

# ANALYTICAL CERTIFICATE

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<b>Sample name</b>	<b>AOD-9604</b>
<b>Batch No.</b>	<b>2025248</b>
<b>Sample No.</b>	<b>01</b>
<b>Sequence</b>	Tyr-Leu-Arg-Ile-Val-Gln-Cys-Arg-Ser-Val-Glu-Gly-Ser-Cys-Gly-Phe Disulfide bridge Cys7-Cys14
<b>Manufacturing date</b>	<b>NA</b>
<b>Submitter of analytical request</b>	<b>Particle s.r.o., Slovakia</b>

## 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 5 $\mu$  C4-BIO 300 A, 4.6 x 100 mm  
Serial No 221258

### 1.3 Sample preparation:

The whole amount of AOD-9604 (5 mg) was dissolved in 1 mL of DMSO.  
Injection: 1.0  $\mu$ L

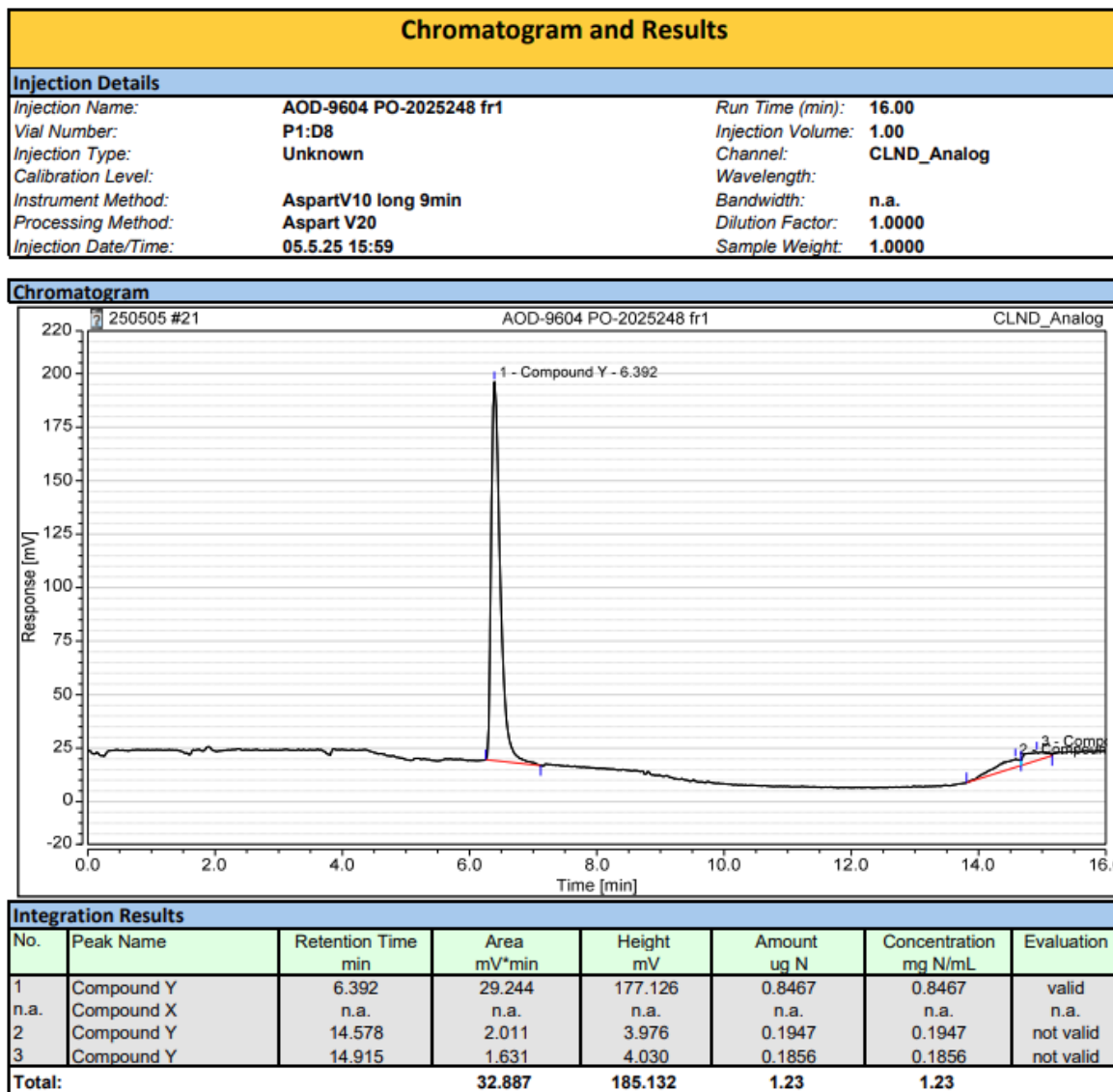
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## 1.4 Chromatograms and calibration curve:

Instrument: CLND-2 Sequence: 250505

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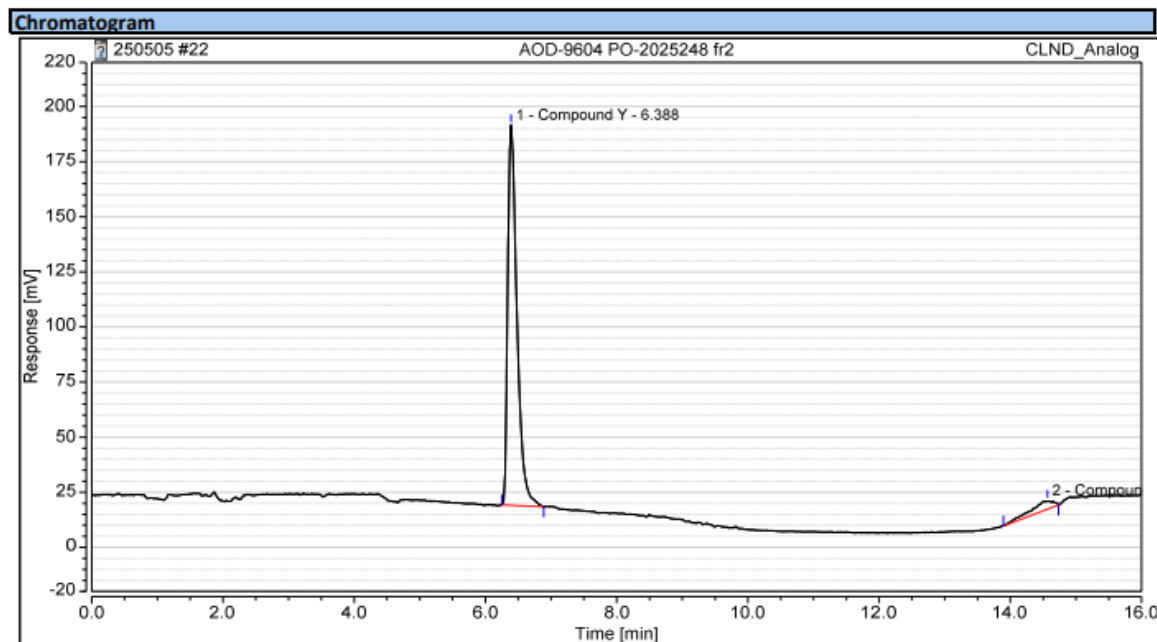
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Instrument:CLND-2 Sequence:250505

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Chromatogram and Results			
Injection Details			
Injection Name:	AOD-9604 PO-2025248 fr2	Run Time (min):	16.00
Vial Number:	P1:D8	Injection Volume:	1.00
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:	05.5.25 16:15	Sample Weight:	1.0000



Integration Results							
No.	Peak Name	Retention Time min	Area mV*min	Height mV	Amount ug N	Concentration mg N/mL	Evaluation
1	Compound Y	6.388	28.049	172.595	0.8181	0.8181	valid
n.a.	Compound X	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
2	Compound Y	14.565	1.684	3.935	0.1869	0.1869	not valid
<b>Total:</b>			<b>29.732</b>	<b>176.531</b>	<b>1.00</b>	<b>1.00</b>	

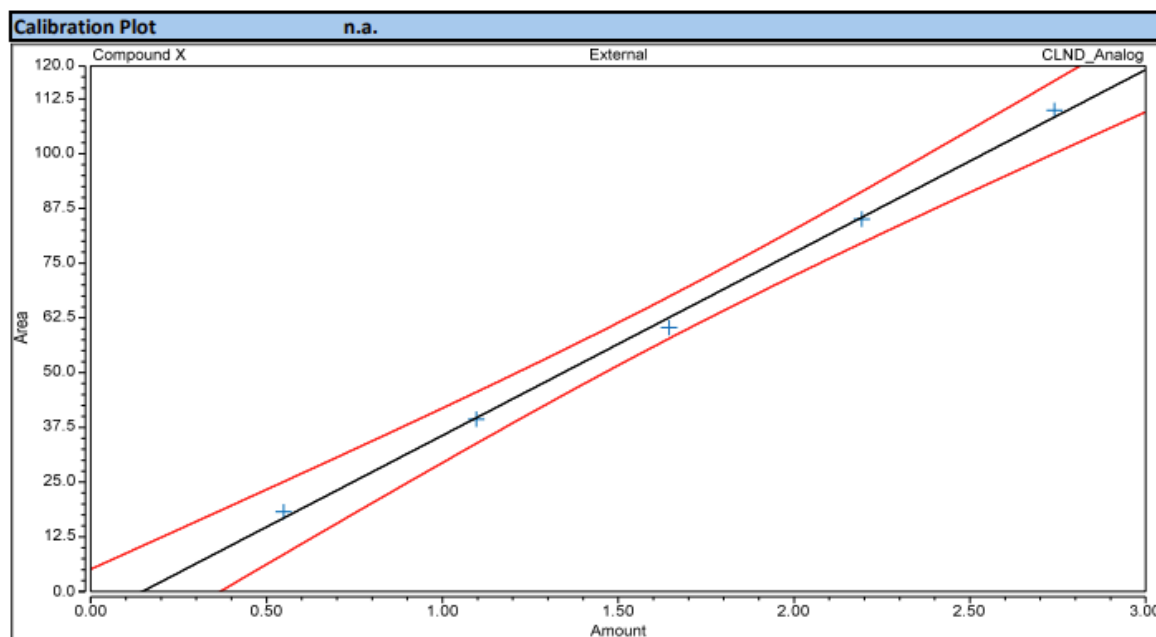
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Instrument:CLND-2 Sequence:250505

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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 Results		n.a.					
No.	Injection Name	Calibration Level	X Value	Y Value	Y Value	Area	Height
			CLND_Analog Compound X	CLND_Analog Compound X	CLND_Analog Compound X	mV*min CLND_Analog Compound X	mV CLND_Analog Compound X
2	Aspart5	1	2.7408	109.8871	109.8871	109.887	651.130
3	Aspart4	1	2.1926	85.0517	85.0517	85.052	516.416
4	Aspart3	1	1.6445	60.2627	60.2627	60.263	345.950
5	Aspart2	1	1.0963	39.3684	39.3684	39.368	243.419
6	Aspart1	1	0.5482	18.2525	18.2525	18.253	104.634

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### 1.4 Results:

NNC: AOD-9604		Salt: AcOH	
MW (calculated) g/mol		N content (calculated) %	N conc. (measured) mg × N/ml
1815,1		17,75	0,8324
Theoretical Volume ml		Lyophilizate amount mg	
1,00		5,00	
Peptide concentration mg/ml		Quantified amount mg	
4,69		4,7	
nmol/ml		nmol	
2584		2 584	
Peptide content assay %			
93,8			

### 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.
AOD-9604	5	48,4	4,7	9,7 %	93,8 %

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### **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:              0.45 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

#### **2.3 Sample preparation:**

An aliquote of AOD-9604 (2,2 mg) was dissolved in 1 mL of MilliQ water.  
Injection:               4.0 µL

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## 2.4 Chromatogram of AOD-9604 (PO-2025248)

### Sample information

#### UPLC5

Channel Description PDA Ch1 214nm@4.8nm

Vial : 1:C,6 Vol. : 4.00 ul

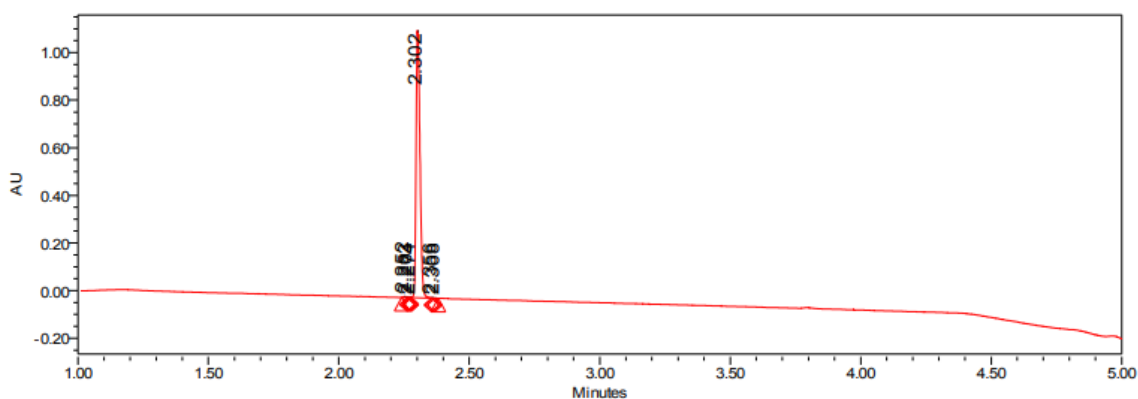
**Sample: AOD-9604 (PO-2025248)**

Date Acquired 4/16/2025 1:24:50 PM CEST

Date Processed 4/16/2025 2:43:14 PM CEST

Acq Method Set :

Gr5\_60\_4mi\_40C\_0\_45ml\_K2\_met\_s



	RT	Area	Height (μV)	% Area
1	2.252	2841	3417	0.24
2	2.302	1156128	1124607	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, 1.7μm, 2.1 x 50 mm column

column oven temp. = 40 °C

## 2.5 Result of purity assessment

The overall purity is 99.51 % at 214 nm.

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### 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 AOD-9604 (2,2 mg) was dissolved in 1 mL of MilliQ water.  
Injection:                      4.0 µL

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## 3.5 Mass Spectra of AOD-9604 (PO-2025248)

### Sample information

#### UPLC1 ZQ

**Sample: AOD-9604 (PO-2025248)**

Channel Description ACQUITY TUV ChA 214nm

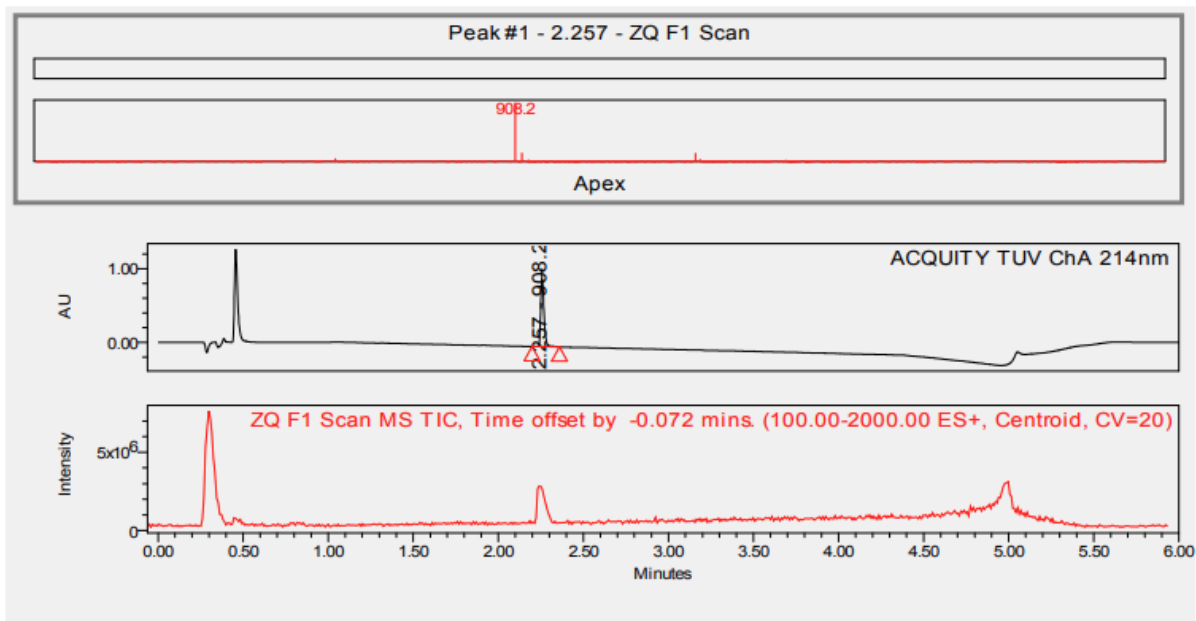
Date Acquired 4/17/2025 9:44:09 AM CEST

Vial : 1:F,2 Vol. : 4.00 ul

Date Processed 4/17/2025 9:59:55 AM CEST

Acq Method Set :

Gr5\_60\_MS\_4min\_0\_45ml\_K2\_me\_s



**MS Result Table**

	Name	RT	Base Peak (m/z)
1		2.257	908.24

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	$[M+2H]^{2+}$	$[M+3H]^{3+}$	$[M+4H]^{4+}$	$[M+5H]^{5+}$	$[M+6H]^{6+}$	$[M+7H]^{7+}$
1813,9	908,0	605,6	454,5	-	-	-
Found	908,2	NA				

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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:

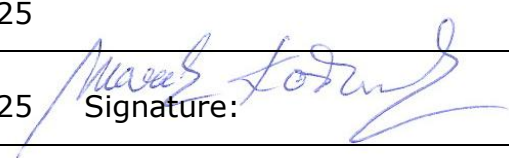
**The sample AOD-9604 (Batch No. 2025248) was analyzed for peptide content, UV purity, identity by MS and endotoxins.**

**Peptide content is 93,8 % (4.7 mg in 5 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.**

<b>ANALYSIS COMPLETED:</b>	Date: 16.04.2025
<b>Issued by QC:</b>	Date: 15.05.2025  Signature:

# Analytical report AR-25-KT-013202-04



## 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: 14.04.2025 Date of Testing: 14.04.2025 - 15.04.2025

Issue date: 28.04.2025

**Sample information:** 104-2025-00014559  
# Sample description: AOD-9604(PO-2025248)  
Material: Peptidy  
Material: Peptidy

## Physical and chemical tests

Parameter	Unit	Allowed Value	Measured Value	Uncertainty of Method measurement*	Testing method	E	SL	TT	
Arsenic (As)	mg/kg	max, 1,5	0,034	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	<0,010	-	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 with 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:

RNDr. Hana Benkovičová  
Deputy Head of Laboratory Turčianske Teplice

Worked out by: Zuzana Kubisová

Validity check of document

**Test Certificate approved by**

RNDr. Hana Benkovičová  
Deputy Head of Laboratory Turčianske Teplice

