

ENZYMES

BY

SORACHIM

Leucine Dehydrogenase from Bacillus sp.

LED-201

SPECIFICATIONS

Product name	L-Leucine: NAD ⁺ oxidoreductase (deaminating)
EC	1.4.1.9
Appearance	White amorphous powder, lyophilized
Activity	Grade II, 20 U/mg-solid or more (contains approx. 70% of stabilizers)
Contaminants	Leucylpeptide decomposing enzymes : (Leu-Val) : $\leq 1.0 \times 10^{-2}\%$, (Leu-Gly-Gly) : $\leq 1.0 \times 10^{-2}\%$ NADH oxidase : $\leq 1.0 \times 10^{-2}\%$
Stabilizers	2-Mercaptoethanol, L-cysteine, dithiothreitol, ethylenediaminetetraacetate
Stability	Stable at -20°C for at least 12 months
Molecular weight	245,000
Michaelis constants	$1.0 \times 10^{-3}\text{M}$ (L-Leucine), $3.9 \times 10^{-4}\text{M}$ (NAD ⁺), $3.5 \times 10^{-5}\text{M}$ (NADH) $3.1 \times 10^{-4}\text{M}$ [α -Ketoisocaproate (α -K I C)], $2.0 \times 10^{-1}\text{M}$ (NH ₃)
Structure	6 subunits per mol of enzyme
Inhibitors	Na ₂ S, Hg ²⁺ , Cu ²⁺ , Co ²⁺ , Mg ²⁺ , p-chloromercuribenzoate
Optimum pH	10.5 - 10.8 (L-Leu → α -K I C), 9.4 (α -K I C → L-Leu)
Optimum temperature	Above 70°C
pH Stability	pH 5.5 - 10.5 (25°C, 20hr)
Thermal stability	Below 60°C (pH 6.9, 10min)

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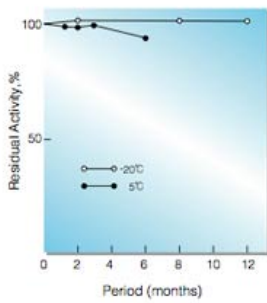


Fig.1. Stability (Powder form)
(kept under dry conditions)

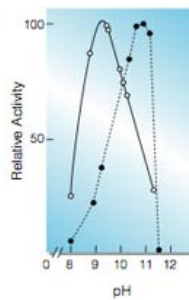


Fig.2. pH-Activity
○ — α-KIC → Leu in 1M ammonium buffer
● — Leu → α-KIC in 0.2M glycine-KOH buffer



Fig.4. pH-Stability
[25°C, 20hr-treatment with 50mM buffer solution: pH4.0-6.0, acetate; pH6.0-8.5, phosphate; pH9.0-11.0, carbonate:
○ — with 0.01% mercaptoethanol
● — without mercaptoethanol



Fig.3. Temperature activity
○ — α-KIC → Leu in 1.0M ammonium buffer pH9.5
● — Leu → α-KIC in 0.2M glycine-KOH buffer pH10.5



Fig.5. Thermal stability
[10min-treatment with 50mM phosphate buffer, pH6.9]

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