NADMED

Certificate of Analysis

Analysis type: NAD+ purity test

Customer: Inside Out Biotech Ltd.

Sample number: 1

Sample state: dry powder in clear sealed glass bottle

Sample reception date: 11.06.2024

Storage conditions before analysis: -20°C, light protected

Purpose of the analysis is to determine content of physiologically active NAD+ as anhydrous base in the provided vial.

Contents of NAD+ purity test:

1. Visual inspection of the sample

2. UV-Vis Spectroscopy analysis of the sample to determine concentration of NAD+-related molecules

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

4. Summary

1. Visual inspection the sample

Description

The vial contained dry fine crystalline powder of white color. Content of the bottle was weighted and reconstituted in 5 ml of deionized water followed by measurement of resulting volume.

Color of the cap	Weight of the powder	Volume of obtained solution	Comment on the appearance of the solution
red	0.61 g	5.2 ml	Colorless clear solution

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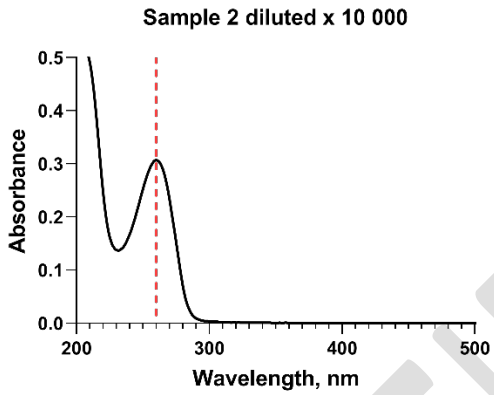
2. UV-Vis Spectroscopy analysis of the sample to determine concentration of NAD+ related molecules

Description

Provided solution was diluted 10 000 times 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+-related compounds in aqueous solutions have absorbance peak at 260 nm with extinction coefficient Λ=18 mM*cm^{-1}, which is used to determine concentration of pool of NAD+-related molecules.

Result: The sample showed NAD+related characteristic absorbance peak with maximum at 260 nm (shown on the left panel in the Table below).

Optical spectrum of NAD+-related content	Parameters
	OD at 260 nm = 0.307 Concentration in original solution = 170 mM

3. Functional assay to determine content of physiologically active NAD+ fraction in the sample

Description

To examine whether determined concentration of NAD+ represents physiologically active form of the compound we used our proprietary calibrated NADMED assay for NAD+. In this assay NAD+-specific enzyme uses selectively NAD+ to produce colored substance, which light absorbance is linearly proportional to NAD+ concentration in the added solution. If solution contains, for example, ADP-ribose, which is a moiety in NAD+ molecule with UV-VIS absorption spectra similar to NAD+, the enzyme will not react with it and, therefore, we will see lower signal than expected from the spectroscopy measurement of the concentration.

To perform this assay we prepared 100 000 dilution of the provided sample using proprietary buffer stabilizing specifically NAD+ and run the assay according to the protocol we use for measurement of NAD+ concentration in biological samples.

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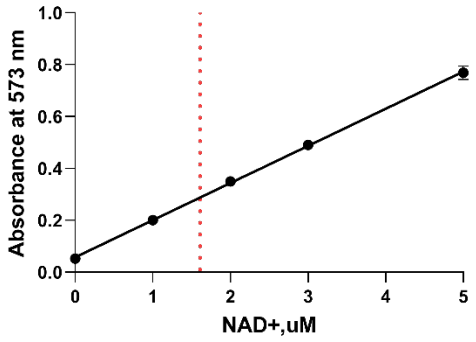
Response in the NAD+ assay	Parameters
	Concentration in x100 000 diluted solution – 1.60 µM Concentration in original solution – 160 mM

4. Summary

Based on measured: 1) weight of the powder in the bottle; 2) concentration of physiologically active NAD+ and 3) molecular weight of anhydrous NAD+ (663.43 g/mol) purity grade was calculated.

Weight of the powder, g	Amount of physiologically active NAD+, g	Purity of the physiologically active NAD+ in the powder, %
0.61	0.554	90.9

Report issue date: 19.06.2024

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

Laboratory: NADMED facility

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