Title:	**NADMED Lot 0003 and 0004**
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Approval date:	2023-03-10
Effective date:	2023-03-10
Next periodic review date:	2025-03-10

1. CERTIFICATE OF ANALYSIS

Product: Q-NADMED blood kit, Catalog number IVD_01

Lot: NADMED - 0003 and 0004

Specification: Performance evaluation upon manufacturing

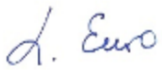
Tested parameters:

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

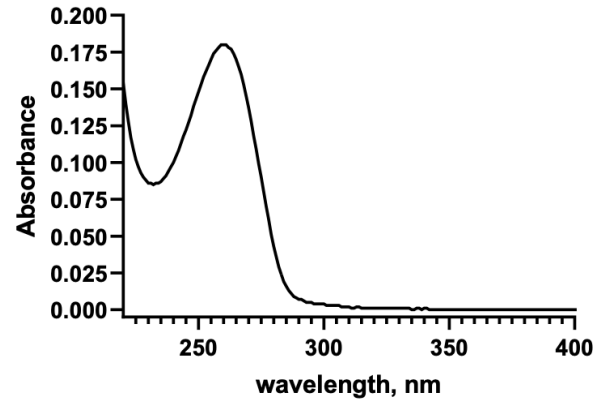
Date: 24.02.2023

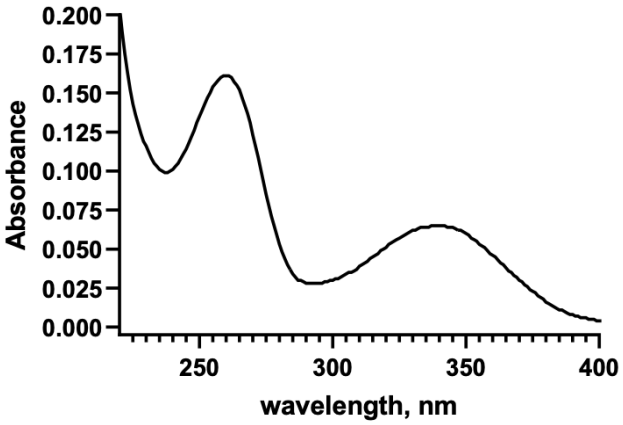
Approved by Liliya Euro, PhD



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



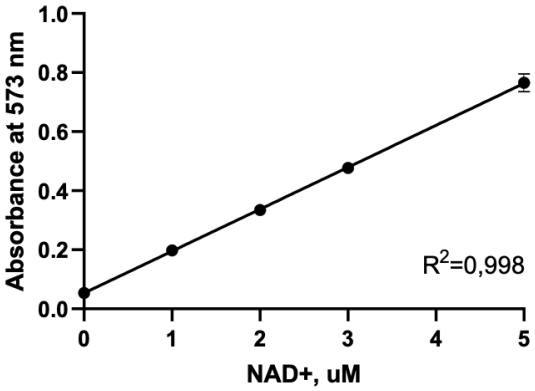


Standard: 1 mM NAD+
Extinction coefficient at 260 nm: 18 mM*cm^{-1}
Dilution: x100 with water to 10 µM
Measurement:1 cm quartz cuvette
Instrument: Shimadzu UV-2401pc
Absorbance at 260 nm: 0.180 Optical Units
Theoretical value for absorbance of 10µM NAD+: 0.180
Accepted range for absorbance measurement based on instrument characteristics: 0.178 - 0.185

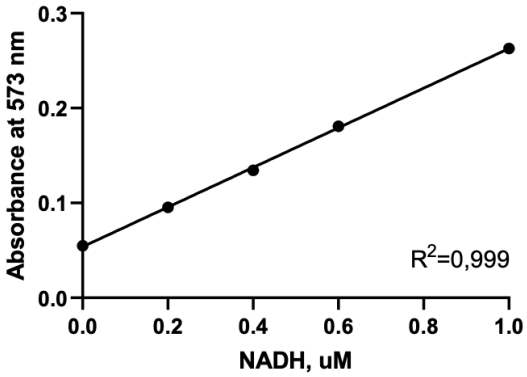
Standard: 1 mM NADH
Extinction coefficient at 340 nm: 6.22 mM*cm^{-1}
Dilution: x100 with water to 10 µM
Measurement:1 cm quartz cuvette
Instrument: Shimadzu UV-2401pc
Absorbance at 260 nm: 0.061 Optical Units
Theoretical value for absorbance of 10µM NADH: 0.065
Accepted range for absorbance measurement based on instrument characteristics: 0.059-0.066

3. Summary of Standard performance in the assays

<table>
<tr><th>Assay</th><th>Absorbance at 573 nm</th><th>Standard curve fitting</th></tr>
<tr><td rowspan="2">NAD+ Standards</td><td>Reaction time – 4 min

<table>
<tr><th>NAD+, uM</th><th>1 meas</th><th>2 meas</th></tr>
<tr><td>0</td><td>0,054</td><td>0,054</td></tr>
<tr><td>1</td><td>0,201</td><td>0,195</td></tr>
<tr><td>2</td><td>0,329</td><td>0,34</td></tr>
<tr><td>3</td><td>0,487</td><td>0,467</td></tr>
<tr><td>5</td><td>0,787</td><td>0,744</td></tr>
</table></td><td> 
</td></tr>
<tr><td>NADH Standards</td><td>Reaction time – 6 min</td><td></td></tr>
</table>

NADH, uM	1 meas	2 meas
0	0,055	0,055
0,2	0,096	0,095
0,4	0,135	0,134
0,6	0,184	0,178
1	0,261	0,265



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. References

Certificate of Analysis 0003 and 0004.docx