Product Information

β-Nicotinamide adenine dinucleotide, reduced disodium salt hydrate

≥94% (HPLC)

N6005

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]

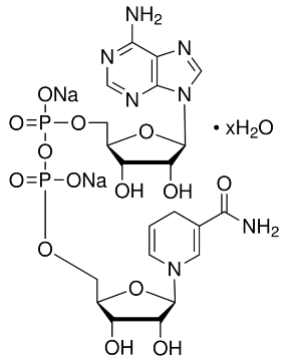
E^{mM} = 6.22 (340 nm)[1] and 14.4 (259 nm, pH 9.5)[2]

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,[10-13] theses,[14-17] and dissertations[18-26] have cited use of N6005 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. Ellis, E.A., and Grant, M.B., *Methods Mol. Biol*., **196**, 3-12 (2002).
11. Rato, L. *et al*., *Biochim. Biophys. Acta*, **1837(3)**, 335-344 (2014).
12. Kanamori, K.S. *et al*., *Bio. Protoc.*, **8(14)**, e2937 (2018).
13. Ahel, J. *et al*., *eLife*, **9**, e56185 (2020).
14. Gustafsson, Marcus, "Spectroscopic Studies of Tissue Using Near-Infrared Raman Microscopy". Lund University, M.Sc. thesis, p. 25 (1997).
15. Mays, Jason Warren, "Bioaccumulation of Platinum Group Metals in the Freshwater Mussel *Elliptio Complanata*". North Carolina State University, M.S. thesis, p. 77 (2007).
16. Perkins, Akeysha A., "Polymeric Polyphenols As Anti-Inflammatory Agents". Miami University, M.S. thesis, p. 25 (2007).
17. Sohail, Afshan, "Characterization of the Trans-plasma Membrane Electron Transport System in the Myelin Membrane". Wilfred Laurier University, M.Sc. thesis, p. 35 (2014).
18. Hung, Chin-Chang, "Nitrate Reductase Activity, Nitrate Uptake and Iodine Speciation in the Marine Environment". Old Dominion University, Ph.D. dissertation, p. 22 (1999).
19. Nicholls, Stephen James, "The Role of Anti-Inflammatory Properties of High Density Lipoproteins in Atheroprotection". University of Adelaide, Ph.D. dissertation, p. 84 (2004).
20. Brachtvogel, Elizeu Luiz, "População de Plantas e Uso de Piraclostrobina na Cultura do Milho: Alterações Agronômicas e Fisiológicas" ("Plant Population and Use of Pyraclostrobin in Corn Crop: Agronomic and Physiological Changes"). Universidade Estadual Paulista "Júlio de Mesquita Filho", Ph.D. dissertation, p. 38 (2010).

21. Da Silva, Ricardo Eustáquio, "Avaliação estrutural e quantitativa dos efeitos do envelhecimento sobre o gânglio trigeminal de ratos Wistar" ("Structural and quantitative evaluation of the effects of aging on the trigeminal ganglion of Wistar rats"). Universidade de São Paulo, Ph.D. dissertation, p. 49 (2010).
22. Kern, Sandra M., "Untersuchungen zur Antioxidativen Kapazität von Bier und Bierinhaltsstoffen sowie zu Absorption und Metabolismus von Hydroxyzimtsäuren im Menschen" ("Investigations on the Antioxidative Capacity of Beer and Beer Ingredients, as well as on the Absorption and Metabolism of Hydroxycinnative Acids in Humans"). Technischen Universität München, Dr. rer. nat. dissertation, p. 11 (2010).
23. Defour, Aurélia, "Fonctions metaboliques de Sirtuine 1 dans le muscle strie squelettique: -Contribution à l'etude de la regulation de l'expression de SREBP-1c, -Role potentiel lors d'un jeune chez des myotubes C2c12" ("Metabolic functions of Sirtuin 1 in the skeletal stria muscle: -Contribution to the study of the regulation of expression of SREBP-1c, -Potential role during youth among c2c12 myotubes"). Université Jean Monnet – Saint Étienne, Ph.D. dissertation, p. 105 (2010).
24. Rincón, Ovalles Rosalba, "Biofuel Cell Anode for NAD-Dependent Enzymes". University of New Mexico, Ph.D. dissertation, pp. 56, 75, 92, 157 (2010).
25. Kratochwil, Manuela, "Effects of ageing, calorie restriction and ageing-associated diseases on the mitochondrial proteome". Technischen Universität Darmstadt, Dr. rer. nat. dissertation, p. 19 (2015).
26. Pimenta, Sara Filomena Ribeiro, "Optical Microsystem for Spectroscopy Signals Extraction Applied to Gastrointestinal Dysplasia Detection". Universidade do Minho, Ph.D. dissertation, pp. 35, 107, 111, 114 (2016).

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