.

Procedure
1. Prepare the following reaction mixture and pipette 3.00 mL of reaction mixture into a cuvette,
   Solution I 15.00mL Solution III 0.93mL
   Solution II 10.00mL \( \text{H}_2\text{O} \) 4.07mL
2. Incubate at 30°C for about 3 minutes.
3. Add 0.01 mL of enzyme solution into the cuvette and mix.
4. Read absorbance change at 340 nm per minute (ΔAbs₃₄₀) in the linear portion of curve.

Calculation

\[
\text{Volume activity (U/mL)} = \frac{\Delta \text{Abs}_{340}}{6.22 \times 0.01} X \text{d.f.}
\]

Specific activity (U/mg protein) = \[
\frac{\text{Volume activity (U/mL)}}{\text{Protein concentration (mg/mL)}*}
\]

\text{d.f. ; dilution factor}
6.22 ; millimolar extinction coefficient of NADH (cm²/μmol)
*Protein concentration ; determined by Bradford's method

REFERENCE
Fig. 1  pH profile

- □ Gly-KOH,
- ○ phosphate

Fig. 2  pH stability

- △ acetate,
- ○ phosphate,
- ● Tris-HCl,
- ■ Gly-KOH

Fig. 3  Thermal stability

- treated for 24 hr at 4 °C in the following buffer solution (0.1 M);
  - △ acetate,
  - ○ phosphate,
  - ● Tris-HCl,
  - ■ Gly-KOH

Fig. 4  Thermal stability

- treated in 0.1M Gly-KOH buffer, pH 9.0
  - ○ 60°C, □ 70°C, ● 80°C