

Q-NAD BLOOD NAD⁺ assay kit

Quantitative assay kit for whole blood

FOR SINGLE USE ONLY

These instructions must be read in their entirety before using this product.

RUO

For Research Use Only.
Not for use in diagnostic procedures.

GENERAL INFORMATION

- A. Proprietary name: Q-NAD Blood NAD⁺ assay kit: quantitative assay kit for NAD⁺ in whole blood
- B. Catalog number: RUO_002, 40 samples (96-well format)
- C. Storage: -80°C
- D. IFU issued: March 2023

Manufacturer:

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INTRODUCTION

NAD metabolites are derivatives of vitamin B3 and exist in cells in four forms – NAD⁺, NADH, NADP⁺ and NADPH. NAD metabolites are essential for functional adaptation of cell metabolism to environmental conditions. Their levels are dynamic and change in response to different endogenous and exogenous stimuli. This kit is designed for measurement of systemic levels of NAD⁺ in whole blood (human or animal).

RESEARCH BACKGROUND

NAD⁺ is known central regulator of human body metabolism and energy homeostasis. Accumulated research data show that systemic levels of NAD⁺ decrease in response to manifested disease, signaling of an imbalance of body energy homeostasis. Degree of NAD⁺ decrease varies in different patients and in different pathologies. Progressive decline of NAD⁺ levels makes it impossible for the body to maintain its basic metabolic functions to survive even in conditions of ongoing therapy. Q-NAD allows screening patients for NAD⁺ deficiency with the aim to correct it and increase efficiency of treatment.

PRINCIPLES OF THE ASSAY

The principle of the assay is a cyclic enzymatic reaction with a colorimetric end-point detection. First, NAD⁺ is extracted from a blood sample followed by a stabilization step. Then, stabilized extract is analyzed using enzymatic reaction coupled to a color change. The intensity of the color change in the assay is linearly proportional to the concentration of NAD⁺ in the reaction mixture.

Q-NAD Blood NAD⁺ assay kit is sufficient for analysis of NAD⁺ in 40 whole blood samples. The number of samples is defined by provided volume of BUFFER A. This kit offers flexibility of NAD⁺ analysis by allowing measurement of two smaller sets of samples at two different time points. For more detail, please see Practical considerations on page 8.

REAGENTS PROVIDED

REAGENTS	DESCRIPTION*	BEFORE THE ASSAY
BUFFER A	28 mL of extraction buffer	Equilibrate to room temperature (15-25°C).
NAD ⁺ stabilizing reagent	2 bottles with 8 mL of buffered solution for NAD ⁺ measurement	Equilibrate to room temperature (15-25°C).
NAD ⁺ standard stock	2 microtubes with 40 µL of 1 mM NAD ⁺	See preparation guide.
BUFFER C	2 bottles with 19 mL of assay buffer	Equilibrate to room temperature (15-25°C).
Assay Color reagent	2 bottles with 3 mL of reagent with the assay color	Equilibrate to room temperature (15-25°C). Should be used within 2 h after equilibration.
Enzyme	2 microtubes with 40 µL of enzyme. One per plate.	Thaw only before adding into Master Mix.
Stop Solution	1 bottle with 3 mL of solution to stop the assay reaction	Equilibrate to room temperature (15-25°C).
Positive Control (buffer)	1 microtube with 200 µL of buffer for preparation of Positive Control	Equilibrate to room temperature (15-25°C). See preparation guide.

*Note: Accepted variation of the filling volume 5%.

PRECAUTIONS AND WARNINGS

Safety

For in vitro diagnostic use only by trained personnel.

The Stop Solution may cause skin, eye and respiratory irritation. Avoid breathing in fumes.

Assay Color reagent may cause skin irritation. Handle with care, use gloves.

BUFFER A can cause eye irritation. Handle with care, use goggles.

Do not smoke, drink, eat or apply cosmetics in the working area. Wear protective gloves, clothing, and eye protection. Wash hands thoroughly after handling.

Identified hazards of the chemicals presented in this kit and the appropriate warning information associated with those hazards are presented in NADMED Safety Data Sheet (SDS).

Disposal of used kit components is described in in NADMED Safety Data Sheet (SDS).

Q-NAD Blood NAD⁺ assay kit is suitable for measurement of NAD⁺ metabolite from whole blood. For measurement of both NAD⁺ and NADH metabolites from whole blood, please use Q-NAD Blood NAD⁺ and NADH assay kit (catalog number RUO_001).

STORAGE AND EXPIRATION DATING OF REAGENTS

- Before opening, all kit components should be stored at -80°C. Avoid temperature fluctuations in the freezer.
- After thawing, BUFFER A, NAD⁺ stabilizing reagent, Positive Control (buffer) and Stop Solution are stable for two weeks at room temperature.
- BUFFER C should be thawed and used on the day of the assay.
- Assay Color reagent is stable for up to 3 hours at room temperature after thawing.
- The Enzyme should be used directly after thawing.
- Assay standards and the Positive Control should be prepared and used on the day of the assay.
- Standards should be protected from light.

OTHER MATERIAL REQUIRED

The following materials are required but not provided in the kit:

- Deionized water
- Microtubes (1.5 mL, e.g., microcentrifuge tubes) for dilution of standards and sample preparation
- Two plastic Multichannel Pipette reservoirs, one for pipetting Master Mix, the other for pipetting Stop Solution
- Two 96-well transparent polystyrene microplates with medium protein binding affinity designed for colorimetric and absorbance assays.
- Dry bath (heat block) with adjustable temperature (up to 80°C)
- Table-top cooling microcentrifuge (max speed 20 000 x g)
- Ice
- Calibrated single channel pipettes (0.5-10 µL, 5-50 µL, 20-200 µL, 100-1000 µL) and multichannel pipettes (5-50 µL, 30-300 µL) and beveled pipette tips, low retention
- Spectrophotometric microplate reader capable of measuring absorbance at 570-573 nm
- Aluminum foil to protect microtubes and plates from light

REAGENT PREPARATION

Thaw one set of reagents to run one 96-well plate of NAD⁺ assay, and BUFFER A, Stop Solution and Positive Control (buffer) **according to instructions in the table on page 5** before the assay.

- A. **BUFFER A** - Ready to use. One bottle is for two sets of samples (40 samples in total). After thawing for extraction of the first set of samples, BUFFER A can be stored at room temperature for two weeks, or re-frozen at -80°C until analysis of the second set of samples.
- B. **NAD⁺ stabilizing reagent** - Ready to use. Single use. Do not re-freeze.
- C. **Enzyme** - Ready to use. Single use. Do not re-freeze.
- D. **Stop Solution** - Ready to use. One bottle is for two assays. If a precipitate forms in Stop Solution upon thawing, it should be re-dissolved by 5 min incubation at +37°C, and then cooled back down to 25°C before use. Do not shake Stop Solution vigorously. After thawing for analysis of the first set of samples, Stop Solution can be stored at room temperature for two weeks, or re-frozen at -80°C until analysis of the second set of samples.
- E. **Positive Control (buffer)** - Ready to use. One bottle is for two assays. For further instructions see preparation of Positive Control. After preparation of Positive Control for the first assay, store the rest of the Positive control buffer at room temperature for two weeks, or re-frozen at -80°C until the second use.
- F. **Assay standards** – Prepared from 1 mM NAD⁺ Standard stock. For further instructions see preparation of standards and preparation of Positive Control.
- G. **Master Mix** – 1 bottle of **Assay Color reagent** (3 mL, Set 1 or Set 2) should be mixed into 1 bottle of **BUFFER C** (Set 1 or Set 2) to create 1 bottle of Master Mix required for 1 plate of NAD⁺ assay. Protect from light by keeping it in the original amber bottle. Do not shake vigorously. Discard leftovers. Do not re-freeze.

SAMPLE COLLECTION AND STORAGE

The kit is only suitable for whole blood samples. Whole blood samples should be collected into either heparin or EDTA collection tubes (e.g. BD K2 EDTA Vacutainer or TASSO+ device[®] with K2 EDTA collection tube) and **properly mixed**. Samples can be analyzed fresh or frozen. Fresh blood can be analyzed within 72 hours if maintained at 4 – 8°C. Fresh samples can be aliquoted (e.g. 120 - 200 µL) within 72 hours and then stored frozen at -20°C for one month or at -80°C for approximately a year. The kit is measuring intracellular NAD⁺ content. Thus, it is very important to adequately mix each fresh sample during the aliquoting to ensure that every aliquot contains approximately the same number of cells. Frozen samples must be maintained frozen before the assay. Time intervals during sample handling should be consistent for all samples. For measurement of NAD⁺, 100 µL of whole blood is needed. Subsequently freeze-thawed blood samples cannot be used for the analysis.

PRACTICAL CONSIDERATIONS

- Q-NAD Blood NAD⁺ assay kit is sufficient for analysis of NAD⁺ in 40 whole blood samples. The number of samples is defined by provided volume of BUFFER A. This kit offers flexibility of NAD⁺ analysis by allowing measurement of two smaller sets of samples at two different time points. For example:
 - Extraction of 30 samples should be followed by NAD⁺ analysis on one 96-well plate the same day using components of Set 1.
 - On a different day, extraction of the remaining 10 samples should be followed by NAD⁺ analysis on the second 96-well plate using components of Set 2.
 - Optionally, all 40 samples can be analyzed at once on one 96-well plate using one set of reagents (Set 1). In this case, the second set of reagents (Set 2) will remain unused or can be utilized to repeat the first measurement during the same day.
- Do not use kit components beyond the expiry date.
- Do not mix materials from different kit lots.
- This assay is NOT suitable for measurement of NAD⁺ in plasma or serum, cultured cells or tissues (human or animal).
- Thoroughly mix all reagents by gentle swirling. Small microtubes should be quickly centrifuged at low speed prior to opening.
- Both fresh and frozen blood samples can be used for the assay. It is very important to have homogenous (properly mixed) sample for analysis.
- We recommend extracting a maximum of eight samples at a time to minimize handling time.
- To avoid cross-contamination, change to new pipette tips between the additions of each standard, sample, and reagent. Also, use separate reservoirs for Master Mix and Stop Solution.
- High precision pipettes and beveled tips with less retention will improve the precision.
- Assay Color reagent is a yellow, light sensitive compound which turns brown upon enzymatic reaction. The exposure to direct sunlight or artificial light causes its unspecific color change to green. To minimize the light interference with the assay, we recommend:
 - pipetting of Master Mix and then Stop Solution into the plate under conditions of natural indirect light. To achieve these conditions, switch off artificial indoor lights and avoid pipetting close to the window.
 - covering the 96-well plate with a lid from an aluminum foil during the reaction time after addition of Master Mix, as well as during the transfer to the plate reader after addition of Stop Solution. Do not wrap the plate with the aluminum foil due to risk of contamination and sample mixing during unwrapping.
- Positive Control is an artificial sample containing known amount of pure NAD⁺.

- Technical requirements for dry bath (heat block):
 - Test the efficiency of heat transfer in the dry bath as follows:
 1. Add 250 μ L of water into a microtube fitting into the dry bath you are using,
 2. Insert a conventional lab thermometer into the microtube with the liquid and place it into the dry bath set to 80°C,
 3. Measure the time needed to increase the temperature of the liquid to 70°-75°C,
 4. All dry bath incubation times mentioned in this protocol are given for a dry bath that heats 250 μ L of water to 75°C in 2 minutes. This is enough for the chemical reaction to reach 70°-75°C in the solution upon incubation in the dry bath.
- Technical requirements for spectrophotometric plate reader:
 - Measurement of light absorption at 570-573 nm,
 - Option to adjust the scanning light brightness/intensity to **low**. In some plate readers, the brightness can be adjusted as the number of flashes per one measurement. In the latter case, set the number of flashes in the range from 5 to 10.
- Master Mix and Stop Solution contain detergents. To avoid bubbles, pipette Master Mix and Stop Solution by pressing the pipette to the first stop position, remove any bubbles in the wells with a small needle. Avoid touching the content of the wells with pipette tips.
- We recommend the following order of steps:
 1. Bring BUFFER A, NAD⁺ stabilizing reagent, Positive Control (buffer) and Stop Solution to room temperature the day before the assay, or use solutions stored at room temperature within two weeks after thawing.
 2. On the day of the assay, first prepare the assay standards and Positive Control.
 3. On the day of the assay, thaw bottles with BUFFER C and Assay Color reagent and bring them to room temperature. It takes about 2 - 3 hours to melt. During this time perform extraction of the samples and stabilization of the obtained extracts for measurement of NAD⁺. Perform NAD⁺ assay on one plate.

EXTRACTION OF NAD⁺ FROM BLOOD

1. Bring BUFFER A and NAD⁺ stabilizing reagent to room temperature before extraction.
2. Use fresh blood cooled on ice or thaw frozen blood samples in the ice-water bath for 12-15 min prior to the extraction. (**Tip:** use occasional quick cycles of warm hands during thawing time). We recommend extracting a maximum of eight samples at a time.
3. Pipette 500 µL of BUFFER A into 1.5 mL microtubes.
4. Heat the aliquots of BUFFER A in a dry bath set at 75° – 80°C for 2-3 min (max 5 min). Keep in the dry bath until step 6.
5. Quickly mix blood samples by couple of up-and-down pipetting cycles with simultaneous rotation of the tip before extraction, avoid foaming.
6. Add 100 µL of blood by one quick firm move directly into the hot BUFFER A in the microtube kept in the dry bath. Immediately mix by 2 - 3 intensive up-and-down pipetting cycles with simultaneous rotation of the tip to efficiently mix the cold sample with the hot BUFFER A.
7. Incubate homogenate at 75° - 80°C for 1 min.
8. Cool the mixture in an ice-water bath for at least 5 min. After cooling on ice, homogenate should polymerize without any free liquid.
9. Centrifuge at 20 000 x g for 10 min at 4°C. Transfer the supernatant into a clean microtube and discard the pellet. Keep obtained total extracts (supernatants) containing NAD⁺ at 4°C, covered with a foil lid until the next step. Continue from step 2 to step 9 with next batch of 8 samples as long as you have samples to extract. Once all samples are extracted continue to step 10. Optionally, the supernatants can be stored at -80°C for one week. In this case, thaw at room temperature for 10 min before preparation of aliquots in step 10.
10. Prepare a separate aliquot from each obtained extract into a clean microtube - 150 µL / tube.
11. To each 150 µL aliquot, add 100 µL of NAD⁺ stabilizing reagent to get stabilized extract with NAD⁺. Vortex and incubate at room temperature for 5 min. If the assay is not performed immediately, keep the stabilized extracts refrigerated until the assay. **Protect from light by covering with an aluminum foil lid.**

NOTE: Final dilution of the original whole blood sample will be 10 times.

NOTE: In case of individual supplementation with NAD precursors, the levels of NAD⁺ may increase in the blood and thus, the NAD⁺ stabilized extract should be further diluted (two times) using deionized water before the assay. In this case, the dilution of the original blood sample will be 20 times for NAD⁺.

PREPARATION OF STANDARDS

Handling notes: Protect the standards from light with aluminum foil.

1. Prepare assay standards on the day of the assay. Thaw one microtube with 1 mM NAD⁺ standard stock for 5 min at room temperature. Protect from light with a foil lid during thawing.
2. Prepare **50 µM stock of NAD⁺** by adding 25 µL of 1 mM NAD⁺ standard stock (provided) into 475 µL of deionized water, vortex. This stock is used for preparation of NAD⁺ standards and Positive Control.
3. Prepare **NAD⁺ assay standards** according to the scheme below by mixing indicated volumes of 50 µM NAD⁺ stock, deionized water, BUFFER A and NAD⁺ stabilizing reagent. Final volume of each standard is 1 mL. (**Tip:** use the same single 20 - 200 µL pipette for pipetting 50 µM NAD⁺ stock and water).
4. Cover the stand with ready assay standards with an aluminum foil lid to protect from light and keep refrigerated prior to pipetting on the plate.

STANDARD NUMBER	CONCENTRATION of NAD ⁺ (µM)	STANDARD PREPARATION			
		50 µM NAD ⁺ stock (µL)	dH ₂ O (µL)	BUFFER A (µL)	NAD ⁺ stabilizing reagent (µL)
ST1	0	0	100	500	400
ST2	1	20	80	500	400
ST3	2	40	60	500	400
ST4	3	60	40	500	400
ST5	5	100	0	500	400

PREPARATION OF POSITIVE CONTROL

Positive Control is prepared before the assay by mixing known amount of NAD⁺ standard with the Positive Control buffer (provided). The volume of the Positive Control and concentration of NAD⁺ mimics a blood sample of a healthy human subject. Prepared Positive Control undergoes the same extraction and stabilization steps as whole blood samples.

1. Thaw the microtube with Positive Control (buffer) for 5 min at room temperature.
2. To prepare Positive Control, add 75 µL of Positive Control buffer into a microtube.
3. Add 75 µL of **50 µM NAD⁺ stock** (for preparation, see page 11) into Positive Control buffer and vortex. Expected concentration of NAD⁺ in the obtained 150 µL of Positive Control is 25 ± 2 µM.
4. Positive Control does not contain proteins and therefore it can be extracted with BUFFER A at room temperature. Add 100 µL of the prepared Positive Control into 500 µL of BUFFER A at room temperature to create an “extract” of Positive Control, vortex, and proceed to step 5.
5. Prepare a separate aliquot of the Positive Control “extract” (from step 4) into a clean microtube - 150 µL / tube.
6. Add 100 µL of **NAD⁺ stabilizing reagent**, vortex, and incubate at room temperature for 5 min. Then keep refrigerated and covered with foil until used in the NAD⁺ assay.

NOTE: Final dilution of the Positive Control will be 10 times.

ASSAY PROCEDURE

Handling notes: Important! Pipette Master Mix and Stop Solution into plates under conditions of natural indirect light (see practical considerations).

- Every assay needs to contain Sample Blanks to correct for all unspecific background signals. Sample Blanks are prepared from the first four stabilized sample extracts (BL UNK1-4 of samples 1-4) to correct for unspecific interaction between the extract components and Assay Color reagent in Master Mix. Positive Control does not require a separate Blank.
- **All Sample Blanks are incubated with Master Mix WITHOUT added enzyme.**

Enzyme should be thawed just before addition into Master Mix. Briefly centrifuge the microtube with enzyme at low speed prior to opening.

1. Incubate ready assay standards, stabilized sample extracts, and stabilized "extract" of Positive Control for 5 min at room temperature before pipetting onto the plate.
2. Pipette 20 µL of each NAD⁺ standard in duplicates starting from ST1 (0 µM) according to the plate scheme on page 21.
3. Pipette 20 µL of stabilized Positive Control and stabilized sample extracts in duplicates (see the scheme on p.21). For the first four stabilized sample extracts, pipette one extra replicate to the indicated well (BL UNK1-4) according to the scheme on p.21. These four wells are Sample Blanks needed for the analysis without enzyme, to correct for unspecific interaction between the extract and Assay Color reagent within the Master Mix.
4. Prepare Master Mix for one 96-well plate by adding the content of one bottle of Assay Color reagent into one bottle of BUFFER C, mix gently by rotation.
5. From this step onwards, turn off the direct indoor lights. Protect Master Mix in the reservoir during pipetting with multichannel pipette by using an aluminum foil lid (please see further instructions in our instructional video at www.nadmed.com).
6. Add 190 µL of Master Mix into each of the four Sample Blank wells (BL UNK1-4).
7. Add 40 µL of Enzyme into the remaining Master Mix. Mix gently, avoid foaming.
8. Add 190 µL of Master Mix with added enzyme to all remaining wells including stabilized Positive Control, using a multichannel pipette, avoid foaming and direct light. Immediately cover the ready plate with the aluminum foil lid.
9. Incubate the covered plate for 4 - 6 min at room temperature.

NOTE: the reaction can be stopped when there is a distinct color gradient in the standards and difference in color intensity between samples with added enzyme and Sample Blanks. The longer the reaction time the more intense signal will be observed.

10. Stop the reactions by adding 10 µL of Stop Solution to each well in the same order as Master Mix using a multichannel pipette. Avoid foaming, gently shake the plate by hand on a table surface, remove bubbles.
11. Measure light absorbance at 573 nm immediately after addition of Stop Solution. If possible, shake the plate inside the microplate reader for 5 sec before the measurement. Note: after addition of stop solution color intensity can slowly increase uniformly in all the wells. This is expected due to non-enzymatic background process in the Master Mix.

CALCULATION OF RESULTS

1. Calculate average of absorbance readings for each standard (ST1 – ST5). Create a standard curve by plotting the mean absorbance for each standard on the Y-axis against the concentration (in μM) on the X-axis and perform a simple linear regression fitting of the standard curve.
2. Find concentration of the metabolite in each well using the formula of linear regression of the standard curve.
3. Calculate the average of four Sample Blanks (BL UNK1-4). The obtained value represents unspecific signal of the stabilized extracts.
4. Calculate the average of duplicates of each stabilized sample extract (UNK).
5. Calculate the average of duplicates of the stabilized Positive Control and multiply by 10 to obtain the concentration (μM) of NAD⁺ metabolite in the original 150 μL of Positive Control.
6. Correct the obtained concentrations in stabilized sample extracts for the Sample Blank value (calculated in step 3) and multiply by 10 to obtain the concentration (μM) of NAD⁺ metabolite in the original blood sample (see the details on page 15). If NAD⁺ stabilized extracts have been additionally diluted, the concentration must be multiplied by the additional dilution factor.

POSITIVE CONTROL

The purpose of Positive Control is to monitor the overall assay performance. Concentration of measured NAD⁺ in the Positive Control is expected to be within the range of 23 - 27 μM .

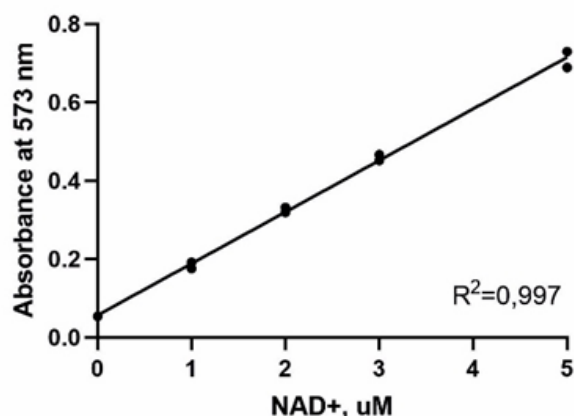
- Absorbance values of stabilized "extract" of Positive Control on the NAD⁺ plate is expected to be between the absorbance of ST3 and ST4.

PERFORMANCE AND LIMITATIONS

A. TYPICAL DATA

The standard curve and the concentrations in the stabilized sample extracts are provided for demonstration only and should never be used instead of the real-time calibration curve.

STANDARD CURVE FOR NAD⁺



Standard	NAD ⁺ (μM)	Absorbance (573 nm) Assay time - 4min
ST1	0	0.054
		0.054
ST2	1	0.176
		0.192
ST3	2	0.319
		0.332
ST4	3	0.452
		0.466
ST5	5	0.689
		0.730

CALCULATION OF RESULTS FOR NAD⁺

Concentration values in the stabilized sample extracts (UNK) and Sample Blanks (BL UNK1-4) are determined from the linear fit formula of the NAD⁺ standard curve.

Unknown	Concentration in stabilized extracts (μM)	Concentration in stabilized extracts corrected by average of Sample Blank (BL UNK 1-4, μM)	Final NAD ⁺ concentration in the original sample (μM)*
UNK 1	2.944 3.151	3.008	30.08
UNK 2	2.841 3.129	2.945	29.45
UNK 3	2.686 2.730	2.668	26.68
UNK 4	1.895 1.999	1.907	19.07
UNK 5	2.346 2.420	2.343	23.43
UNK 6	3.432 3.499	3.425	34.25
BL UNK 1	0.040	-	
BL UNK 2	0.048		
BL UNK 3	0.026		
BL UNK 4	0.048		

*Corrected by dilution factor x10

B. LIMITS OF DETECTION

The Limit of Blank (LOB) for Q-NAD Blood is presented in the table below (LOB \pm standard deviation (SD)).

Limit of Blank	
	pmol/well
NAD+	1.84 \pm 0.9

The Limit of Detection (LOD) was calculated from NAD⁺ standard curve and is presented in the table below (LOD \pm SD).

Low detection limit	
	μ M in whole blood
NAD+	0.33 \pm 0.2

The Limit of Quantitation (LOQ) is presented in the table below (LOQ \pm SD).

Limit of Quantitation	
	μ M in whole blood
NAD+	0.66 \pm 0.3

C. PRECISION AND REPRODUCIBILITY

Precision of the assay performance was determined by Intra-assay variation in measurement. Intra-Assay precision is presented in the table below (CV = coefficient of variation).

Intra-assay precision	
	CV (%) \pm SD
NAD+	1.48 \pm 0.8

Results of the assay reproducibility are summarized in the table below (N = number, * 3 aliquots of the same sample were analyzed in triplicates).

Reproducibility	NAD+		
Sample	Ctr1	Ctr2	Ctr3
N of measurements *	9	9	9
Mean (μ M)	27.41	29.41	22.00
Standard deviation	0.62	1.31	0.87
CV (%)	2.28	4.45	3.95

D. ACCURACY

The accuracy of the assay was calculated from samples with known amount of pure NAD⁺. The results are summarized in the table below (assay accuracy \pm SD).

Accuracy (%)		
NAD ⁺	N = 32	97.13 \pm 7.6

E. ASSAY CUT-OFF

The low and high cut-off values represent the smallest and the highest concentration observed in 5 - 7% individuals of given population extract. The cut-off values are summarized in the table below.

Cut-off value		
	Low	High
NAD ⁺ (μ M)	20	36

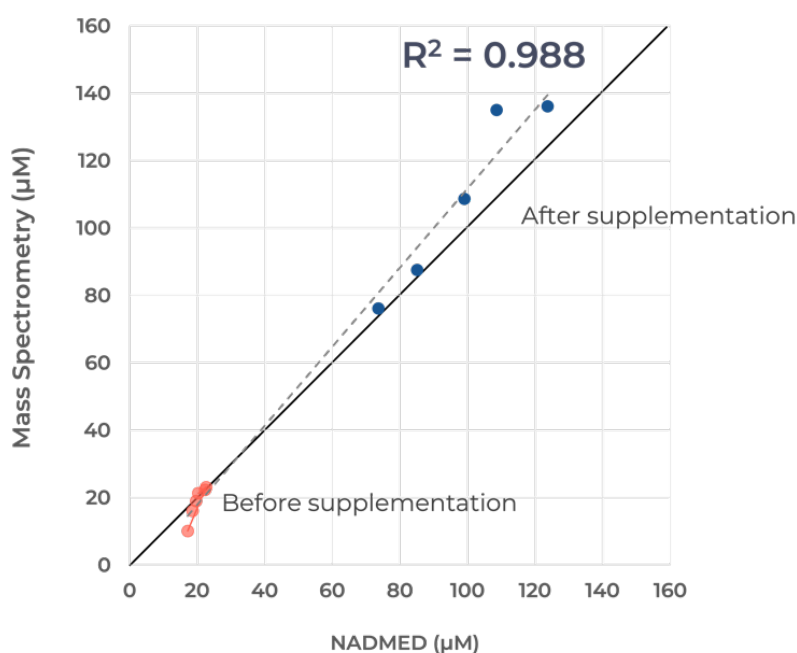
F. PERFORMANCE CHARACTERISTICS

Interference of other metabolites in the extract was not separately investigated, as their contribution is low and taken into account by performing Blank analysis without added enzyme.








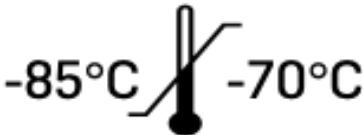
Warning: presence of potassium sorbate, borate, pyridine, bismuth in a sample can cause enzyme inhibition, and thus causing underestimation of the results.





G. METHOD COMPARISON

To validate the performance of NADMED, we measured NAD⁺ concentration in a set of control human blood samples that were also analyzed by mass spectrometry. Frozen blood samples of five healthy subjects - before and after 16 weeks of niacin supplementation - were analyzed in parallel by NADMED and mass spectrometry. Results from NADMED were concordant with those obtained by mass spectrometry.



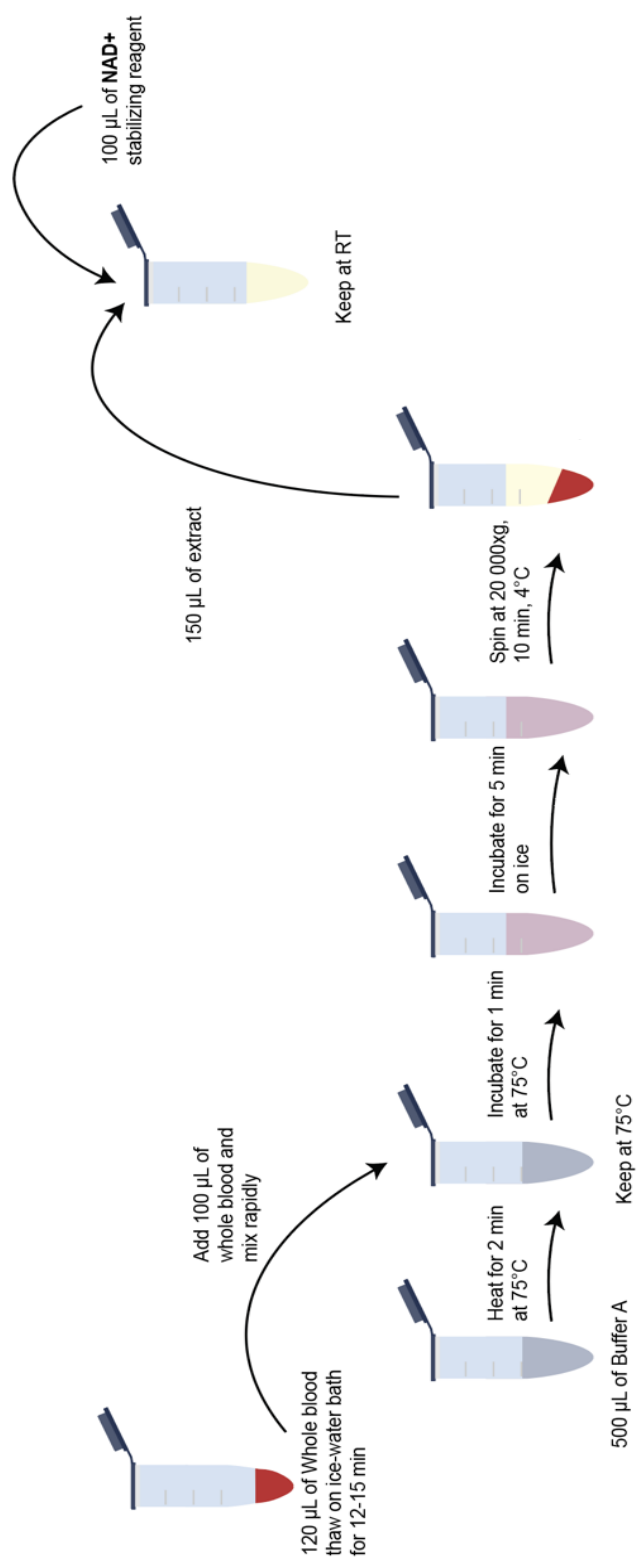
SYMBOLS

Symbol	
	Flammable liquid and vapor
	Warning/Danger
	Consult instructions for use
	Use-by date
	Catalogue number
	Batch code
	Manufacturer
	Upper limit of temperature

	Do not use if package is damaged
	Keep dry
	Number of reactions
	For Research Use Only
	Protect from direct light

SCHEMATIC PICTURES

A. EXTRACTION OF NAD⁺ FROM BLOOD



B. RECOMMENDED PLATE LAYOUT FOR NAD⁺ MEASUREMENT

1	2	3	4	5	6	7	8	9	10	11	12
St1	St1	UNK1	UNK1	UNK9	UNK9	UNK17	UNK17	UNK25	UNK25	UNK33	UNK33
St2	St2	UNK2	UNK2	UNK10	UNK10	UNK18	UNK18	UNK26	UNK26	UNK34	UNK34
St3	St3	UNK3	UNK3	UNK11	UNK11	UNK19	UNK19	UNK27	UNK27	UNK35	UNK35
St4	St4	UNK4	UNK4	UNK12	UNK12	UNK20	UNK20	UNK28	UNK28	UNK36	UNK36
St5	St5	UNK5	UNK5	UNK13	UNK13	UNK21	UNK21	UNK29	UNK29	UNK37	UNK37
PosCtr	PosCtr	UNK6	UNK6	UNK14	UNK14	UNK22	UNK22	UNK30	UNK30	UNK38	UNK38
BL UNK1	BL UNK2	UNK7	UNK7	UNK15	UNK15	UNK23	UNK23	UNK31	UNK31	UNK39	UNK39
BL UNK3	BL UNK4	UNK8	UNK8	UNK16	UNK16	UNK24	UNK24	UNK32	UNK32	UNK40	UNK40

Plate layout for NAD⁺ assay: St – standard, BL - blanks with indicated samples used for the blank reading, UNK – unknown samples, stabilized extracts from the samples with unknown metabolite concentration, PosCtr – stabilized Positive Control. Use 20 µL of standards, Positive Control and stabilized sample extracts per well. Sample Blanks of the first four samples are analyzed in the Master Mix without added enzyme.

NOTES

PLATE LAYOUT

Use this plate layout to record standards and samples assayed.

12								
11								
10								
9								
8								
7								
6								
5								
4								
3								
2								
1								
	A	B	C	D	E	F	G	H