ANALYTICAL CERTIFICATE

Sample name	**SS-31**
Batch No.	**2024252**
Sample No.	**01**
Sequence	DArg-Dmt-Lys-Phe-NH_2
Manufacturing date	**NA**
Submitter of analytical request	Adria Peptides d.o.o.

1. Peptide content by HPLC/CLND:

1.1 HPLC Instrument:

Pump: Agilent 1200 Series, Quat Pump G1311A
Sampler: Agilent 1260 Series, Hip ALS G1367E
Degasser: Agilent 1200 Series, Degasser G1379B
Detectors: Agilent 1200 Series, VWD G1314B
Nitrogen detector Antek 8060

1.2 HPLC conditions:

Eluents: A – MilliQ water
B – isopropanol
D – 1% TFA in MilliQ water
Flow rate: 1 mL/min
Gradient:

Time	A (%)	B (%)	D (%)
0	90	0	10
1	90	0	10
9	10	80	10
10	10	80	10
11	90	0	10
15	90	0	10

Column: ARION 5µ C4-BIO 300 A, 4.6 x 100 mm
Serial No 221258

1.3 Sample preparation:

The whole amount of SS-31 (50 mg) was dissolved in 10 mL of MilliQ water.
Injection: 2.0 µL

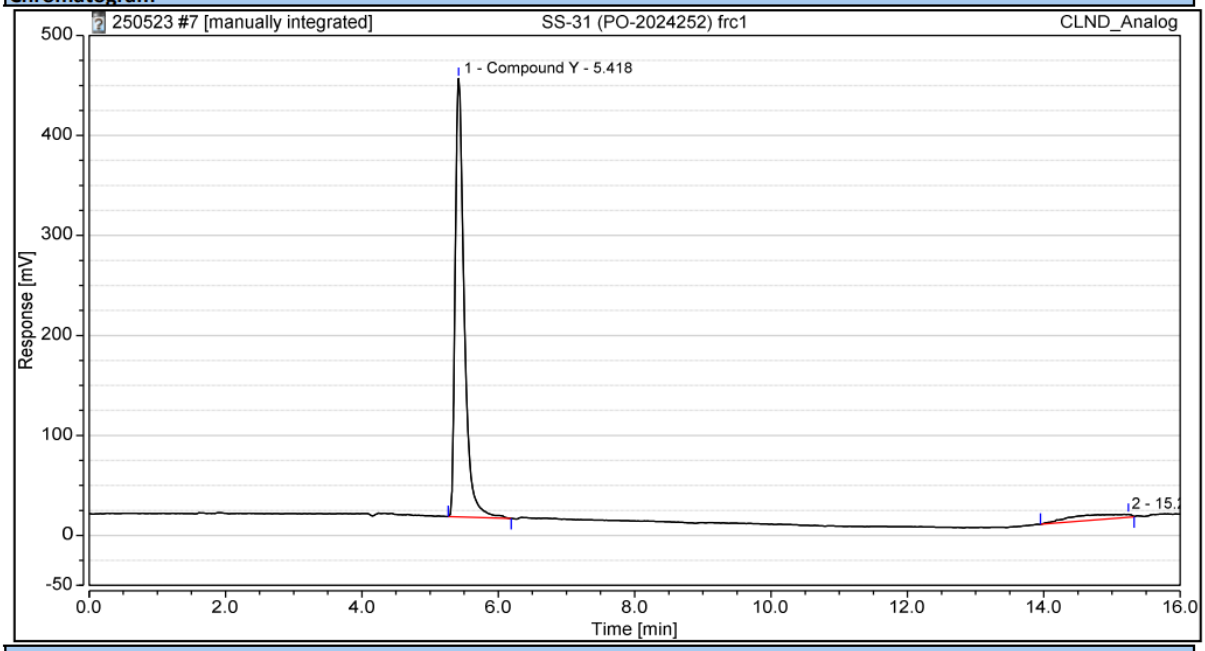
1.4 Chromatograms and calibration curve:

Chromatogram and Results

Injection Details

Injection Name:	**SS-31 (PO-2024252) frc1**	*Run Time (min):*	**16.00**
Vial Number:	**P1:E1**	*Injection Volume:*	**2.00**
Injection Type:	**Unknown**	*Channel:*	**CLND_Analog**
Calibration Level:		*Wavelength:*	
Instrument Method:	**AspartV10 long 9min**	*Bandwidth:*	**n.a.**
Processing Method:	**Aspart V20**	*Dilution Factor:*	**1.0000**
Injection Date/Time:	**23.5.25 11:56**	*Sample Weight:*	**1.0000**

Chromatogram



Integration Results

No.	Peak Name	Retention Time min	Area mV*min	Height mV	Amount ug N	Concentration mg N/mL	Evaluation
1	Compound Y	5.418	67.627	438.664	1.9458	0.9729	valid
n.a.	Compound X	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
2		15.242	4.936	3.023	n.a.	n.a.	n.a.
Total:			**72.563**	**441.687**	**1.95**	**0.97**	

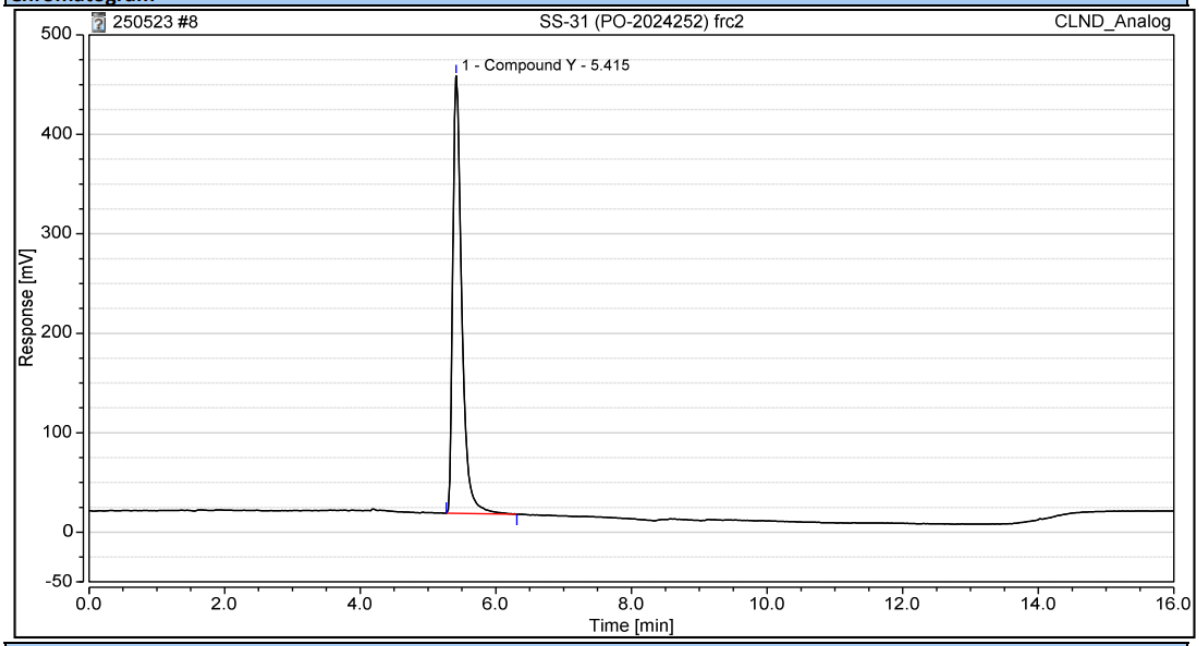
ANALYTICAL CERTIFICATE

Chromatogram and Results

Injection Details

Injection Name:	**SS-31 (PO-2024252) frc2**	*Run Time (min):*	**16.00**
Vial Number:	**P1:E1**	*Injection Volume:*	**2.00**
Injection Type:	**Unknown**	*Channel:*	**CLND_Analog**
Calibration Level:		*Wavelength:*	
Instrument Method:	**AspartV10 long 9min**	*Bandwidth:*	**n.a.**
Processing Method:	**Aspart V20**	*Dilution Factor:*	**1.0000**
Injection Date/Time:	**23.5.25 12:13**	*Sample Weight:*	**1.0000**

Chromatogram



Integration Results

No.	Peak Name	Retention Time min	Area mV*min	Height mV	Amount ug N	Concentration mg N/mL	Evaluation
1	Compound Y	5.415	68.040	439.948	1.9575	0.9788	valid
n.a.	Compound X	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Total:			**68.040**	**439.948**	**1.96**	**0.98**	

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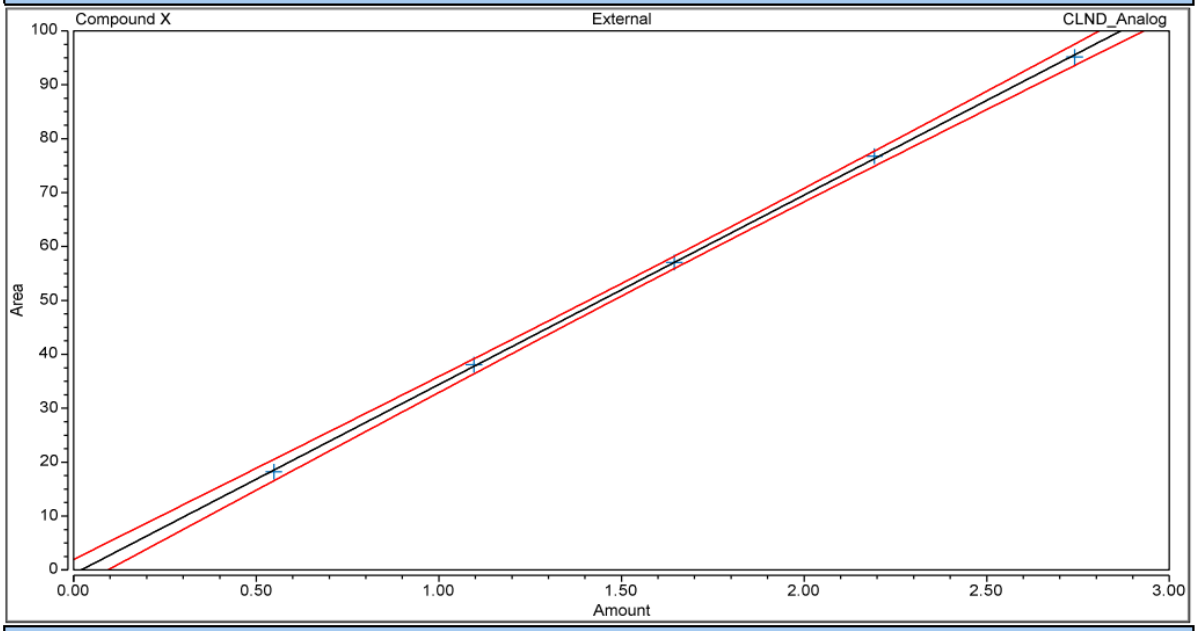
Calibration

Calibration Details	n.a.		
Calibration Type	Lin, WithOffset	*Offset (C0)*	n.a.
Evaluation Type	Area	*Slope (C1)*	n.a.
Number of Calibration Points	n.a.	*Curve (C2)*	n.a.
Number of disabled Calibration Points	n.a.	*R-Square*	n.a.

Calibration Plot n.a.



Calibration Results n.a.

No.	Injection Name	Calibration Level	X Value CLND_Analog Compound X	Y Value CLND_Analog Compound X	Y Value CLND_Analog Compound X	Area mV*min CLND_Analog Compound X	Height mV CLND_Analog Compound X
2	Aspart5	1	2.7408	95.1396	95.1396	95.140	555.193
3	Aspart4	1	2.1926	76.7674	76.7674	76.767	441.850
4	Aspart3	1	1.6445	57.0328	57.0328	57.033	329.763
5	Aspart2	1	1.0963	38.0617	38.0617	38.062	215.626
6	Aspart1	1	0.5482	18.2048	18.2048	18.205	103.476

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1.4 Results:

NNC: SS-31 (PO-2024252)	Salt:	0

MW *(calculated)* g/mol	N content *(calculated)* %	N conc. *(measured)* mg × N/ml
639,8	**19,7**	**0,9759**

Theoretical Volume ml	Lyophilizate amount mg
10,00	50,00

Peptide concentration		Quantified amount	
mg/ml	nmol/ml	mg	nmol
4,95	**7742**	**49,5**	**77 423**

Peptide content assay %
99,1

Summary table:

Peptide	Aliquoting (mg)	Total weight of sample (mg)	Content of the peptide by CLND (mg)	Content of the peptide in the sample (%)	Content of the peptide against the amount on label.
SS-31	50	--	**49,54**	--	99,1 %

2. Purity assessment by UPLC:

2.1 HPLC Instrument:

LC-System	Waters Acquity UPLC
Detectors:	UV or DAD at 214 nm

2.2 HPLC conditions:

Eluents:	A – MilliQ water + 0.05% TFA
	B – acetonitrile + 0.05% TFA
Flow rate:	0.40 mL/min
Gradient:	from 5% B to 60% B in 16 min, according to chromatogram results
Column:	Waters Acquity BEH, C-18, 1.7µm, 2.1mm x 150mm

2.3 Sample preparation:

An aliquote of SS-31 (1 mg) was dissolved in 1 mL of water.

Injection:	1.0 µL

2.4 Chromatogram of SS-31 (PO-2024252)

Sample information

UPLC5

Channel Description PDA Ch1 214nm@4.8nm

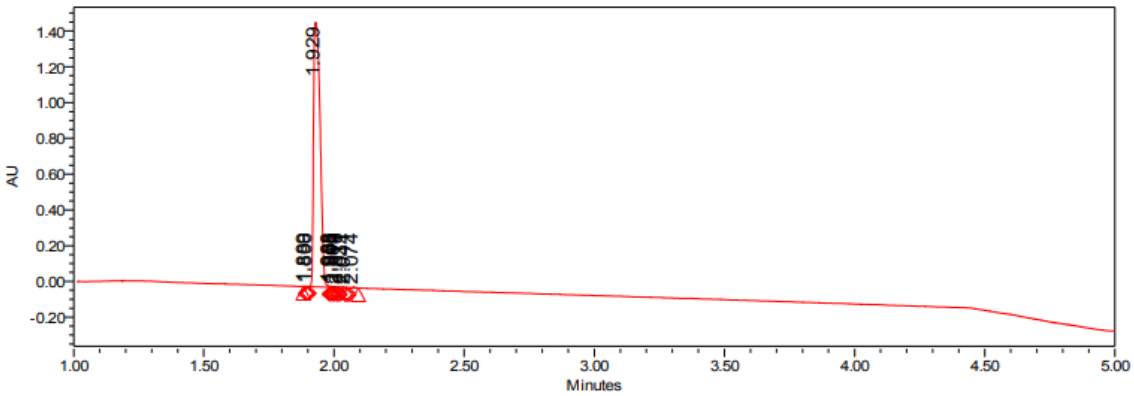
Vial : 1:A,3 Vol. : 1.00 ul

Sample: SS-31 (PO-2024252)

Date Acquired 5/26/2025 1:04:03 PM CEST

Date Processed 5/26/2025 2:53:36 PM CEST

Acq Method Set :
Gr5_60_4mi_40C_0_45ml_K2_met_s



	RT	Area	Height (µV)	% Area
1	1.929	2599351	1477324	99.51

A: 0.05% TFA in water
B: 0.05% TFA in acetonitrile
Gradient :
0.0 - 0.5min 5 - 5 % B
0.5 - 4 min 5 - 60 % B
4.0 - 4.5 min 60 - 100 % B
4.5 - 5.0min 100 % B
5.0 - 5.5min 100 - 5 % B
6min 5 % B
0.45ml/min
Acquity UPLC BEHC18, 1.7um, 2.1 x 50 mm column
column own temp. = 40 °C

2.5 Result of purity assessment

The overall purity is 99.51 % at 214 nm.

3. Peptide identity by UPLC/MS:

3.1 HPLC Instrument:

LC-System	Waters Acquity UPLC
Detectors:	UV or DAD at 214 nm

3.2 HPLC conditions:

Eluents:	A – MilliQ water + 0.05% TFA
	B – acetonitrile + 0.05% TFA
Flow rate:	0.40 mL/min
Gradient:	from 5% B to 60% B in 4 min, according to chromatogram results
Column:	Waters Acquity BEH, C-18, 1.7µm, 2.1mm x 50mm
	Part No 186002353

3.3 MS Detector:

Detector	Waters (Micromass) ZQ 2000
Ionisation method:	ES+
Scanning range:	200 – 2000 amu
Capillary voltage:	3.0 kV
Cone Voltage:	20 V
Scantime:	0.9 s
Interscan delay:	0.1 s
Detection method:	quadrupole

3.4 Sample preparation:

An aliquote of SS-31 (1 mg) was dissolved in 1 mL of water.

Injection:	0.8 µL

3.5 Mass Spectra of SS-31 (PO-2024252)

Sample information

UPLC1 ZQ **Sample: SS-31 (PO-2024252)**

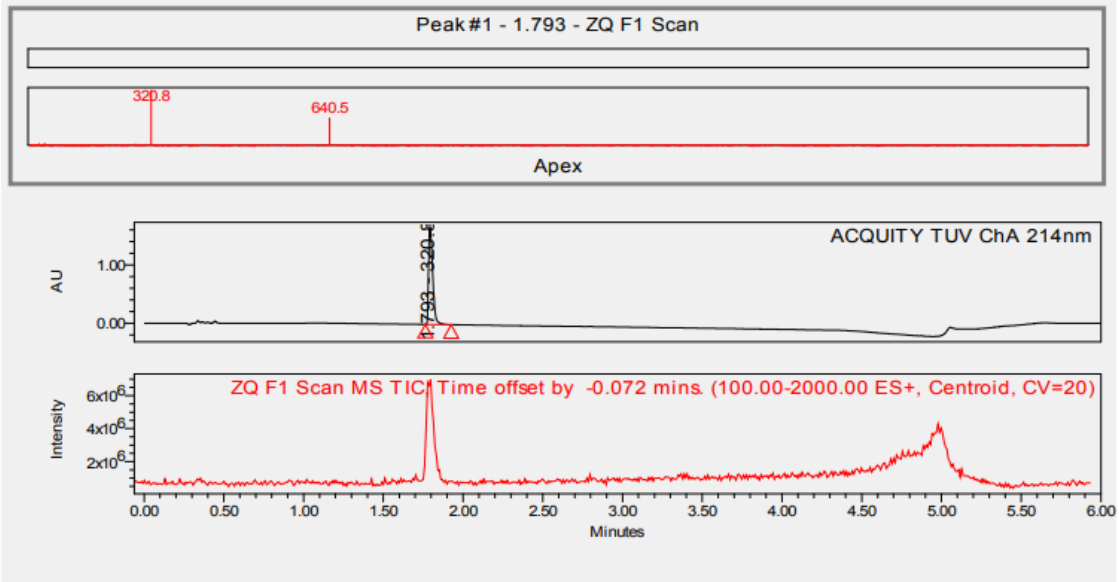
Channel Description ACQUITY TUV ChA 214nm

Vial : 1:A,2 Vol. : 0.80 ul

Date Acquired 5/26/2025 1:10:13 PM CEST

Date Processed 5/26/2025 2:55:10 PM CEST

Acq Method Set : Gr5_60_MS_4min_0_45ml_K2_me_s



MS Result Table

	Name	RT	Base Peak (m/z)
1		1.793	320.82

UPLC conditions:

A: 0.05% TFA in water
B: 0.05% TFA in acetonitrile
Gradient :
0.0 - 0.5min 5 - 5 % B
0.5 - 4 min 5 - 60 % B
0.45ml/min
Acquity UPLC BEHC18, 1.7um, 2.1 x 50 mm column
column temp. = 40 °C

Theoretical values of m/z:

Peptide MW	$[M+1H]^{2+}$	$[M+2H]^{3+}$	$[M+3H]^{4+}$	$[M+4H]^{5+}$	$[M+5H]^{6+}$	$[M+6H]^{7+}$
639,8	640,8	320,9				
Found	**640,5**	**320,8**				

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4. Endotoxin test:

4.1 Description:

Test tubes: Gel Clot Lyophilized Amebocyte Lysate Single Test in Vial
Manufacturer: Xiamen Bioendo Technology Co., Ltd.
Lot: 24061152
Content: 0.2 ml endotoxin-specific Amebocyte Lysate which includes beta-glucan inhibitor in the formulation
Sensitivity of test: 0.5 EU/mL
Sample sensitivity level: 5 EU/mg

4.2 Sample preparation and test:

A sample peptide is dissolved in endotoxin-free water to form a concentration of 0.5 mg/mL. 200 µL of this solution is then transferred to the Amebocyte Lysate Single Test tube and incubated at 37 °C for 60 min. Immediately after incubation the test tube is slowly turned upside down.
A solid gel clot which doesn't come down immediately indicates **positive** result (meaning that endotoxins are above the current sensitivity level).
An absence of solid gel clot so the solution freely flows down from the bottom of test tube indicates **negative** result (meaning that endotoxin are below the current sensitivity level).

4.3 Result:

NEGATIVE (-)

CONCLUSION:
The sample SS-31 (Batch No. 2024252) was analyzed for peptide content, acetic acid content, UV purity, identity by MS and endotoxins.

Peptide content is 99.08 % (49.54 mg in 50 mg)
Purity is 99.51 % (UPLC at 214 nm).
MS identity complies with theoretical calculation of m/z values.
Endotoxin test (sensitivity level 5 EU/mg) - NEGATIVE.

ANALYSIS COMPLETED:	Date: 26.05.2025
Issued by QC:	Date: 27.05.2025 Signature:

Certificate Of Analysis

LIQUILABS
Analytical services

Client:

Particle Peptides
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+421 917 381 743

Laboratory:

Liquilabs s.r.o.
Ohradní 24B
14000 Michle
Czechia
www.liquilabs.cz

Sample Identification

Sample Name	SS-31 50 mg	**Batch Number**	2025252	**Date Published**	2025-07-29 12:36

Results for Lyo-0065

Bioburden	Result	Unit	Uncertainty	Reporting Limit	
Total Aerobic Microbial Count USP <61> Plate Count Method	Not detected	*CFU/g*		>= 1000	⚠
Total Yeast and Mold Count USP <61> Plate Count Method	Not detected	*CFU/g*		>= 100	⚠

Attachments for Lyo-0065

LIQUILABS Analytical services	Method Specification	
Determination of bioburden of lyophilized samples		
Document number MIC_001_2025	*Superseded document* -	*Number of pages* 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 innoculation

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 containter 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 innoculation 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 sterilze.
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 innoculated 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.

1

Attachment for Lyo-0065
Filename: Bioburden-images-0.jpg

2.3 Total Yeast and Mold count innoculation 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 sterilze.
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 innoculated 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:

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

$$CFU_{avg} = average\ CFU\ counted\ form\ n\ innoculations$$
$$CFU_n = CFU\ counted\ per\ innoculation$$
$$n = number\ of\ innoculations$$

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

$$CFU_{avg} = Average\ CFU\ counted\ from\ n\ innoculations$$
$$m_s = mass\ of\ sample\ (mg)$$
$$DF = Dilution\ factor$$

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

Attachment for Lyo-0065
Filename: Bioburden-images-1.jpg

Responsibles

Mr. Ján Galbavý
Founder/Manager

Analysis results relate only to the samples tested.
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Analytical report AR-25-KT-018095-02

Testing laboratory:
Eurofins Environment Testing Slovakia s.r.o.
Robotnícka 820/36, 039 01 Turčianske Teplice
IČO: 53 248 376
Place of work:
Accredited testing laboratory Turčianske Teplice
Robotnícka 820/36, 039 01 Turčianske Teplice
tel: 043/490 1562
RegistrationEnviroSK@etcee.eurofins.com, www.eurofins.sk

Customer:
PARTICLE s.r.o.
Kolonáda 4490/18
984 01 Lučenec
SLOVAKIA

Date of Sample Receipt: 20.05.2025 **Date of Testing**: 20.05.2025 - 22.05.2025 **Issue date:** 22.05.2025

Information about Sampling:
Sampler: customer

Sample information: **104-2025-00019379**
Sample description: SS-31 (PO-2024252)
Material: Peptidy

Physical and chemical tests

Parameter	Unit	Allowed Value	Measured Value	Uncertainty of measurement*	Method	Testing method	E	SL	TT
Arsenic (As)	mg/kg	max, 1,5	0,039	25%	ICP-MS	LS-PP-CH-85	S	TR	A
Cadmium (Cd)	mg/kg	max, 0,2	<0,10	-	ICP-MS	LS-PP-CH-85	S	TR	A
Lead (Pb)	mg/kg	max, 0,5	<0,30	-	ICP-MS	LS-PP-CH-85	S	TR	A
Mercury (Hg)	mg/kg	max, 0,3	0,018	25%	ICP-MS	LS-PP-CH-85	S	TR	A

Notes:
E - evaluation
S - satisfied
NS - not satisfied
(A) - accredited sampling
(SA) - accredited sampling executed under the subcontract
ŠPP - Standard operation procedure
ND - not detected by given method
LOQ, LQ – limit of quantification
CFU - Colony forming unit
NM - necessary quantity
m - the highest allowed value at the case of one sample
M, c - "M" highest allowed value for the number "c" at the case of 5 sample`s evaluation
* - measurement uncertainty – sampling and analysis – determined by extension coefficient k=2 (with probability of 95%). If sample is taken by the customer uncertainty of sampling is not available.
- uncertainty given in % reflects the uncertainty from the result of measurement.
** - Acceptable to consumers and no abnormal change
SL - analysis laboratory: NZ-Nové Zámky, TR-Turčianske Teplice, RK-Ružomberok, TV-Trebišov

TT - type of test
A - accredited test executed at the own test laboratory
N - non accredited test executed at the own test laboratory
SA - accredited test executed under the subcontract
SN - unaccredited test executed under the subcontract
(TM) - testing outside the laboratory at the customer

Disclaimer: Laboratory is a disclaimer when the information is supplied by the customer (#) and can affect the validity of results. If the sample has been provided by the customer, the results refer to the sample as it was received. Gauges and measuring equipment used for testing were calibrated or attested in accordance with the valid metrological instructions. The above mentioned test results refer to the tested sample only! The result given in this Analytical report and marked as non accredited test shall not be a subject of accreditation. The result given in this Analytical report and marked as sub- delivery is the result of a Subcontractors gauging made under the terms and conditions of a contract concluded eith him. This Analytical report shall not be reproduced except in full colour version, without written approval of the laboratory. SNAS is a Signatory to the Multilateral Agreement MRA ILAC.

Responsible for correctness:

Michaela Ruttkayová
Specialist worker

Worked out by: Zuzana Kubisová

Validity check of document

Eurofins Environment Testing Slovakia s.r.o. - 1 - AKREDITOVANÉ SKÚŠOBNÉ LABORATÓRIUM SNAS č. S-406

Test Certificate approved by

Michaela Ruttkayová
Specialist worker