NADMED

Ref: PT-2025-NAD-06

Certificate of Analysis

Analysis type: NAD+ purity test

Customer: Inside Out Biotech

Sample description: colorless solution in a narrow clear glass tube with sealed mouth and rubber plug as a bottom.

Information on the tube: number 1 was written on the bottom rubber plug.

Sample reception date: 15.04.2025

Analysis date: 16.04.2025

Storage conditions before analysis: +6°C, light protected

Purpose of the analysis: to determine content (in mg) of physiologically active NAD+ as an anhydrous base in the vial content.

Result

Sample ID	Sample volume, ml	Physiologically active NAD+, mg
1	3,118	439,0

Description of performed analysis

1. Inspection of the sample

2. UV-Vis Spectroscopy analysis of the sample to determine amount of total NAD+-related content.

3. Functional assay to validate UV-VIS spectroscopy analysis and determine the amount of physiologically active NAD+.

4. Summary

Haartmaninkatu 4
02900 Helsinki, FINLAND

Tel: +358 44 242 3751
info@nadmed.fi

Business ID
3259265-3

1. Visual inspection the sample

Description

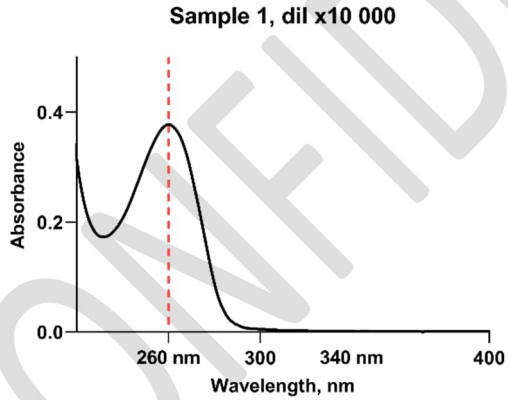
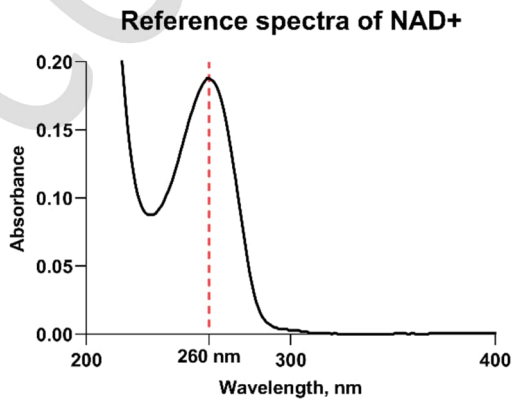
Clear narrow glass tube with colorless solution. The tube was closed with rubber cap with metal sealing. Bottom was made from rubber plug. Sample was withdrawn using a syringe and volume of the solution was measured. V = 3,118 ml.

2. UV-Vis Spectroscopy analysis of the sample to identify NAD related molecules

Description

Solution was diluted 10 000 fold with deionized water and UV-Vis spectra was measured using Shimadzu UV-2401pc Spectrophotometer and compared with Reference spectra of NAD+ (Sigma Cat#N1636). UV-VIS spectra of NAD+ in aqueous solutions have absorbance peak at 260nm with extinction coefficient Ɛ=18 $mM*cm^{-1}$, which is used to determine concentration of NAD+-related molecules in the sample.

Result: Sample showed characteristic spectrum of NAD+ with absorbance maximum at 260 nm.

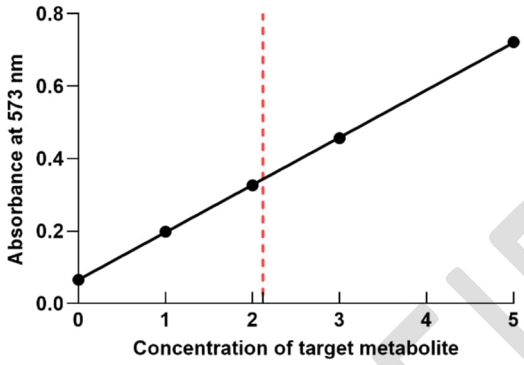
Sample ID	UV-VIS spectrum	Parameters
Test sample		OD of x10 000 diluted sample at 260 nm = 0,383 This equals to 212,8 mM concentration in solution with solubilized powder
Reference NAD+ spectra, Sigma Cat#N1636		Absorbance spectra of 10µM NAD+ solution is shown OD at 260nm = 0.180

3. Functional assay to determine content of physiologically active NAD+ (mg) in the sample

Description

To examine whether determined concentration of NAD+ represent physiologically active form of the compound we used our proprietary calibrated NADMED assay for NAD+. In this assay NAD+-specific enzyme uses NAD+ to produce colored substance, which light absorbance is linearly proportional to NAD+ concentration in the added solution. If solution contains adducts of NAD+, originated from NAD+ molecule and having overlapping UV-VIS absorbance spectrum, the enzyme will not react with them and, therefore, we will see lower signal than expected from the spectroscopy measurement of the concentration.

For the analysis sample was diluted 100 000 fold with a proprietary buffer stabilizing exclusively NAD+ followed by the NAD+ assay.

Response in the NAD assay	Parameters
	Concentration of NAD+ in x 100 000 diluted solution - 2,12 µM This equals to 212,2 mM concentration in solution with solubilized powder

4. Summary

Based on measured concentration of the physiologically active NAD+ in the sample and measured volume, mass of NAD+ as anhydrous base in the tube was calculated.

Compound	Mass of total NAD+ related compounds in the vial, mg	Mass of physiologically active NAD+ as anhydrous base in the vial, mg	Purity as % of physiologically active NAD+ in total NAD+-related compounds
NAD+	440,1	439,0	99,7

Interpretation of the results

Determined amount of physiologically active NAD+ as anhydrous base in the provided solution is almost identical to the one measured using UV-VIS Absorbance spectra. This is considered as a very pure NAD+ preparation.

Report issue date: 21.04.2025

Report validated by: Liliya Euro, PhD, Chief Scientist in NADMED Oy

Laboratory: NADMED facility

Address: Haartmaninkatu 4, 00290 Helsinki, Finland