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ProductInformation

β-Nicotinamide adenine dinucleotide, reduced, disodium salt hydrate

Product Number **N 7535**
Storage Temperature 2-8 °C

Product Description
Molecular Formula: $C_{21}H_{27}N_7Na_2O_{14}P_2$
Molecular Weight: 709.4
CAS Number: 606-68-8
λ_{max}: 340 nm[1] and 259 nm (pH 9.5)[2]
Extinction Coefficient: E^{mM} = 6.22 (340 nm)[1] and 14.4 (259 nm, pH 9.5)[2]
Fluorescent Properties:
Excitation Wavelength: 340 nm[3]
Emission Wavelength: 460 nm[3]
Synonyms: β-NADH, NADH, β-DPNH, DPNH, Diphosphopyridine nucleotide, reduced form

Product N 7535 is a preweighed vial of 3.75 mg of β-NADH, disodium salt, per vial.

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] β-NADH should be stored desiccated and protected from light.[1]

Precautions and Disclaimer
For Laboratory Use Only. Not for drug, household or other uses.

Preparation Instructions
This product is soluble in 0.01 M potassium phosphate buffer, pH 7.5 (0.375 mg/ml), yielding a clear, colorless solution.

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
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 does 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

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