SIGMA-ALDRICH®

sigma-aldrich.com

3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

# Product Information

**β-Nicotinamide adenine dinucleotide, reduced disodium salt hydrate**

Catalog Numbers **N1161, N6005, and N8129**
Storage Temperature –20 °C

CAS RN 606-68-8 (anhydrous)
Synonyms: β-NADH, NADH, β-DPNH, DPNH, Diphosphopyridine nucleotide, reduced form

**Product Description**
Molecular Formula: $C_{21}H_{27}N_7Na_2O_{14}P_2 \cdot xH_2O$
Molecular Weight: 709.40 (anhydrous basis)

$\lambda_{max}$: 340 nm[1] and 259 nm (pH 9.5)[2]
$E^{mM}$ = 6.22 (340 nm)[1] and 14.4 (259 nm, pH 9.5)[2]
Fluorescent Properties:[3]
Excitation Wavelength = 340 nm
Emission Wavelength = 460 nm

β-NADH, a pyridine nucleotide and biologically active form of nicotinic acid, is a coenzyme necessary for the catalytic reaction of certain enzymes. β-$NAD^+$ is a carrier for hydride ion, forming β-NADH. The hydride ion is enzymatically removed from a substrate molecule by the action of dehydrogenases such as, malic dehydrogenase and lactic dehydrogenase. These enzymes catalyze the reversible transfer of a hydride ion from malate or lactate to β-$NAD^+$, forming the reduced product, β-NADH. Unlike β-$NAD^+$, which has no absorbance at 340 nm, β-NADH absorbs at 340 nm. The increase in absorbance (with the formation of β-NADH) or the decrease in absorbance (with the formation β-$NAD^+$) is the basis for measurement of activity of many enzymes at 340 nm.[4]

Many metabolites and enzymes of biological interest are present in tissues at low concentrations. With the use of β-NADH as a cofactor and several enzymes in a multistep system, known as enzyme cycling, much greater sensitivity for detection of these components is achieved. β-NADH is fluorescent; whereas, β-$NAD^+$ is not. This difference in fluorescence provides a sensitive fluorescent measurement of the oxidized or reduced pyridine nucleotides at concentrations down to $10^{-7}$ M.[5,6] Discussion of optimizing the fluorescence intensity and identification of interfering substances has been reported.[6]

**Precautions and Disclaimer**
This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

**Preparation Instructions**
This product is soluble in 0.01 M NaOH (100 mg/ml).

Water alone should not be used to prepare solutions since it tends to be acidic and would decompose β-NADH. If solutions must be stored for any length of time, phosphate buffers should be avoided since they accelerate the destruction of β-NADH.[6,7] Trizma® (0.01 M, pH 8.5) and MES buffers are better options. Since β-NADH solutions are susceptible to oxidation even at low temperatures, solutions should be prepared at concentrations no greater than 5 mM, at a pH of 9–11, and stored at 4 °C.[6] The presence of light and heavy metals can accelerate the oxidation process[1] If a low temperature freezer is available (temperatures at –40 °C or colder), more concentrated solutions can be prepared and stored for years without any loss of activity.[6]

**Storage/Stability**
Store the product at –20 °C. β-NADH should be stored desiccated and protected from light.[1]

Solutions should be freshly prepared and used promptly unless extreme care is taken. Potent enzyme inhibitors have been reported to form in frozen solutions and even in damp powder. These inhibitors have the same absorbance at 340 nm as β-NADH and they cannot be detected in this manner.[8] Two identified inhibitors of lactate dehydrogenase generated during β-NADH storage have been isolated by chromatography. One is a dimer of the dinucleotide where the AMP moiety is unmodified. The other is generated from β-$NAD^+$ in the presence of a high concentration of phosphate ions at alkaline pH. This compound was formed through the addition of one phosphate group to position C-4 of the nicotinamide ring of β-$NAD^+$.[9]

## References


1. Methods of Enzymatic Analysis, Vol. 1, Bergmeyer, H.U., Academic Press (New York, NY:1974), pp. 545-546.
2. Siegel, J.M. et al., Ultraviolet absorption spectra of DPN and analogs of DPN. Arch. Biochem. Biophys., **82(2)**, 288-299 (1959).
3. Enzymatic Analysis. A Practical Guide, Passonneau, J.V., and Lowry, O.H., Humana Press (Totowa, NJ:1993), pp. 9-10.
4. Methods of Enzymatic Analysis, Vol. 4, Bergmeyer, H.U., Academic Press (New York, NY:1974), pp. 2066-2072.
5. Enzymatic Analysis. A Practical Guide, Passonneau, J.V., and Lowry, O.H., Humana Press (Totowa, NJ:1993), pp. 85-110.
6. Enzymatic Analysis. A Practical Guide, Passonneau, J.V., and Lowry, O.H., Humana Press (Totowa, NJ:1993), pp. 3-20.
7. Alivisatos, S.G.A. et al., Spontaneous reactions of 1,3-substituted 1,4-dihydropyridines with acids in water at neutrality. 1. Kinetic analysis and mechanism of the reactions of dihydro-nicotinamide adenine dinucleotide with orthophosphates. Biochemistry, **4(12)**, 2616-2630 (1965).
8. Fawcett, C.P. et al., Inhibition of dehydrogenase reactions by a substance formed from reduced diphosphopyridine nucleotide. Biochim. Biophys. Acta, **54**, 210-212 (1961).
9. Biellmann, J.F. et al., Structure of lactate dehydrogenase inhibitor generated from coenzyme. Biochemistry, **18(7)**, 1212-1217 (1979).


Trizma is a registered trademark of Sigma-Aldrich Co. LLC.

KA,RC,ARO,CMH,KMR,RXR,MAM 01/12-1

©2012 Sigma-Aldrich Co. LLC. All rights reserved. SIGMA-ALDRICH is a trademark of Sigma-Aldrich Co. LLC, registered in the US and other countries. Sigma brand products are sold through Sigma-Aldrich, Inc. Purchaser must determine the suitability of the product(s) for their particular use. Additional terms and conditions may apply. Please see product information on the Sigma-Aldrich website at www.sigmaaldrich.com and/or on the reverse side of the invoice or packing slip.