

Title (en)

DEHYDROGENASES WITH IMPROVED NAD-DEPENDENCE, THE PRODUCTION THEREOF, AND THEIR USE

Title (de)

DEHYDROGENASEN MIT VERBESSERTER NAD-ABHÄNGIGKEIT, DEREN HERSTELLUNG UND VERWENDUNG

Title (fr)

DESHYDROGENASES A DEPENDANCE NAD AMELIOREE, LEUR PRODUCTION ET LEUR UTILISATION

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Application

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Abstract (en)

[origin: DE19812004A1] The NADH specificity of preferred NADPH-dependent dehydrogenases can be improved by basicity reduction in the coenzyme docking area through corresponding genetically engineered modification of the relevant amino acid sequence. Dehydrogenases with NADH-dependence suitable for preparative purposes which correspond to a kcat/KM value for NAD<+>=20 can be microbially obtained. To this end, a microorganism is used which codes for the enzyme and which has a gene sequence in the genetic code. The gene sequence codes for the enzyme with an amino acid sequence whose basic amino acid(s) are at least partially replaced on the coenzyme binding site or sites by uncharged amino acids. Alternatively or additionally, basic or positively charged or uncharged amino acid(s) can be replaced by negatively charged amino acid(s). The inventive method is especially used for obtaining short-chain dehydrogenases with coenzyme binding sites on the N-terminus. According to the example, alcohol-dehydrogenases were obtained with the aid of E.coli HB101+ (pUBS 520) which contains the mutated gene in an overexpressed manner. Said gene codes for the dehydrogenase and is cloned in the expression plasmid pKK-117-3HB.

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