

ENZYMES

BY

SORACHIM

D-3-Hydroxybutyrate Dehydrogenase from Pseudomonas sp. HBD-301

SPECIFICATIONS

Product name	(R)-3-hydroxybutanoate: NAD ⁺ oxidoreductase
EC	1.1.1.30
Appearance	white amorphous powder lyophilized
Activity	Grade III, 100 U/mg-solid or more
Contaminants	NADH oxidase ≤ 2.0×10 ⁻³ % Malate dehydrogenase ≤ 2.0×10 ⁻³ % Lactate dehydrogenase ≤ 2.0×10 ⁻³ %
Stabilizers	Sucrose, mannitol, BSA
Stability	Stable at - 20°C for at least 12 months
Molecular weight	approx. 130,000 (by gel filtration)
Isoelectric point	5.6±0.1
Michaelis constants	4.2×10 ⁻⁴ M (25°C, pH8.3), 7.0×10 ⁻⁴ M (37°C, pH8.3) (D-3-Hydroxybutyrate) 4.9×10 ⁻⁵ M (25°C, pH8.3), 7.2×10 ⁻⁵ M (37°C, pH8.3) (NAD ⁺) 8.1×10 ⁻⁵ M (25°C, pH7.1), 2.4×10 ⁻⁴ M (37°C, pH7.1) (Acetoacetate) 8.4×10 ⁻⁶ M (25°C, pH7.1), 1.5×10 ⁻⁵ M (37°C, pH7.1) (NADH)
Inhibitors	PCMB, MIA, IAA, Ag ⁺ , Hg ²⁺ , SDS, DAC
Optimum pH	8.3
Optimum temperature	55°C
pH Stability	pH 5.0 - 8.5 (25°C, 20hr)
Thermal stability	below 40°C (pH 6.5, 15min)

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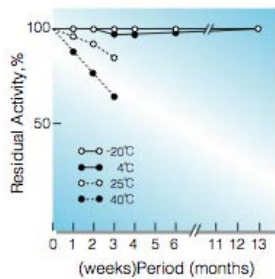


Fig.1. Stability (Powder form)
(kept under dry conditions)

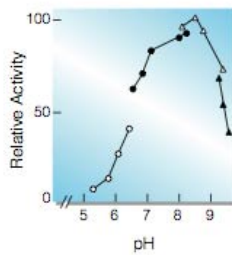


Fig.2. pH-Activity

[37°C,5min-reaction in 0.1M buffer solution:
pH5,3-6.5, dimethylglutaric acid-NaOH;pH5.9-
8.3,K-phosphate;pH7.9-9.1,Tris-HCl;
pH8.9-9.2,K₂CO₃-NaHCO₃]

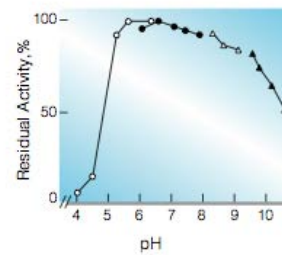


Fig.4.pH-Stability

[25°C,20hr-treatment with 50mM buffer
solution:pH4.0-6.0, dimethylglutaric acid-
NaOH;pH6.0-8.0, K-phosphate;pH8.0-9.0,
Tris-HCl;pH9.0-10.5, K₂CO₃-NaHCO₃]

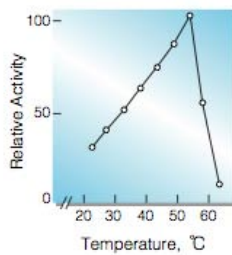


Fig.3. Temperature activity

(in 0.1M Tris-HCl buffer, pH8.3)

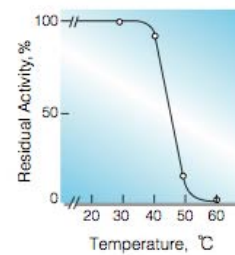


Fig.5. Thermal stability

[15min-treatment with 50mM K-phosphate
buffer,pH6.5. enzyme concn.:50U/ml]