

Product Information

β -Nicotinamide adenine dinucleotide, reduced disodium salt hydrate

≥94% (HPLC)

N6005

Product Description

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

Molecular Formula: $C_{21}H_{27}N_7Na_2O_{14}P_2 \cdot xH_2O$

Formula Weight: 709.40 (anhydrous)

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

λ_{max} : 340 nm¹ and 259 nm (pH 9.5)²

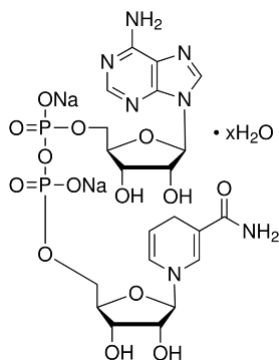
$E^{mM} = 6.22$ (340 nm)¹ and 14.4 (259 nm, pH 9.5)²

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 dissertations¹⁸⁻²⁶ 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

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