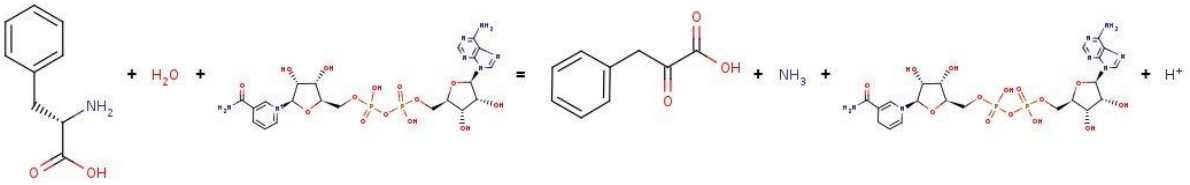
L-Phenylalanine-dehydrogenase

L-Phenylalanine: NAD^+ oxidoreductase, E.C. 1.4.1.20

Description: Partially purified enzyme preparation for the oxidative deamination of L-phenylalanine or the enantioselective reductive amination of phenylpyruvate.

Catalysed reaction:



L-Phenylalanine + H_2O + NAD^+ → Phenylpyruvate + NH_3 + NADH + H^+

Origin: *Rhodococcus spec. Strain M4 (not pathogen)*

Application: Synthesis and determination of L-Phenylalanine

Clinical diagnostics: monitoring of phenylketonuria, determination of L-Phenylalanine in blood and urine

Activity: > 120 U/mL
(Method: ASA Spezialenzyme GmbH)

Specific activity: > 20 U/ mg Protein

Parameter:		
	pH oxidative deamination	Optimum: 10.1 (L-Phenylalanine)
	pH reductive amination	Optimum: 9.25 (Phenylpyruvate)
	Temperature	Optimum: 45°C

Molecular weight: 69 000 (determined via high performance gel filtration)

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Michaelis-constants:

Oxidative deamination:

Substrate	KM [mM]
NAD+	0.22
L-Phenylalanine	0.75
L-Methionine	4.3
L-Tryptophane	10.5

Reductive amination:

substrate	KM [Mm]
NADH	0.08
Phenylpyruvate	0.16
p-Hydroxyphenylpyruvate	2.4
Indolepyruvate	7.7
2-Keto-4 methyl-mercaptobutyric acid	2.1

Inhibitors: The enzyme is completely inhibited by p-mercuric benzoic acid and $HgCl_2$.

The following components lead to a loss of activity between 10 – 20%

EDTA	(1.0 – 10 mM)
1.10-Phenanthrolin	(0.1 – 10 mM)
2.2-Dipyridyl	(0.1 – 10 mM)
2-Mercaptoethanol	(10 mM)
DTE	(1.0 mM)
GSH	(10 mM)

DTE at a concentration of 10 mM causes a 59% loss of activity.

$MgCl_2$, $NiCl_2$, $ZnCl_2$, $CaCl_2$ and Na_2MoO_4 in a concentration of 1 mM in combination with Me^{2+} at a temperature of 30°C do not have an inhibitory effect.

Substrate specificity: Phenylpyruvate, p-hydroxyphenylpyruvate, indolpyruvate or 2-keto-4 mercurymethylmercaptobutyric acid lead to a reaction into the corresponding L-amino acids in the presence of ammonium and NADH.

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Article-no.:	1420
Form of delivery:	Grey to white lyophilizate, stabilized with NAD
Stability:	Stable at -20°C Temperatures over 48°C lead to a fast thermic inactivation pH: stable at 4°C in 100 mM buffer (pH 7.5) The enzyme is stable for app. a hour at pH values from 5.5 – 7. Long thermic stability (app. a week) at positive temperatures from 4 – 8 °C can just be achieved at pH values over 9.
Storage:	-20°C

Literature:

[1] Hummel, W., Weiss, N., Kula, M.-R. (1984), Arch. Microbiol., 137, 47-52

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