Product Information

β-Nicotinamide adenine dinucleotide, reduced disodium salt hydrate

≥97% (HPLC)

N8129

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]

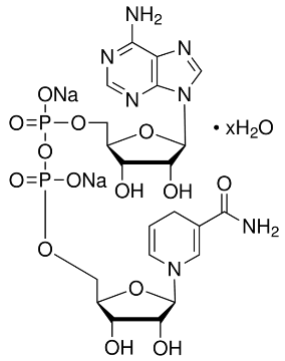
E^{mM} = 6.22 (340 nm)[1] and 14.4 (259 nm, pH 9.5)[2]

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,[10-17] theses,[18-22] and dissertations[23-32] have cited use of N8129 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

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