Leucine Dehydrogenase from Bacillus sp.

L1493003

Storage temperature: -20℃.

Introduction

Leucine Dehydrogenase is a member of the amino acid dehydrogenase family. Leucine Dehydrogenase is a nicotinamide adenine dinucleotide hydrogen (NADH)-dependent oxidoreductase. It is involved in catalyzing the reductive amination of aliphatic 2-oxo-acids to their respective L-amino acids.

PREPARATION and SPECIFICATION

Appearance	White amorphous powder, lyophilized
Activity	≥20U/mg enzyme powder (containing approx. 70 % of stabilizers)
Contaminants	Leucylpeptide decomposing enzymes
(Leu-Val)	≤1.0×10^{-2} %
(Leu-Gly-Gly)	≤1.0×10^{-2} %
NADH oxidase	≤ 1.0×10^{-2} %
Stabilizers	2-Mercaptoethanol, L-cysteine, dithiothreitol, ethylenediaminetetraacetate

PROPERTIES

Stability	Stable at −20 ℃ for at least one year	(Fig1)
Molecular weight	245,000	
Michaelis constants	1.0×10^{-3} M (L-Leucine), 3.9×10^{-4} M (NAD^+), 3.5×10^{-5} M (NADH), 3.1×10^{-4} M [α-Ketoisocaproate (α-KIC)], 2.0×10^{-1} M (NH_3)	
Structure	6 subunits per enzyme molecule	
Inhibitors	Na_2S, Hg^{2+}, Cu^{2+}, Co^{2+}, Mg^{2+}, p-chloromercuribenzoate	
Optimum pH	10.5–10.8 (L-Leu→α-KIC), 9.4 (α-KIC→L-Leu)	(Fig3)
Optimum temperature	above 70 ℃	(Fig4)
pH Stability	pH 5.5–10.5 (25 ℃, 20 hr)	(Fig5)
Thermal stability	below 60 ℃ (pH 6.9, 10 min)	(Fig6)
Substrate specificity	(Table 1)	

APPLICATIONS

This enzyme is useful for enzymatic measurement of L-leucine and the activity of leucine amino-peptidase.

ASSAY

1.Principle

$$\text{L-Leucine} + NAD^+ + H_2O \xrightarrow{\text{leucine dehydrogenase}} \alpha\text{-Ketoisocaproate} + NADH + NH_3 + H^+$$

The formation of NADH is measured at 340 nm by spectrophotometry.

2. Unit definition

One unit causes the formation of one micromole of NADH per minute under the conditions detailed below.

3. Method

3.1 Reagents

A. L-Leucine solution: 20mM L-leucine in 0.2M glycine-KCl-KOH buffer, pH 10.5 (Prepare freshly)

B. NAD^+ solution: 12.5mM (Should be prepared fresh)

C. Enzyme diluent: 25mM K-phosphate buffer, pH 7.2

3.2 Procedure

① Prepare the following reaction mixture in a cuvette (d＝1.0cm) and equilibrate at 37 ℃ for approximately 5 minutes.

3.0 mL	Substrate solution	(A)
0.3 mL	NAD^+ solution	(B)

Concentration in assay mixture:

Glycine buffer	0.18 M
L -Leucine	18 mM
NAD^+	1.1mM

② Add 0.05 mL of the enzyme solution* and mix by gentle inversion.

③ Record the increase in optical density at 340 nm against water for 2 to 3 minutes with a spectrophotometer thermostated at 37 ℃, and calculate the ΔOD per minute from the initial linear portion of the curve (ΔOD test).

At the same time, measure the blank rate (ΔOD blank) using the same method as the test except that the enzyme diluent (C) is added instead of the enzyme solution.

*Dissolve the enzyme preparation in ice-cold enzyme diluent (C) (approx. 5 mg/mL) and dilute to 0.25−0.33 U/mL with the same buffer, immediately before the assay.

3.3 Calculation

Activity can be calculated by using the following formula:

$$\text{Volume activity (U/ml)} = \frac{\Delta OD/min\,(\Delta OD\ test - \Delta OD\ blank) \times Vt \times df}{6.22 \times 1.0 \times Vs} = \Delta OD/min \times 10.77 \times df$$

$$\text{Weight activity (U/mg)} = (U/ml) \times 1/C$$

Vt	Total volume (3.35 mL)
Vs	Sample volume (0.05 mL)
6.22	Millimolar extinction coefficient of NADH (cm²/micromole)
1.0	Light path length (cm)
df	Dilution factor
C	Enzyme concentration in dissolution (c mg/mL)

Table 1. Substrate Specificity of Leucine dehydrogenase

Substrate(10mM)	Relative activity(%)	Substrate(10mM)	Relative activity(%)
L-Leucine	100	α-Ketoisocaproate	100
L-Valine	74	α-Ketoisovalerate	126
L-Isoleucine	58	α-Ketovalerate	76
L-Norvaline	41	α-Ketobutyrate	57
L-Norleucine	10	α-Ketocaproate	46
L-Methionine	0.6	Inert: Pyruvate, α-Ketoglutarate, Phenylpyruvate, Oxaloacetate, Glyoxylate	
L-Cysteine	0.3		
Inert: L-Ala, L-Glu, L-Thr, L-Ser, Gly, L-Phe, L-Lys, L-Arg, D-Leu, D-Val, D-Ile			

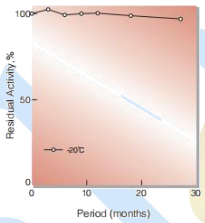

Fig.1. Stability (Powder form)
(kept under dry conditions)

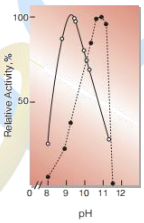

Fig.3. pH-Activity
○—○: α-KIC→Leu in 1M ammonium buffer
●---●:Leu→α-KIC in 0.2M glycine-KOH buffer

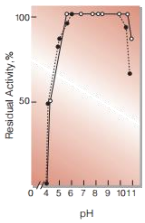

Fig.5. pH-Stability
25℃,20hr-treatment with 50mM buffer solution:pH4.0-6.0,acetate; pH6.0-8.5,phosphate;pH9.0-11.0, carbonate:
○—○:with 0.01% mercaptoethanol
●---●:without mercaptoethanol

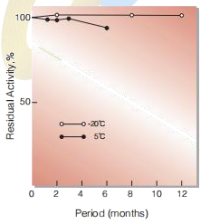

Fig.2. Stability (Powder form)
(kept under dry conditions)

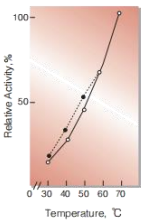

Fig.4. Temperature activity
○—○: α-KIC→Leu in 1.0M ammonium buffer pH9.5
●---●:Leu→α-KIC in 0.2M glycine-KOH buffer pH10.5

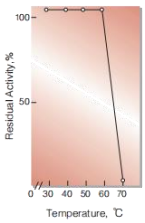

Fig.6. Thermal stability
10min-treatment with 50mM phosphate buffer,pH6.9