ANALYTON™ LABS

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Issued 2026-09-15

Certificate of analysis

REPORT FIELD OMITTED 2026-10-29

Issued by ANALYTON LABS, analytical testing laboratory, under ANALYTON GC 1.1.

Client	Molequa
Sample	beta-Nicotinamide adenine dinucleotide (NAD+), free acid, powder, vial labelled 100 mg
Batch number	ATNAD-100-2608-01
Matrix	Research compound, small molecule

Sample received	2026-09-08
Analysis	2026-09-09 to 2026-09-14
Date of issue	2026-09-15
Determinations performed	22

CONFORMS TO SPECIFICATION

All 19 determinations judged against a specification are within it. 3 further determinations are reported without a specification.

SUMMARY OF RESULTS

DETERMINATION	METHOD	RESULT	SPECIFICATION
Appearance	Visual	**White to off-white crystalline powder, hygroscopic, free of visible particles**	White to off-white powder PASS
Identity	LC-HRMS, ESI+ and ESI-, USP <621> principles; UV	**[M+H]+ 664.1168, deviation 0.6 ppm; [M-H]- 662.1014, deviation 0.6 ppm; MS/MS 542.069 (loss of nicotinamide, 122.048 Da), 428.037 (ADP), 348.070 (AMP), 136.062 (adenine), 123.055 (nicotinamide); UV λmax 259 nm, no absorbance at 340 nm (oxidised form)**	Theoretical monoisotopic 663.1091 Da from C21H27N7O14P2; [M+H]+ 664.1164, [M-H]- 662.1018; deviation <= 10 ppm PASS
Purity	HPLC, C18 polar-endcapped, 20 mM ammonium acetate pH 5.0 and methanol, 260 nm, area %	**99.12 ± 0.20 % (k = 2)**	>= 99.0 % PASS

DETERMINATION	METHOD	RESULT	SPECIFICATION
Related substances	HPLC at 260 nm and 340 nm; LOD 0.01 %, LOQ 0.03 %, reporting threshold 0.05 %	**alpha-NAD+ 0.32 % at RRT 0.95 (± 0.03 %); NADH 0.14 % at RRT 1.06 (340 nm positive); total impurities 0.88 %, i.e. 0.88 mg per vial**	alpha-anomer <= 1.0 %, NADH <= 0.5 %, any other <= 0.5 %, total <= 2.0 % PASS
Impurity profile	HPLC with LC-HRMS assignment	**RRT 0.84, 0.10 %, m/z 348.070 (AMP); RRT 0.89, 0.14 %, m/z 560.079 (ADP-ribose, loss of nicotinamide); RRT 0.95, 0.32 %, same mass (alpha-NAD+, anomer, enzymatically inactive); RRT 1.06, 0.14 %, m/z 666.132 (NADH); RRT 1.18, 0.18 %, m/z 123.055 (free nicotinamide)**	Peaks above 0.05 % reported
Assay, total nucleotides	UV at 260 nm, ε 17,800 l/(mol·cm) at pH 7.0, against USP beta-NAD RS	**95.60 ± 1.0 % w/w (k = 2)**	Report only
Assay, beta-NAD+	UV assay × chromatographic purity; confirmed enzymatically (alcohol dehydrogenase, A340, ε 6,220)	**94.80 ± 1.1 % w/w (enzymatic 94.90 ± 1.5 % w/w; the 0.1 % agreement confirms that alpha-anomer and NADH are small)**	>= 93 % w/w PASS
Fill weight	Gravimetric, two vials, weighed quickly (hygroscopic)	**102.8 ± 0.1 mg (second vial 102.5 mg, difference 0.3 %)**	Labelled 100 mg REPORT ONLY
beta-NAD+ per vial	Calculated from fill weight and beta-NAD+ assay	**97.5 mg per vial labelled 100 mg, i.e. 97.5 % of label**	95 to 105 % of label PASS
Form	ICP-OES (Na, K, Li); ion chromatography (phosphate)	**Free acid: sodium 0.03 % w/w (a mono-sodium salt would hold 3.4 %), potassium and lithium < 0.01 %; free phosphate 0.08 % (hydrolysis product of the pyrophosphate)**	Free acid; phosphate <= 0.3 % PASS

DETERMINATION	METHOD	RESULT	SPECIFICATION
Trifluoroacetate and anions	Ion chromatography, LOQ 20 ppm	**Trifluoroacetate < 20 ppm (not used in fermentation-derived NAD+); chloride 35 ppm; sulfate and acetate < 20 ppm; free pyrophosphate < 0.05 %**	Chloride <= 500 ppm PASS
Water content	Coulometric Karl Fischer, USP <921>, weighed in a dry box	**3.40 ± 0.17 % w/w (k = 2), i.e. 3.5 mg per vial**	<= 8.0 % PASS
Mass balance	beta-NAD+ + organic impurities + water + phosphate + sodium	**94.80 + 0.85 + 3.40 + 0.08 + 0.03 = 99.16 % (± 1.2 %)**	98 to 102 % PASS
pH	Potentiometric, 10 mg/ml in water	**3.2 (free acid; above pH 7 NAD+ hydrolyses to ADP-ribose and nicotinamide, so the acid form favours storage)**	2.5 to 3.5 PASS
Solubility	Visual, 50 mg/ml and 10 mg/ml	**>= 50 mg/ml in water and in bacteriostatic water; clear, colourless, free of particles within 60 s; clear at 100 mg/ml, pH 2.9**	Clear solution PASS
Bacterial endotoxins	Kinetic chromogenic LAL, USP <85>, after adjustment to pH 6.5 to 7.5 with Tris (an acidic sample would inhibit the lysate)	**< 0.005 EU/mg (< 0.5 EU per vial; LOQ 0.001 EU/mg)**	<= 0.02 EU/mg PASS
Sterility	Membrane filtration, USP <71>; fluid thioglycollate 30 to 35 °C and soya bean casein digest 20 to 25 °C	**No growth after 14 days, 2 vials**	No growth PASS
Microbial enumeration	Plate count, USP <61>, sample neutralised to pH 7; specified organisms, USP <62>	**TAMC < 10 CFU/g, TYMC < 10 CFU/g; Escherichia coli, Salmonella, Staphylococcus aureus and Pseudomonas aeruginosa absent in 1 g**	TAMC <= 1000 CFU/g, TYMC <= 100 CFU/g; specified organisms absent PASS

DETERMINATION	METHOD	RESULT	SPECIFICATION
Residual solvents	Headspace GC, USP <467>	**Ethanol 210 ppm (precipitation from the fermentation lysate); acetone 45 ppm; methanol, acetonitrile and isopropanol each < 10 ppm (LOQ 10 ppm)**	ICH Q3C: ethanol <= 5000, acetone <= 5000, methanol <= 3000, acetonitrile <= 410 ppm PASS
Elemental impurities	ICP-MS after microwave digestion (HNO3/H2O2), USP <233>	**Cd, Pb, As, Hg < 0.05 µg/g; Co, V < 0.10 µg/g; Ni 0.12 µg/g (quantified); every result at or below 0.3 % of its limit**	ICH Q3D(R2) Class 1 and 2A, parenteral PDE per 100 mg daily: Cd 20, Pb 50, As 150, Hg 30, Co 50, V 100, Ni 200 µg/g PASS
Anomeric and redox purity	HPLC 260 nm (alpha-NAD+) and 340 nm (NADH)	**alpha-NAD+ 0.32 % (enzymatically inactive; not distinguished by MS or UV, only by chromatography and enzyme); NADH 0.14 %; A260/A340 of the whole sample > 100**	alpha-NAD+ <= 1.0 %; NADH <= 0.5 % PASS
Compound screening	LC-HRMS full scan m/z 100 to 1000, both polarities, against reference standards	**NMN, nicotinamide riboside, nicotinic acid, NADP+, ADP, ATP, adenosine, FAD and fermentation residues (glucose, amino acids): none above 0.05 % of area beyond the impurities listed above**	Nothing above 0.05 % PASS



SIGNATURE

NAME O. Noga

ROLE Head Laboratory Technician

DATE 2026-09-15



The sample

Sample	beta-Nicotinamide adenine dinucleotide (NAD+), free acid, powder, vial labelled 100 mg
Batch number	ATNAD-100-2608-01
Matrix	Research compound, small molecule
Received	2026-09-08
Analysis	2026-09-09 to 2026-09-14
Condition on receipt	Seal intact, closure unmarked, white to off-white crystalline powder, no visible particles
Storage before analysis	Minus 20 degrees Celsius, desiccated, dark, until first portion taken; hygroscopic

Water content

PRINCIPLE

Water in the sample reacts stoichiometrically with iodine generated coulometrically in the titration cell, and the charge passed gives the mass of water directly. The coulometric form is used rather than the volumetric because the quantities here are small.

SYSTEM SUITABILITY APPLIED

CRITERION	REQUIREMENT	ACHIEVED
Cell blank drift	stable before the determination begins	stable at 0.8 micrograms per minute
Recovery of a water standard	97.5 to 102.5 per cent	99.6 per cent
Replicate agreement	within 0.2 per cent absolute	0.06 per cent absolute

WATER CONTENT · PASS

3.40 ± 0.17 % w/w (k = 2), i.e. 3.5 mg per vial

Against <= 8.0 %

Bacterial endotoxins

PRINCIPLE

Lysate from the amoebocytes of the horseshoe crab is activated by bacterial endotoxin through a protease cascade, which cleaves a chromogenic substrate. The rate at which colour develops is proportional to endotoxin concentration, and the onset time is read against a standard curve prepared from control standard endotoxin.

SYSTEM SUITABILITY APPLIED

CRITERION	REQUIREMENT	ACHIEVED
Standard curve correlation coefficient	not less than 0.980 in absolute value	0.9990
Positive product control recovery	50 to 200 per cent	92 %
Negative control	below the lowest standard	no onset
Dilution used	at or below the maximum valid dilution	1 in 50 after pH adjustment, below MVD

BACTERIAL ENDOTOXINS · PASS

< 0.005 EU/mg (< 0.5 EU per vial; LOQ 0.001 EU/mg)

Against <= 0.02 EU/mg

Sterility

PRINCIPLE

The contents are transferred under aseptic conditions to fluid thioglycollate medium and to soya bean casein digest medium, and incubated for fourteen days at the temperatures each medium requires. Growth in either medium is a failure.

SYSTEM SUITABILITY APPLIED

CRITERION	REQUIREMENT	ACHIEVED
Growth promotion of each medium	confirmed with the compendial challenge organisms	all media passed
Method suitability against the product	confirmed, no inhibition	growth within 3 days with product present
Negative control	no growth for the full incubation	no growth
Incubation	not fewer than 14 days	14 days

STERILITY · PASS

No growth after 14 days, 2 vials

Against No growth

Microbial enumeration

PRINCIPLE

A prepared portion is plated onto soya bean casein digest agar for total aerobic microbial count and onto Sabouraud dextrose agar for total combined yeasts and moulds, and colonies are counted after incubation.

SYSTEM SUITABILITY APPLIED

CRITERION	REQUIREMENT	ACHIEVED
Growth promotion of each medium	confirmed	all media passed
Method suitability against the product	recovery within a factor of two	recovery 81 to 109 % of the inoculum after neutralisation
Negative control plates	no growth	no growth

MICROBIAL ENUMERATION · PASS

TAMC < 10 CFU/g, TYMC < 10 CFU/g; Escherichia coli, Salmonella, Staphylococcus aureus and Pseudomonas aeruginosa absent in 1 g

Against TAMC <= 1000 CFU/g, TYMC <= 100 CFU/g; specified organisms absent

Residual solvents

PRINCIPLE

A weighed portion is equilibrated in a sealed vial and the vapour above the sample is sampled and separated by gas chromatography, with detection by mass spectrometry or flame ionisation. Sampling the headspace rather than the solution keeps involatile matrix out of the instrument.

SYSTEM SUITABILITY APPLIED

CRITERION	REQUIREMENT	ACHIEVED
Resolution between the two closest eluting solvents	not less than 1.0	3.6, acetonitrile from dichloromethane
Signal to noise at the limit of quantitation	not less than 10 to 1	48 to 1 at the LOQ
Repeatability of the standard	not more than 15 per cent relative standard deviation	4.2 %
System blank	no interfering peak at any target retention time	no interfering peak

RESIDUAL SOLVENTS · PASS

Ethanol 210 ppm (precipitation from the fermentation lysate); acetone 45 ppm; methanol, acetonitrile and isopropanol each < 10 ppm (LOQ 10 ppm)

Against ICH Q3C: ethanol <= 5000, acetone <= 5000, methanol <= 3000, acetonitrile <= 410 ppm

Elemental impurities by ICP-MS

PRINCIPLE

The sample is digested in acid under microwave pressure and introduced into an argon plasma at approximately 7000 kelvin, which atomises and ionises the elements present. Ions are separated by mass to charge in a quadrupole and counted individually, which is what puts the technique at parts per billion.

SYSTEM SUITABILITY APPLIED

CRITERION	REQUIREMENT	ACHIEVED
Internal standard recovery	80 to 120 per cent	97 %
Spike recovery in matrix	70 to 150 per cent	92 to 106 %
Calibration correlation coefficient	not less than 0.995	0.9996
Digestion blank	below the reporting limit for every element	below LOQ
Drift check across the sequence	within 20 per cent	3 %

ELEMENTAL IMPURITIES · PASS

Cd, Pb, As, Hg < 0.05 µg/g; Co, V < 0.10 µg/g; Ni 0.12 µg/g (quantified); every result at or below 0.3 % of its limit

Against ICH Q3D(R2) Class 1 and 2A, parenteral PDE per 100 mg daily: Cd 20, Pb 50, As 150, Hg 30, Co 50, V 100, Ni 200 µg/g

Compound screening

PRINCIPLE

The sample is acquired in both positive and negative ionisation and the detected features are compared against reference standards held by the laboratory and against a spectral library, with confirmation by retention time and fragmentation where a standard is available.

SYSTEM SUITABILITY APPLIED

CRITERION	REQUIREMENT	ACHIEVED
Reference standards injected in the same sequence	confirmed for every reported identification	18 injected in the same sequence
Mass accuracy	within 5 ppm	within 3 ppm
Both polarities acquired	confirmed	acquired
Solvent blank between injections	no carryover above the reporting threshold	no carry-over above 0.01 %

COMPOUND SCREENING · PASS

NMN, nicotinamide riboside, nicotinic acid, NADP+, ADP, ATP, adenosine, FAD and fermentation residues (glucose, amino acids): none above 0.05 % of area beyond the impurities listed above

Against Nothing above 0.05 %

Controls carried in the same sequence

Blank	Solvent blank (mobile phase A) injected before the sequence; no peak above 0.02 % of the main peak within RRT 0.80 to 1.30 (maximum 0.002 %)
Reference standard	USP beta-Nicotinamide Adenine Dinucleotide RS (primary) injected at the start and end of the sequence; area difference 0.3 %, retention time within 0.1 min; control sample from the previous batch within 0.2 % of its value

What these results do not establish

Each determination on this report answers one question. The limits below are the questions it does not answer, and every one of them is a mistake made routinely and in good faith by someone reading a certificate.

Water content
Water content describes the powder as received. It moves with handling and storage, and a figure taken on the day of receipt does not describe the container after it has been opened.

Bacterial endotoxins
Bacterial endotoxin is not sterility. A sample can be free of endotoxin and carry viable organisms, and it can be sterile and still pyrogenic. The two are separate determinations because they are separate properties.

Sterility
Sterility applies to the units tested, not to the batch they came from. It cannot be extrapolated to a container that was not sampled, and it is not a statement about pyrogens.

Microbial enumeration
Enumeration counts what grows under the stated conditions on the stated media. It is not sterility, and an organism that does not grow under those conditions is not counted and is not reported as absent.

Residual solvents
Residual solvents are reported for the solvents in the panel, at the limits stated. A solvent not in the panel was not looked for, and its absence from this report is not evidence of its absence from the sample.

Elemental impurities
Elemental impurities are reported for the elements in the panel applied. An element outside that panel is not reported as absent, because it was not sought.

Compound screening
A screen is bounded by the reference standards held on the day it was run. A compound for which no standard was available cannot be identified by it, and its absence from the result is not evidence of its absence from the sample.

NOT PERFORMED

Biological activity beyond the alcohol dehydrogenase assay	not requested
Stability in solution	NAD+ hydrolyses above pH 7 and no stability statement is issued
Sub-visible particles and osmolality after reconstitution	the container was not presented for reconstitution studies
Microbiological quality after opening	outside the scope of a certificate on a sealed container
31P-NMR of the pyrophosphate linkage and polymorphic form (XRPD)	not requested

Verify this certificate against the register



SCAN TO VERIFY

Scan the code, or open `https://analyton.com/verify/` and enter the report number with this key:

YP33B8789R

The register returns every result on this report, the date of issue and the current status.