

## ANALYTICAL CERTIFICATE

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<b>Sample name</b>	<b>Modified GRF 1-29</b>
<b>Batch No.</b>	<b>2025247</b>
<b>Sample No.</b>	<b>NA</b>
<b>Sequence</b>	Tyr-D-Ala-Asp-Ala-Ile-Phe-Thr-Gln-Ser-Tyr-Arg-Lys-Val-Leu-Ala-Gln-Leu-Ser-Ala-Arg-Lys-Leu-Leu-Gln-Asp-Ile-Leu-Ser-Arg-NH <sub>2</sub>
<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 1120 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

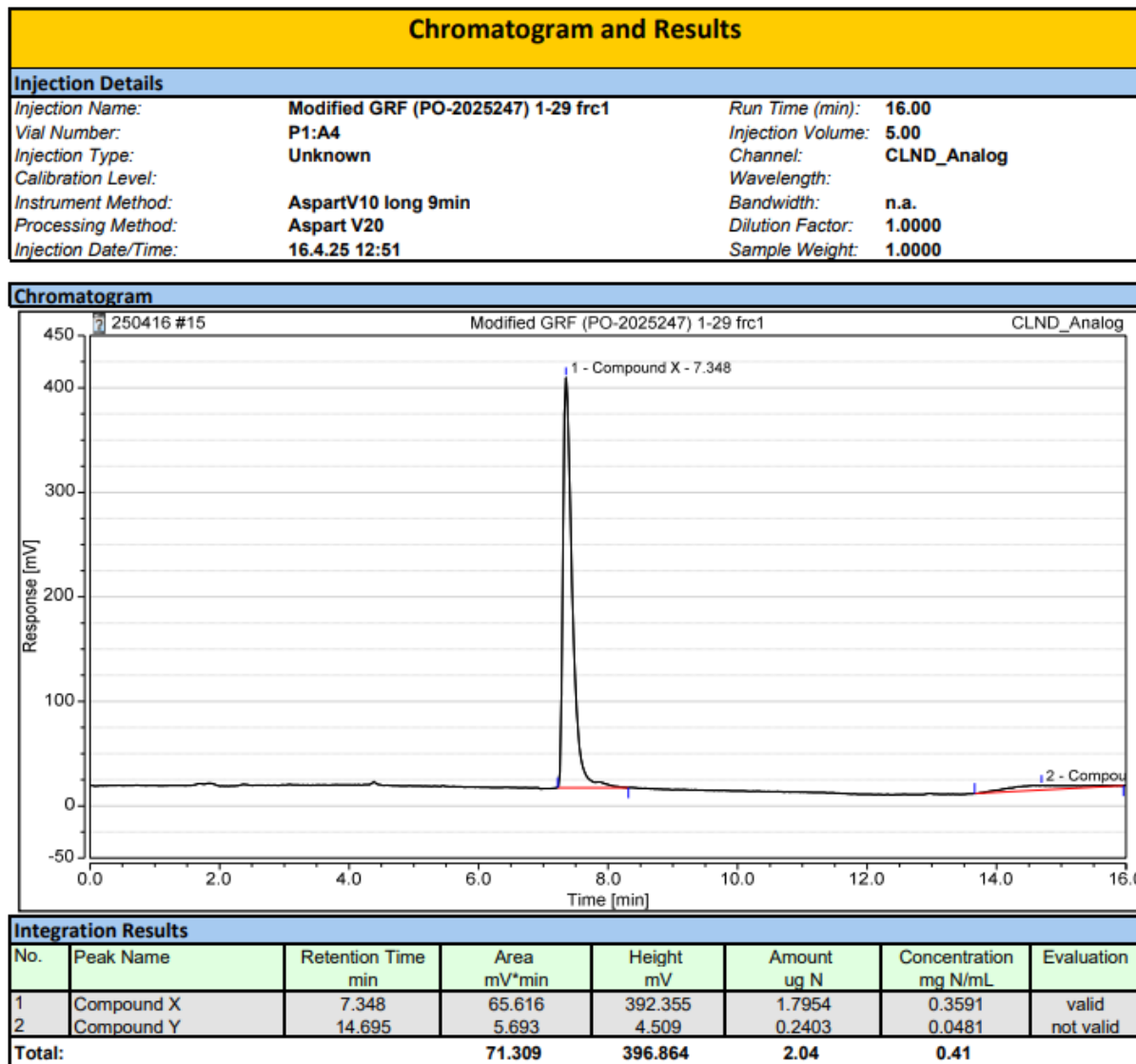
#### 1.3 Sample preparation:

The whole amount of Modified GRF 1-29 (2 mg) was dissolved in 1 mL of DMSO.  
Injection: 5  $\mu$ L

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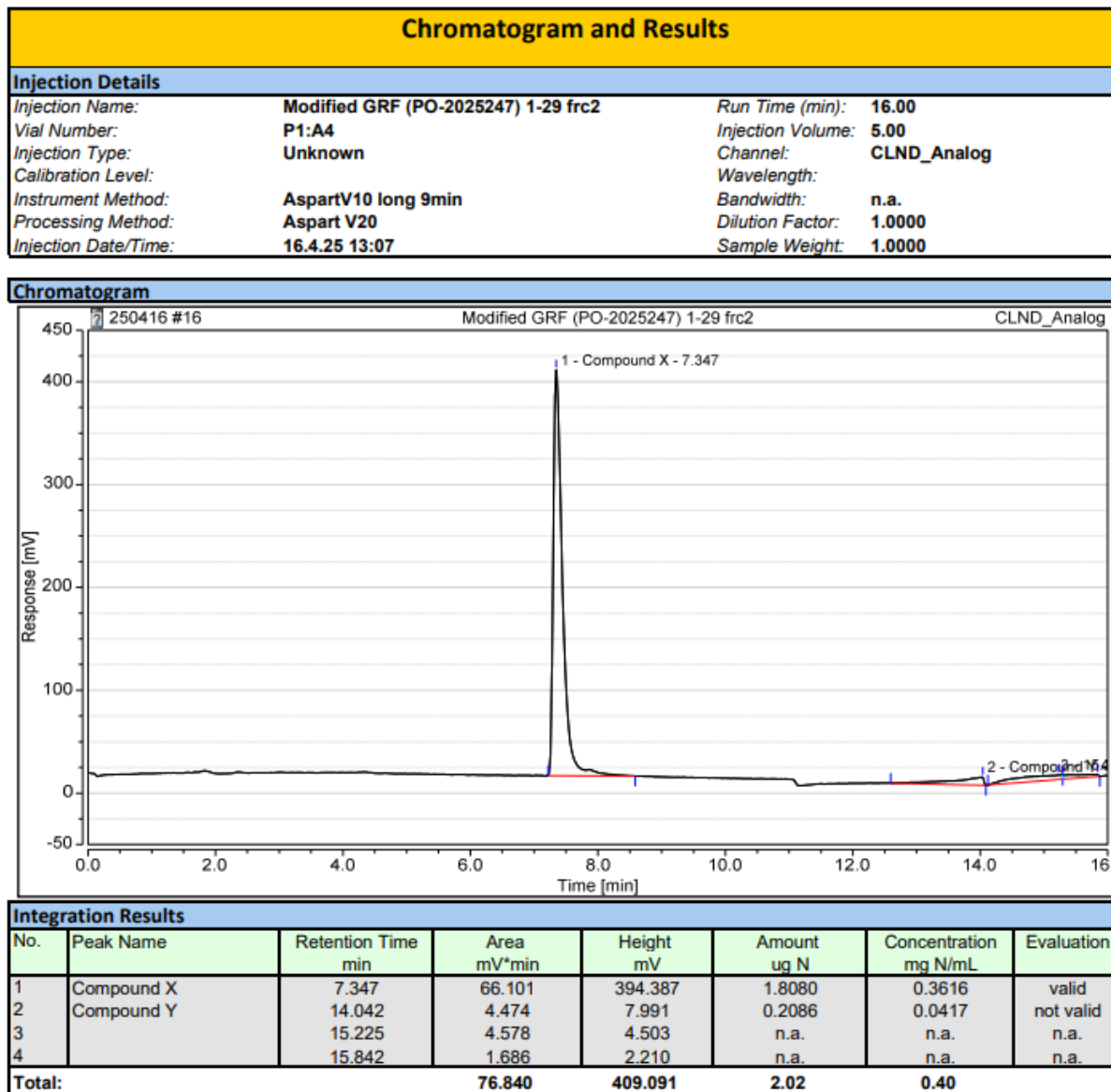
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## 1.4 Chromatograms and calibration curve:



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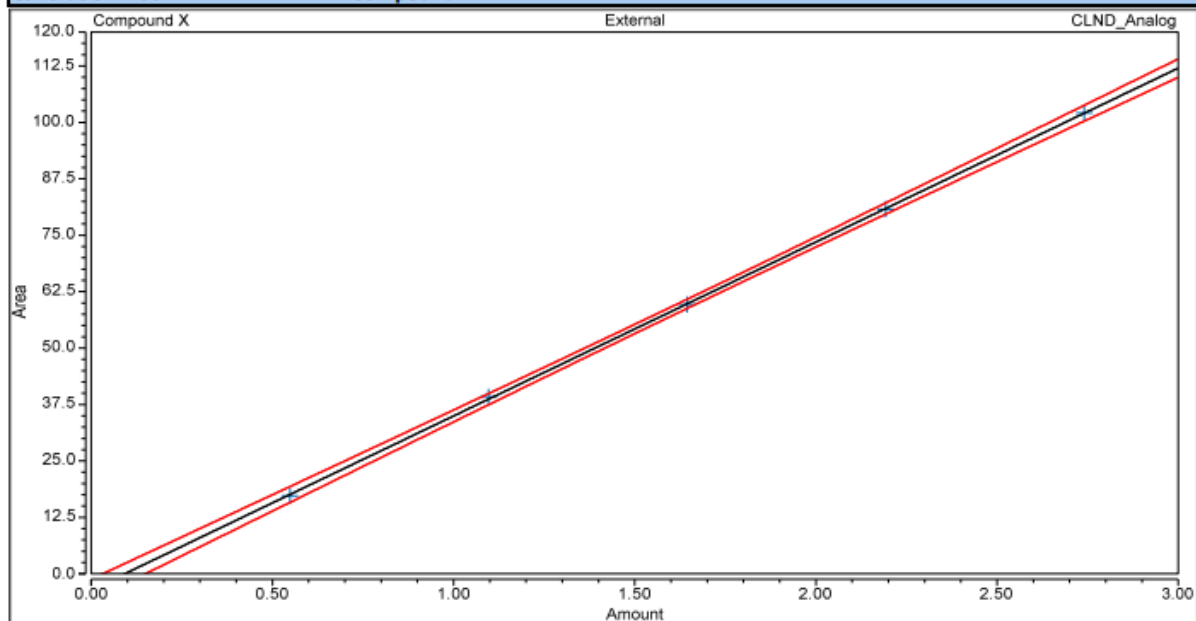
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## Calibration

Calibration Details		Compound X	
Calibration Type	Lin, WithOffset	Offset (C0)	-3.5650
Evaluation Type	Area	Slope (C1)	38.5315
Number of Calibration Points	5	Curve (C2)	0.0000
Number of disabled Calibration Points	0	R-Square	0.9999

## Calibration Plot



Calibration Results		Compound X					
No.	Injection Name	Calibration Level	X Value	Y Value	Y Value	Area mV*min	Height mV
			CLND_Analog Compound X	CLND_Analog Compound X	CLND_Analog Compound X	CLND_Analog Compound X	CLND_Analog Compound X
2	Aspart4	1	2.1926	80.7591	80.7591	80.759	496.121
3	Aspart3	1	1.6445	59.6558	59.6558	59.656	371.578
4	Aspart2	1	1.0963	39.2332	39.2332	39.233	242.457
5	Aspart1	1	0.5482	17.2502	17.2502	17.250	105.652
6	Aspart5	1	2.7408	102.0927	102.0927	102.093	631.759

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### 1.4 Results:

NNC: Modified GRF 1-29 (PO-		Salt:	AcOH
MW (calculated) g/mol	N content (calculated) %	N conc. (measured) mg x N/ml	
3367,95	18,3	0,3604	
Theoretical Volume ml		Lyophilizate amount mg	
1,00		2,00	
Peptide concentration mg/ml                  nmol/ml		Quantified amount mg                          nmol	
1,97	585	2,0	585
Peptide content assay %			
98,5			

### 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.
ModGRF 1-29	2.00	39.80	1.97	4,95 %	98,50 %

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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:                    0.40 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

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

The whole amount of Modified GRF 1-29 (2,2 mg) was dissolved in 1 mL of MilliQ water.  
Injection:                      10.0 µL

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## 2.4 Chromatogram of Modified GRF 1-29 (PO-2025247)

### Sample information

#### UPLC5

Channel Description PDA Ch1 214nm@4.8nm

Vial : 1:C,5 Vol. : 10.00 ul

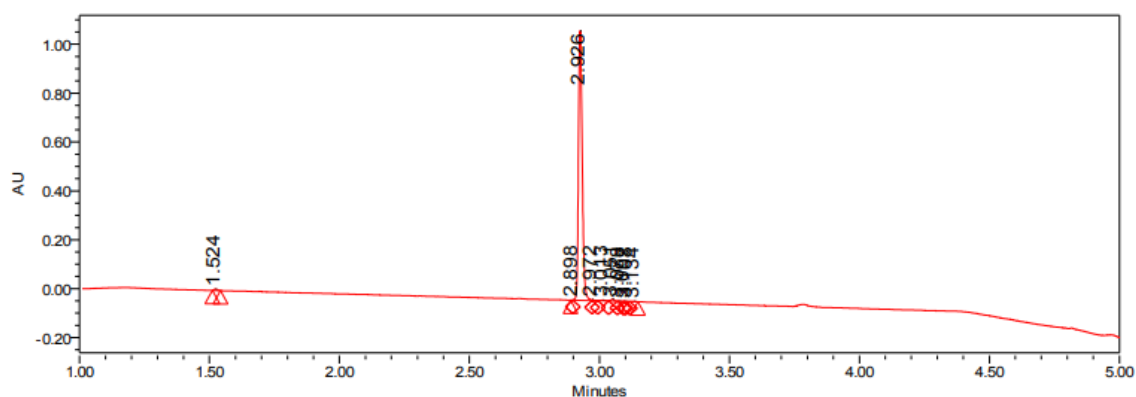
**Sample: Modified GRF 1-29 (PO-2025247)**

Date Acquired 4/16/2025 1:17:49 PM CEST

Date Processed 4/16/2025 2:42:07 PM CEST

Acq Method Set :

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



	RT	Area	Height (μV)	% Area
1	1.524	4737	5266	0.42
2	2.926	1100566	1103865	98.17
3	2.972	2586	3259	0.23
4	3.013	4109	3330	0.37
5	3.051	6767	8073	0.60

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 own temp. = 40 °C

## 2.5 Result of purity assessment

The overall purity is 98.17 % 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:

The whole amount of Modified GRF 1-29 (2,2 mg) was dissolved in 1 mL of MilliQ water.  
Injection:                      10.0 µL

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## 3.5 Mass Spectra of Modified GRF 1-29 (PO-2025247)

### Sample information

#### UPLC1 ZQ

**Sample: Modified GRF 1-29 (PO-2025247)**

Channel Description ACQUITY TUV ChA 214nm

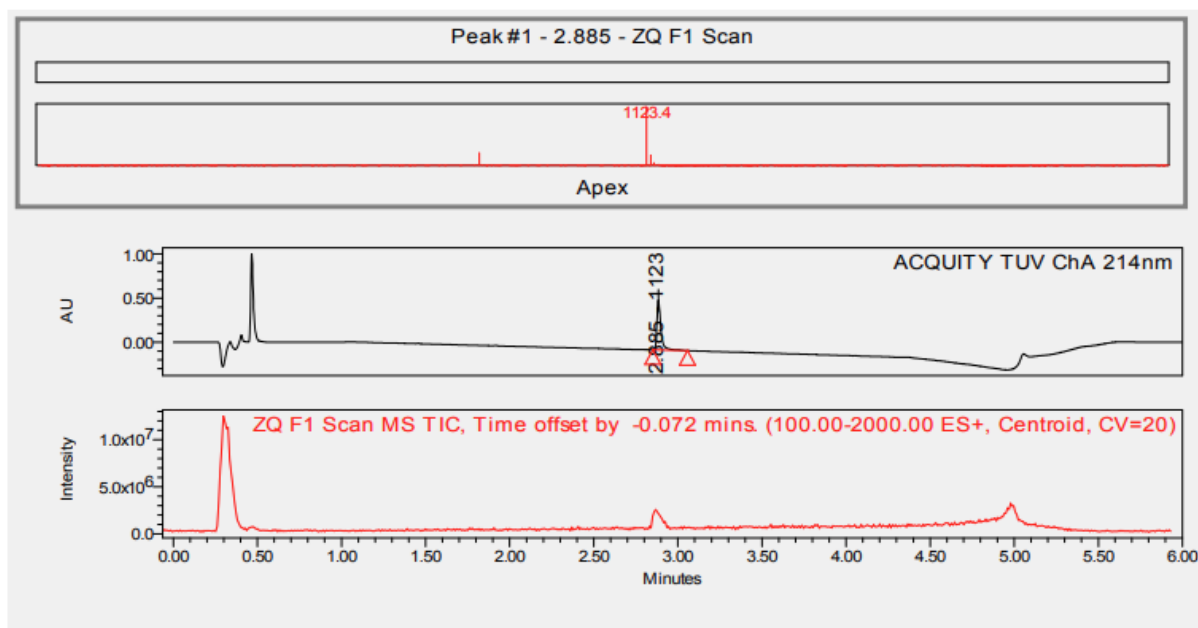
Date Acquired 4/17/2025 9:37:06 AM CEST

Vial : 1:F,1 Vol. : 10.00 ul

Date Processed 4/17/2025 9:59:09 AM CEST

Acq Method Set :

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



**MS Result Table**

	Name	RT	Base Peak (m/z)
1		2.885	1123.42

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] <sup>2+</sup>	[M+3H] <sup>3+</sup>	[M+4H] <sup>4+</sup>	[M+5H] <sup>5+</sup>	[M+6H] <sup>6+</sup>	[M+7H] <sup>7+</sup>
3366,9	1684,5	1123,3	842,8	674,4	562,2	482,0
Found	NA	1123,4				

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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:

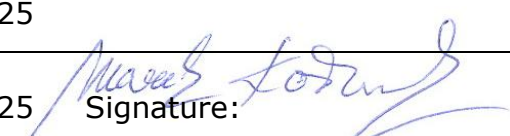
**The sample Modified GRF 1-29 (Batch No. 2025247) was analyzed for peptide content, UV purity, identity by MS and endotoxins**

**Peptide content is 98.50 % (1.97 mg in 2 mg)**

**Purity is 98.17 % (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: 17.04.2025 Signature: 

## Analytical report AR-25-KT-013203-02



## 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: 14.04.2025 Date of Testing: 14.04.2025 - 15.04.2025

Issue date: 15.04.2025

## Sample information: 104-2025-00014560

# Sample description: ModGRF 1-29  
 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	-	<1,5	-	ICP-MS	-	TR	A
Cadmium (Cd)	mg/kg	-	<0,2	-	ICP-MS	-	TR	A
Lead (Pb)	mg/kg	-	<0,5	-	ICP-MS	-	TR	A
Mercury (Hg)	mg/kg	-	<0,3	-	ICP-MS	-	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:

Michaela Ruttkayová  
Specialist worker

Worked out by: Zuzana Kubisová

Validity check of document

**Test Certificate approved by**Michaela Ruttkayová  
Specialist worker