

# Certificate of Analysis

**Grail Formula B.V.**  
Grail Formula B.V.  
Hingenderstraat 39  
6111 AB Sint Joost  
Netherlands  
[info@24peptides.com](mailto:info@24peptides.com)

**Liquilabs s.r.o.**  
Inovační 122  
252 41 Zlatníky-Hodkovice  
Czechia  
[www.liquilabs.cz](http://www.liquilabs.cz)




[Verify Results Online](#)

## Sample Identification

**Sample Name** NAD+ 1000 mg  
**Batch Number** GF-NAD1000-B242  
**Date Published** 2026-04-20 10:21

## Results for LYO-0088

Peptides	Result	Unit	Uncertainty	Acceptable Range
NAD+ Assay Peptide Screening 10mM ammonium formate buffer	1106	mg	[± 6]	
NAD+ Purity Peptide Screening 0.1% TFA	99.0	%	[± 0.5]	
NAD+ Identification by Spectrum Peptide Screening 0.1% TFA	1000		[± 5]	
NAD+ Identification by RT Peptide Screening 0.1% TFA	0.993		[± 0.005]	
Microbiology	Result	Unit	Uncertainty	Acceptable Range
Total Aerobic Microbial Count USP <61>/Eur. Ph. 2.6.12. Plate Count Method	0	CFU/g	[± ]	0 - 1000
Total Yeast and Mold Count USP <61>/Eur. Ph. 2.6.12. Plate Count Method	0	CFU/g	[± ]	0 - 100
Bacterial Endotoxin Chromgenic USP<85>/ Eur. Ph. 2.6.14. Bacterial Endotoxin Chromgenic Test	< 0.001	EU/mg		0 - 0.5
Elemental Impurities	Result	Unit	Uncertainty	Acceptable Range
Arsenic Elemental Impurities screening USP (232) / Ph. Eur. 5.20 / 2.4.20	< 0.001	ppm		0 - 1.5
Cadmium Elemental Impurities screening USP (232) / Ph. Eur. 5.20 / 2.4.20	< 0.001	ppm		0 - 0.5
Quicksilver Elemental Impurities screening USP (232) / Ph. Eur. 5.20 / 2.4.20	< 0.001	ppm		0 - 1.5
Lead Elemental Impurities screening USP (232) / Ph. Eur. 5.20 / 2.4.20	< 0.001	ppm		0 - 1.5
Nickel Elemental Impurities screening USP (232) / Ph. Eur. 5.20 / 2.4.20	< 0.001	ppm		0 - 25
Vanadium Elemental Impurities screening USP (232) / Ph. Eur. 5.20 / 2.4.20	< 0.001	ppm		0 - 25
Cobalt Elemental Impurities screening USP (232) / Ph. Eur. 5.20 / 2.4.20	< 0.001	ppm		0 - 25

	<b>Method Specification</b>	
<b>Determination of identity, content and purity of NAD<sup>+</sup></b>		
<i>Document number</i> NAD_003_2026	<i>Superseded document</i> -	<i>Number of pages</i> 3

## 1. Content Assesment

### 1.1. Instrumentation

Module	Name	Serial Number
System Controller	Shimadzu CBM-20A	L20235355693
Degassing Unit	Shimadzu DGU-14A	NA
Pump A	Shimadzu LC-20AD	L20104350216
Pump B	Shimadzu LC-20AD	L20104451348
Autosampler	Shimadzu SIL-10ADvp	C21054109114
Colum Thermostat	Shimadzu CTO-10ACvp	C21033770144
Detector	Shimadzu SPD-10ADvp	C20994233588

### 1.2. Chromatographic conditions

Chromatographic conditions	
Eluent A	10mM ammonium formate in 0.05% Formic acid in Water (HPLC, Gradient Grade)
Eluent B	0.05% Formic acid in Acetonitrile (HPLC, Gradient Grade)
Flow rate	1.0 mL/min
Program	Gradient elution
Injection volume	0.5 µL
Colum Temperature	40°C
Column	Phenomenex Kinetex 5u C18 100x4.6 mm 100A
Detection wavelength	260nm

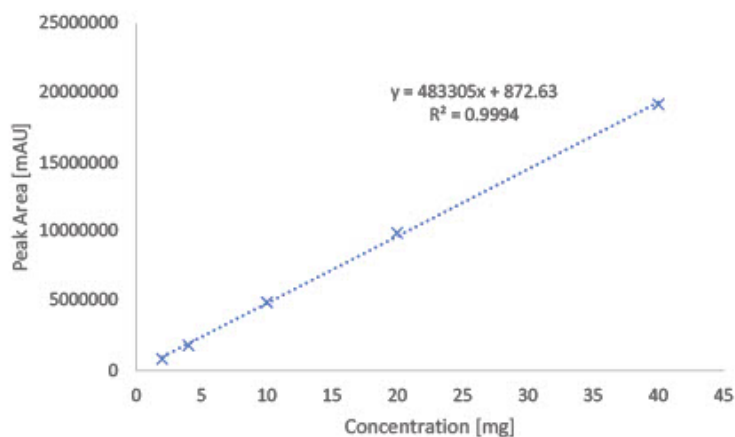
Gradient Program		
Time [min]	A [%]	B [%]
2	95	5
6	50	50
8	50	50
10	95	5
14	end	

### 1.3. Sample preparation

Whole amount of container was dissolved in 10mL of water (HPLC, Gradient Grade). Aliquote part of 0.1 mL was dispensed into HPLC vial, diluted by 0.9 mL of Water (HPLC, Gradient Grade) and. Diluted sample was used for HPLC analysis.

### 1.4. Calibration curve

Calibration curve detail	
Quantitative method	External Standard
Calibration Type	Linear
Number of calibration points	5
Force through Zero	Disabled
Weighting Method	None



## 2. Purity assessment

### 2.1 Instrumentation

Module	Name	Serial Number
System Controller	Shimadzu CBM-20A	L20235355693
Degassing Unit	Shimadzu DGU-14A	NA
Pump A	Shimadzu LC-20AD	L20104350216
Pump B	Shimadzu LC-20AD	L20104451348
Autosampler	Shimadzu SIL-10ADvp	C21054109114
Colum Thermostat	Shimadzu CTO-10ACvp	C21033770144
Detector	Shimadzu SPD-10ADvp	C20994233588

### 2.2 Chromatographic conditions

Chromatographic conditions	
Eluent A	10mM ammonium formate in 0.05% Formic acid in Water (HPLC, Gradient Grade)
Eluent B	0.05% Formic acid in Acetonitrile (HPLC, Gradient Grade)
Flow rate	1.0 mL/min
Program	Gradient elution
Injection volume	0.5 µL
Colum Temperature	40°C
Column	Phenomenex Kinetex 5u C18 100x4.6 mm 100A
Detection wavelength	260nm

Gradient Program		
Time [min]	A [%]	B [%]
2	95	5
6	50	50
8	50	50
10	95	5
14	end	

### 1.5. Sample preparation

Whole amount of container was dissolved in 10mL of water (HPLC, Gradient Grade). Aliquote part of 0.1 mL was dispensed into HPLC vial, diluted by 0.9 mL of Water (HPLC, Gradient Grade) and. Diluted sample was used for HPLC analysis.

### 1.6. Purity assesment

Purity of compound assesed by area normalization method, comparing area of each peak to sum of area of all peaks detected at wavelenght of 214 nm.

# Analysis Report

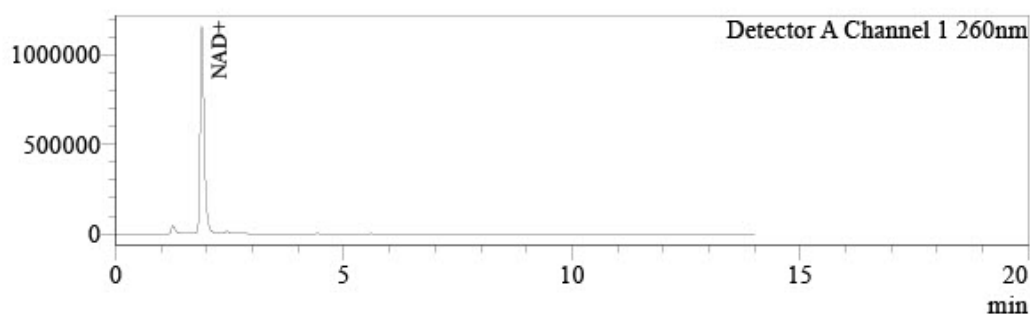


## Sample Information

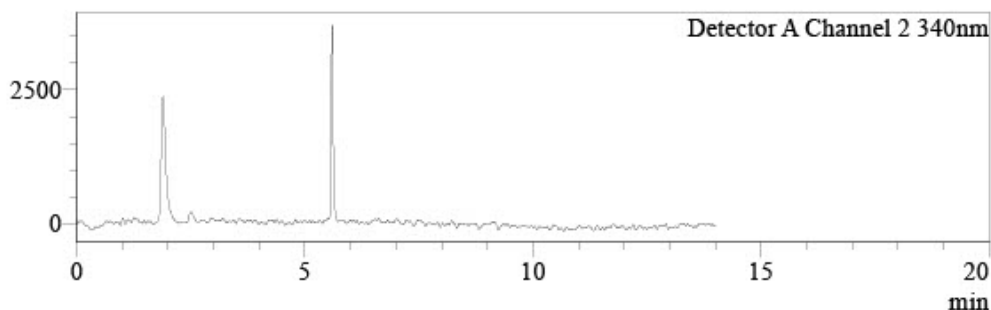
Injection Volume : 0,5  
 Data File : LYO-0088\_003.lcd  
 Method File : NUC\_HILIC.lcm  
 Date Acquired : 08.04.2026 14:34:12

## Chromatogram

uV



uV



### Peak Table


Detector A Channel 1 260nm

Peak#	Name	Ret. Time	Conc.	Unit	Area%
1		1,597	0,000		0,072
2	NAD+	1,886	1105,791	mg/L	99,050
3		2,440	0,000		0,879
Total					100,000

### Peak Table

Detector A Channel 2 340nm

Peak#	Name	Ret. Time	Conc.	Unit
Total				

	<b>Method Specification</b>	
<b>Determination of bacterial endotoxin content of lyophilized samples</b>		
<i>Document number</i> ENDOTOX_0402_2026	<i>Superseded document</i> -	<i>Number of pages</i> 2

## 1. Chromgenic LAL Assay Determination of Bacterial Endotoxin content of sample

### 1.1. Instrumentation

- Pipette set 1-1000 µL
- Thermostatically controlled water bath
- UV VIS spectrometer ( Shimadzu UV-1601)
- GenScript ToxinSensor Chromgenic LAL Endotoxin Assay kit

### 1.2. Chemicals

- LAL Reagent water (endotoxin free)
- Limulus Amoebocyte Lysate
- LAL Substrate
- Color Stabilizer #1
- Color Stabilizer #2
- Color Stabilizer #3
- 35% HCl (p.a.)

### 1.3. Sample preparation

1. Sample container was weighed prior to dissolution and measured weight was marked.
2. Sample was completely dissolved in its container by 2 mL of LAL Reagent water.
3. 100 µL of the sample was aliquoted for analysis.
4. After analysis container was emptied and dried.
5. Dry mass of container was measured and exact weight of dissolved content was determined as:

$$m_{dc} = m_{sample} - m_{container}$$

### 1.4. Toxin sensor Chromgenic LAL Endotoxin Assay kit preparation

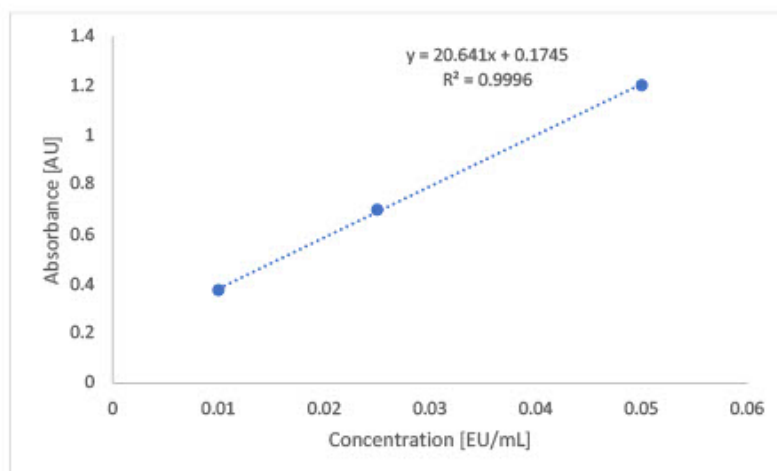
Procedures regarding preparation of reaction solutions possible to find in:

[https://www.genscript.com/site2/document/5292\\_20080806231827.PDF](https://www.genscript.com/site2/document/5292_20080806231827.PDF)

### 1.5. Measurement procedure

	Standards	Samples	Blank
Standards (mL)	0.1	-	-
Samples (mL)	-	0.1	-
LAL Reagent Water (mL)	-	-	0.1
LAL Solution (mL)	0.1	0.1	0.1
Mix well and incubate at 37°C for 27 min			
Substrate solution (mL)	0.1	0.1	0.1
Mix well and incubate at 37°C for 6 min			
Color Stabilizer #1 solution	0.5	0.5	0.5
Color Stabilizer #2 solution	0.5	0.5	0.5
Color Stabilizer #3 solution	0.5	0.5	0.5
Mix well and read the absorbance at 545nm			

### 1.6. Calibration curve



### 1.7. Calculation of endotoxin content

Endotoxin content of the sample was calculated from the calibration curve as:

$$\text{Endotox}[EU/mg] = \frac{\left(\frac{ABS_{sample}}{S_{calib}}\right) * 20}{m_{sample}}$$

$ABS_{sample}$  = Measured absorbance of sample

$S_{calib}$  = Slope of calibration curve

$m_{sample}$  = real measured mass of sample

20 = dilution factor of measured sample

	<b>Method Specification</b>		
<b>Determination of bioburden of lyophilized samples</b>			
<i>Document number</i> MIC_001_2025	<i>Superseded document</i> -	<i>Number of pages</i> 2	

## 1. Instrumentation and chemicals

### 1.1. Instruments used

- Sterile Syringe 2mL Luer
- Sterile needles
- Ready made PCA Plate ROTI Aquatest
- Ready made Sab4 Plate ROTI Aquatest

### 1.2. Chemicals

Sterile physiological solution (0.9% NaCl)

## 2. Sample preparation and inoculation

### 2.1 Sample preparation

1. Fresh sterile needle and syringe was used for measuring exactly 2 mL of sterile physiological solution.
2. Needle was changed and by new needle rubber top of peptide container was penetrated and 2 mL of sterile physiological solution was dispensed.
3. Content of container was completely dissolved and left for 5 minutes to settle potentially created bubbles.
4. This procedure is repeated for two vials.

### 2.2 Total Aerobic microbial count inoculation and cultivation

1. By sterile needle 1 mL of solution was filled into the sterile syringe.
2. Needle was placed above the flame for few seconds to sterilize.
3. Consequently 1 mL of solution was poured into the ready to use sterile petri dish filled with PCA agar and petri dish was closed.
4. Proces was repeated for two petri dishes.
5. With sterile needle, 1 mL of sterile physiological solution was filled into the sterile needle and was inoculated onto one sterile petri dish filled with PCA agar as negative control sample.
6. Samples and negative control sample were placed in incubator at temperature 37°C for 120h.

### 2.3 Total Yeast and Mold count inoculation and cultivation

1. By sterile needle 1 mL of solution was filled into the sterile syringe.
2. Needle was placed above the flame for few seconds to sterilize.
3. Consequently 1 mL of solution was poured into the ready to use sterile petri dish filled with Sab4 agar and petri dish was closed.
4. Proces was repeated for two petri dishes.
5. With sterile needle, 1 mL of sterile physiological solution was filled into the sterile needle and was inoculated onto one sterile petri dish filled with Sab4 agar as negative control sample.
6. Samples and negative control sample were placed in incubator at temperature 25°C for 72h.

## 3. Evaluation of results

After incubation time, colonies are counted as cfu (colonies forming units) and result per 1g of sample is determined as:

$$CFU_{avg} = \frac{\sum CFU_n}{n}$$

$CFU_{avg}$  = average CFU counted from  $n$  inoculations

$CFU_n$  = CFU counted per inoculation

$n$  = number of inoculations

$$CFU \text{ per gram} = \frac{CFU_{avg}}{m_s} * DF$$

$CFU_{avg}$  = Average CFU counted from  $n$  inoculations

$m_s$  = mass of sample (mg)

$DF$  = Dilution factor

If negative control sample is evaluated as positive, process have to be repeated due to possible contamination in the process of inoculation or incubation.

## Responsibles



**Mr. Ján Galbavý**  
*CEO*

Analysis results relate only to the samples tested.

This document shall not be reproduced except in full, without the written approval of Liquilabs s.r.o.