Title:	**NADMED-0008 and -0009**
Document type:	**Certificate of Analysis**
Document ID:	**COA-4**
Author:	Saara Forsström
Owner:	Saara Forsström
Approver(s):	Riikka Äänismaa *approved at 2024-04-12 14:43 (UTC +0200)*
Approval date:	2024-04-12
Effective date:	2024-04-12
Next periodic review date:	2026-04-12

1. CERTIFICATE OF ANALYSIS

Product: Q-NADMED blood NAD+ and NADH assay kit, Q-NADMED blood NAD+ assay kit

Catalog numbers: IVD_001; IVD_001/TH; IVD_001_01_40; IVD_001_01_40/TH

Lots: 0008 and 0009

Specification: Performance evaluation upon manufacturing

Tested parameters:

1. UV-Vis spectra of NAD+ and/or NADH standard stocks
2. Performance of the Standards in the assay
3. Volume of individual components in the kit

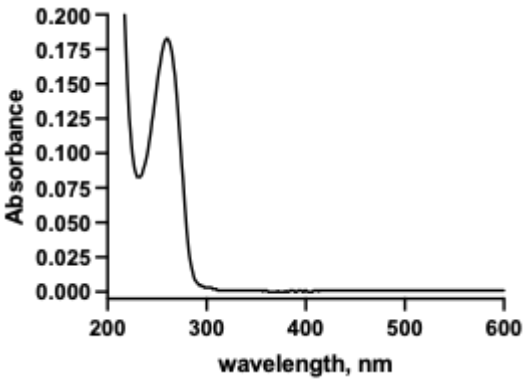
Status: All parameters are within reference range

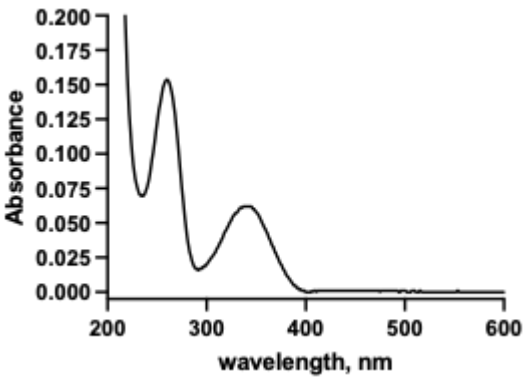
Date: 26.3.2024

Approved by Chief Scientific Officer, Liliya Euro, PhD

L. Euro

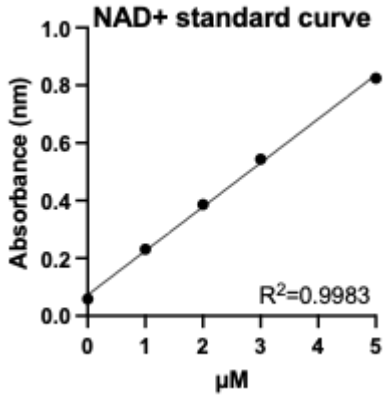
2. Summary of UV-Vis spectroscopy analysis of kit standards

Summary of UV-Vis spectroscopy analysis of kit standards	
Purpose: measurement of compound concentration in NAD+ and NADH standard stocks	
	Standard: 1 mM NAD+ Extinction coefficient at 260 nm: 18 $mM*cm^{-1}$ Dilution: x100 with water to 10 µM Measurement:1 cm quartz cuvette Instrument: Shimadzu UV-2401pc Absorbance at 260 nm: 0.183 Optical Units Theoretical value for absorbance of 10 µM NAD+: 0.180 Accepted range for absorbance measurement based on instrument characteristics: 0.178 - 0.185 Standard: 1 mM NADH

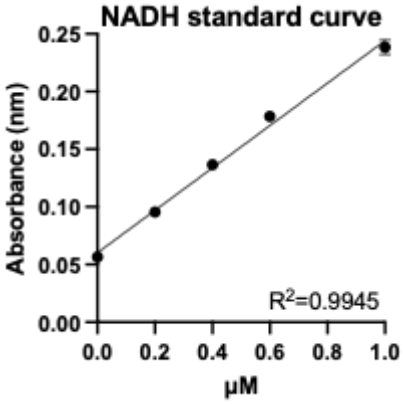


Extinction coefficient at 340 nm: 6.22 mM*cm^{-1}
Dilution: x100 with water to 10 µM
Measurement:1 cm quartz cuvette
Instrument: Shimadzu UV-2401pc
Absorbance at 340 nm: 0.062 Optical Units
Theoretical value for absorbance of 10 µM NADH: 0.0622
Accepted range for absorbance measurement based on instrument characteristics: 0.059-0.066

3. Summary of Standard performance in the assays

Assay	Absorbance at 573 nm	Standard curve fitting
NAD+ Standards	Reaction time – 4 min	

NAD+, uM	1 meas	2 meas
0	0.061	0.056
1	0.23	0.233
2	0.381	0.391
3	0.536	0.551
5	0.814	0.835

Assay	Absorbance at 573 nm	Standard curve fitting
NADH Standards	Reaction time – 6 min	

NADH, uM	1 meas	2 meas
0	0.058	0.055
0.2	0.097	0.094
0.4	0.14	0.133
0.6	0.179	0.178
1	0.243	0.234

4. Criteria for acceptance assay performance results

Parameter	Reference values	Quality Control
Absorbance range for 0 - 5 µM NAD+ Assay Standards (assay time 4 min)	0.04 - 0.800 Accepted variation between replicates - 0.05 Optical Units	passed
Absorbance range for 0 - 1 µM NADH Assay Standards (assay time 6 min)	0.04 - 0.260 Accepted variation between replicates - 0.05 Optical Units	passed
R^2 of liner fit for NAD+ standard curve	>0.99	passed
R^2 of liner fit for NADH standard curve	>0.99	passed
Volumes of single components were enough to perform two 96-well plate assays	Yes	passed

5. Attachments