

L-Leucine Dehydrogenase

L-leucine : NAD⁺ oxidoreductase, Leu-DH
EC 1.4.1.9

Description: L-Leucine Dehydrogenase catalyzes the oxidative deamination of L-leucine, L-valine und L-isoleucine as well as of amino acids with unbranched-chained aliphatic carbonic chain.

Caused by the reverse reaction a lot of 2-ketoacids in particular 2-isocaproate, 2-ketoisovalerate and 2-keto-3-methylvalerate with 0.9 M ammonium were aminated reductively to the corresponding amino acid.

Origin: *Bacillus cereus*

Application:

- syntesis of L-leucine as well as a lot of other aminoacids with unbranched-chained and branched-chained carbonic chain, e.g. L-tert-leucine and L-methionine
- amination of 2-ketoacids under continuous conditions in enzyme-membran-reactors (usage of formiat dehydrogenase in order to the regeneration of the coenzyme).
- synthesis of radioactively marked aminoacids

Molecular weight: 310 000 (± 10 000) Da [1]

Structure: The enzyme is composed of six identical subunits with a molecular weight 39 000 D each. [1]

Isoelectric point: pH 5.75 (native enzyme)
pH 4.0 (subunits) [2]

Activity: > 50 U/ml (method: ASA Spezialenzyme GmbH)

Specific activity: > 20 U/mg

Definition Unit: One international unit will be defined as the amount of L-leucine dehydrogenase that catalyses the production of 1 µmol NADH per minute under standard conditions. [1]

Parameters of
reaction :

pH-optima

oxidative deamination: 10,7 (with L- leucine, L-valine and L-
isoleucine as substrate)

reductive amination: 9,0 – 9,5 (with 2-ketoisocaproate, 2-
ketomethionine as substrate)
8,5 (with 2-ketovalerate as substrate) [1]

temperature-optimum

60°C (oxidative deamination) [1]

Michaelis-
Constants:

oxidative deamination:

<u>substrate</u>	<u>K_M [mM]</u>
NAD ⁺	0,34
L- α -aminobutyrate	22,0
L-norvaline	2,9
L-norleucine	1,5
L-valine	2,5
L-leucine	1,5
L-isoleucine	1,0
L-methionine	23,0

reductive amination:

<u>substrate</u>	<u>K_M [Mm]</u>
NADH	0,034
2-ketobutyrate	1,5
2-ketovalerate	0,4
2-ketocaproate	1,2
2-ketoisovalerate	2,1
2-ketoisocaproate	0,45
2-keto-3-methylvalerate	0,9
2-keto-4-mercaptopbutyrat	2,1

Order-no.: 1410

Form of delivery: suspension with 50% glycerine

Storage: -20°C

Stability: no loss of activity with 50 % glycerine per year at -20°C
loss of activity < 12 % per 24 h at 25°C

Literature: [1] Schütte H., Hummel W., Tsai H., Kula M.R.:
Appl. Microbiol. Biotechnol., 22, 306 – 317 (1985)
[2] Kärst U., Schütte H., Baydoun H., Tsai H.
Proc. 4th European Congress on Biotechnology, Vol.2 (1987)