Product Information

β-Nicotinamide adenine dinucleotide, reduced disodium salt hydrate

~98%, pkg of 100 mg

N1161

Product Description

CAS Registry Number: 606-68-8 (anhydrous)

Molecular Formula: $C_{21}H_{27}N_7Na_2O_{14}P_2 \bullet xH2O$

Formula Weight: 709.40 (anhydrous)

Synonyms: β-NADH, NADH, β-DPNH, DPNH, Diphosphopyridine nucleotide, reduced form

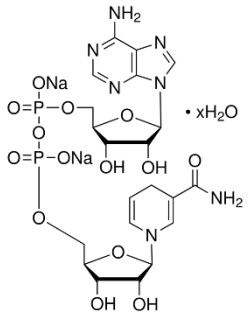
λ_{max}: 340 nm[1] and 259 nm (pH 9.5)[2]

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

Structure:



β-NADH is a pyridine nucleotide and biologically active form of nicotinic acid. β-NADH is a coenzyme required 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 β-NADH formation) or the decrease in absorbance (with β-NAD^+ formation) 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** fluorescent. This difference in fluorescence provides a sensitive 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] Several publications,[10-14] theses,[15] and dissertations[16] have cited use of N1161 in their research protocols.

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Reagent

This product is supplied as a lyophilized powder, packaged by solid weight.

Storage/Stability

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

Preparation Instructions

This product is soluble in 0.01 M NaOH (100 mg/mL).

Solutions should be freshly prepared and used promptly unless extreme care is taken. **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] Tris (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] If a low temperature freezer is available (-40 °C or colder), more concentrated solutions can be prepared and stored for years.[6]

The presence of light and heavy metals can accelerate the oxidation process.[1]

Potent enzyme inhibitors have been reported to form from β-NADH in frozen solutions and even in damp powder. These inhibitors have the same absorbance at 340 nm as β-NADH and thus cannot be detected in this manner.[8] Two inhibitors of lactate dehydrogenase which were generated during β-NADH storage have been identified:[9]

- 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^+.

References

1. Bergmeyer, H.-U. *et al*., *Methods of Enzymatic Analysis*, 2nd ed., Vol. 1 (Bergmeyer, H.-U., ed.). Verlag Chemie/Academic Press, pp. 545-546 (1974).
2. Siegel, J.M. *et al*., *Arch. Biochem. Biophys*., **82(2)**, 288-299 (1959).
3. Passonneau, J.V., and Lowry, O.H., *Enzymatic Analysis. A Practical Guide*. Humana Press (Totowa, NJ), pp. 9-10 (1993).
4. Bergmeyer *et al*., Vol. 4, pp. 2066-2072.
5. Passonneau and Lowry, pp. 85-110.
6. Passonneau and Lowry, pp. 3-20.
7. Alivisatos, S.G. *et al*., *Biochemistry*, **4(12)**, 2616-2630 (1965).
8. Fawcett, C.P. *et al*., *Biochim. Biophys. Acta*, **54**, 210-212 (1961).
9. Biellmann, J.F. *et al*., *Biochemistry*, **18(7)**, 1212-1217 (1979).
10. Betteridge, T. *et al*., *RNA*, **13(9)**, 1594-1601 (2007).
11. Russell, P. *et al*., *J. Enzyme Inhib. Med. Chem.*, **23(3)**, 411-417 (2008).
12. Rosell, F.I., *et al*., *J. Biol. Chem*., **286(33)**, 29273-29283 (2011).
13. Qian, X. *et al*., *Mol. Cell*, **76(3)**, 516-527.e7 (2019).
14. Szibor, M. et al., *J. Biol. Chem*., **295(14)**, 4383-4397 (2020).
15. Hovland, Henrikke Nilsen, "Functional characterization of rare variants of SCHAD, a protein involved in unregulated insulin secretion". University of Bergen, M.Sc. thesis, p. 32 (2018).
16. Pfelzer, Nina, "Experimentelle Bestimmung zellulärer Energiezustände und Analyse des Glykogenstoffwechsels in *Corynebacterium glutamicum*" ("Experimental determination of cellular energy states and analysis of glycogen metabolism in *Corynebacterium glutamicum*"). RWTH Aachen University, Dr. rer. nat. dissertation, p. 154 (2016).

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