

ANALYTICAL CERTIFICATE

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Sample name	Thymosin beta-4
Batch No.	2025238
Sample No.	NA
Sequence	Ac-Ser-Asp-Lys-Pro-Asp-Met-Ala-Glu-Ile-Glu-Lys-Phe-Asp-Lys-Ser-Lys-Leu-Lys-Lys-Thr-Glu-Thr-Gln-Glu-Lys-Asn-Pro-Leu-Pro-Ser-Lys-Glu-Thr-Ile-Glu-Gln-Glu-Lys-Gln-Ala-Gly-Glu-Ser
Manufacturing date	NA
Submitter of analytical request	Particle s.r.o., Slovakia

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
10	10	80	10
11	10	80	10
12	90	0	10
16	90	0	10

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

1.3 Sample preparation:

The whole amount of Thymosin beta-4 (10 mg) was dissolved in 2 mL of H₂O.
Injection: 2 μ L

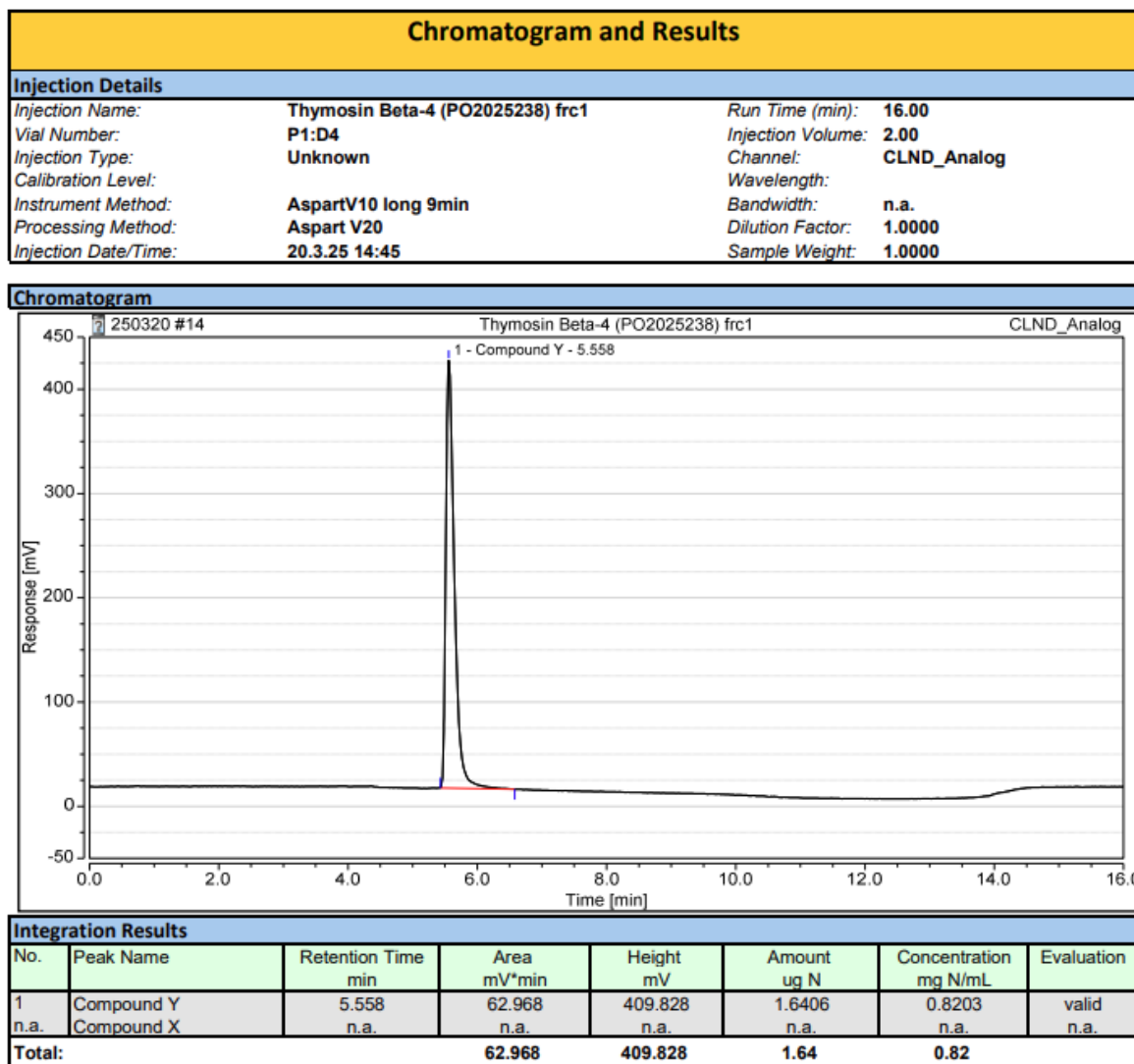
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1.4 Chromatograms and calibration curve:

Instrument:CLND-2 Sequence:250320

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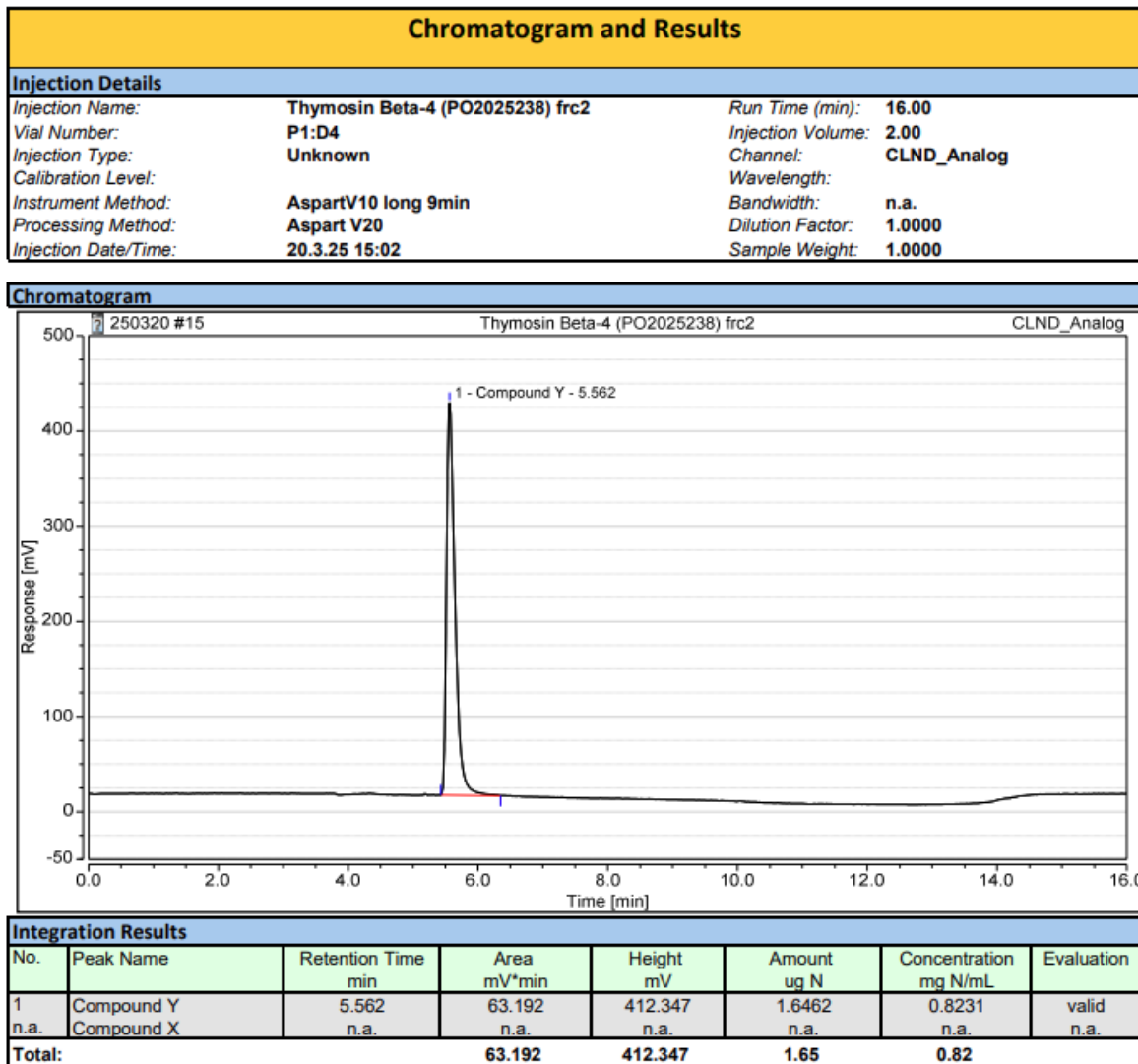


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Instrument: CLND-2 Sequence: 250320

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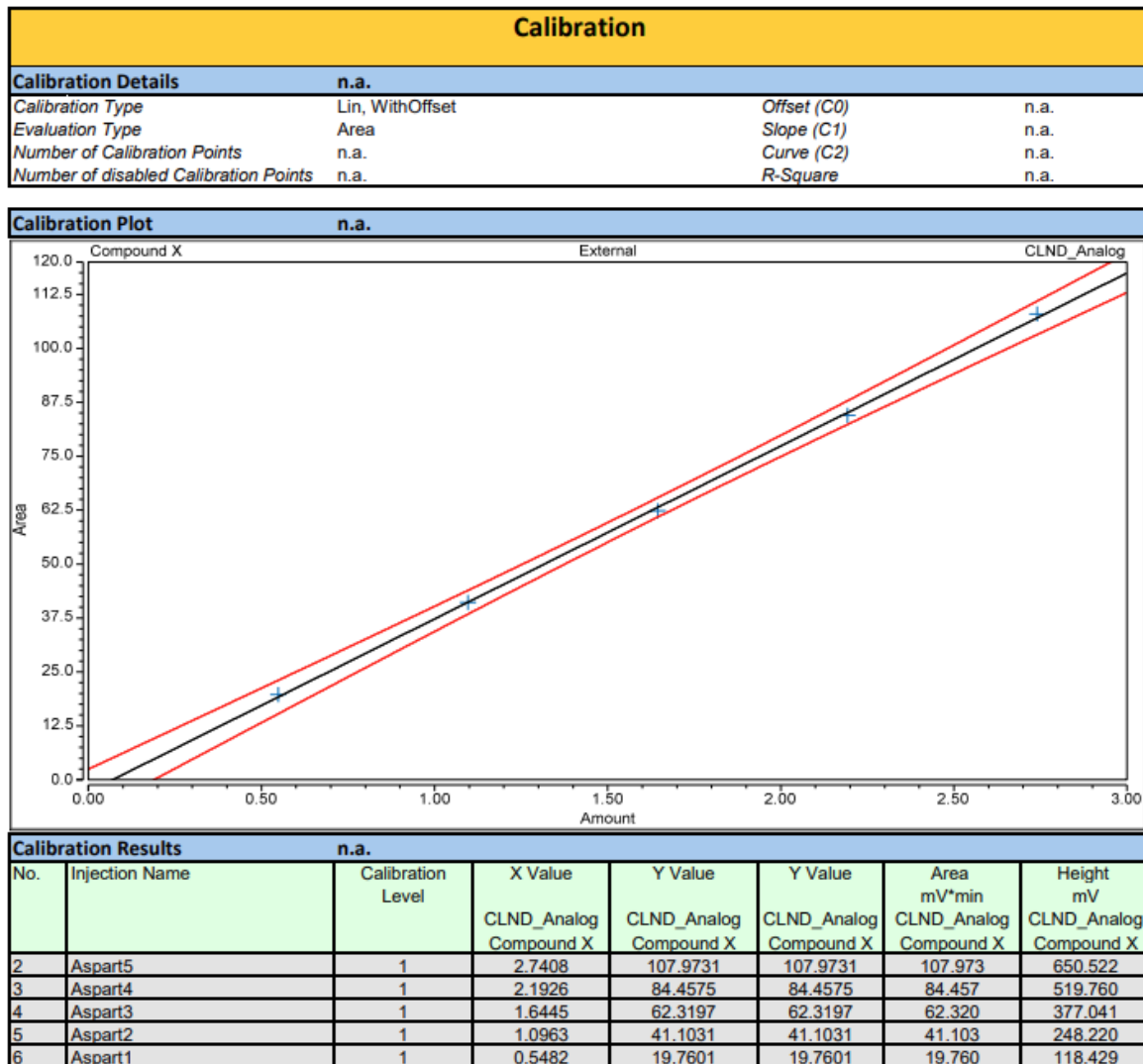


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Instrument: CLND-2 Sequence: 250320

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1.4 Results:

NNC: Thymosin Beta-4 (PO-2)		Salt:	0
MW <i>(calculated)</i> g/mol	N content <i>(calculated)</i> %	N conc. <i>(measured)</i> mg × N/ml	
4963,51	15,8	0,8217	
Theoretical Volume ml		Lyophilizate amount mg	
2,00		10,00	
Peptide concentration mg/ml nmol/ml		Quantified amount mg nmol	
5,20 1048		10,4 2 096	
Peptide content assay %			
104,0			

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.
Thymosin β-4	10	NA	10,4	NA	104,0 %

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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 aliquot of Thymosin beta-4 (1 mg) was dissolved in 1 mL of H₂O.
Injection: 2.4 µL

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2.4 Chromatogram of Thymosin Beta-4 (PO-2025238)

Sample information

UPLC5

Channel Description PDA Ch1 214nm@4.8nm

Vial : 1:C,4 Vol. : 2.40 ul

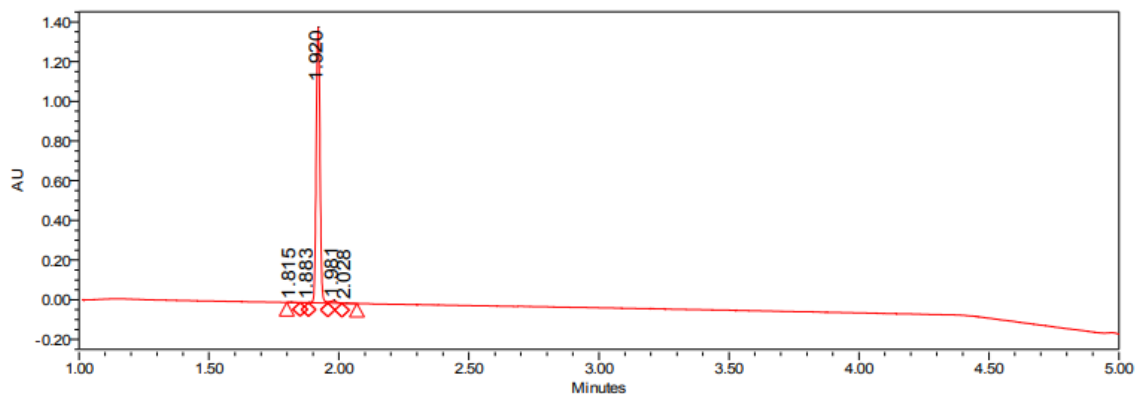
Sample: Thymosin Beta-4 (PO-2025238)

Date Acquired 3/18/2025 4:33:17 PM CET

Date Processed 3/19/2025 2:17:55 PM CET

Acq Method Set :

Gr5_60_4mi_40C_0_45ml_K2_met_s



	RT	Area	Height (μV)	% Area
1	1.815	2514	2366	0.17
2	1.920	1413133	1390784	97.74
3	1.981	24286	14372	1.68
4	2.028	4782	2387	0.33

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 purity is 97.74 % 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
Ionization 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 aliquot of Thymosin beta-4 (1 mg) was dissolved in 1 mL of H₂O.
Injection: 3.8 µL

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3.5 Mass Spectra of Thymosin Beta-4 (PO 2025238)

UPLC1 ZQ

Sample: Thymosin Beta-4 (PO-2025238)

Channel Description ACQUITY TUV ChA 214nm

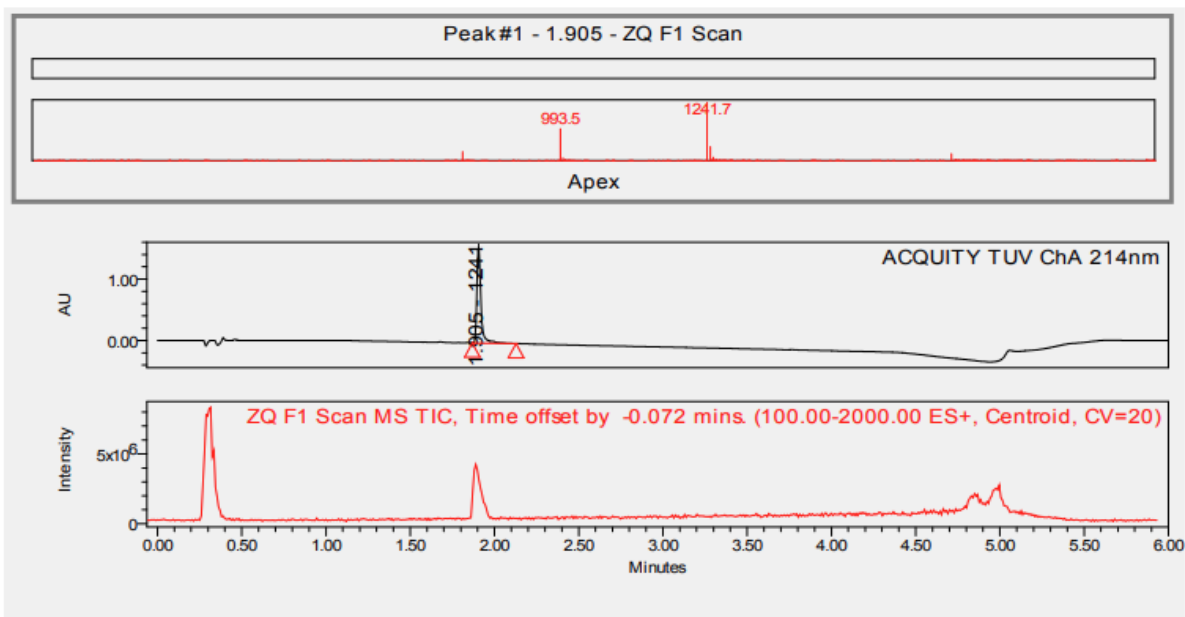
Date Acquired 3/27/2025 8:42:42 AM CET

Vial : 1:F,1 Vol. : 3.80 ul

Date Processed 3/27/2025 9:51:34 AM CET

Acq Method Set :

Gr5_60_MS_4min_0_45ml_K2_me_s



MS Result Table

	Name	RT	Base Peak (m/z)
1		1.905	1241.65

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⁺
4963,5	2482,3	1655,2	1241,6	993,5	828,1	709,9
Found			1241,7	993,5		

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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:

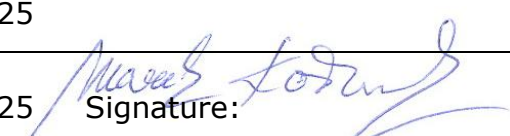
The sample Thymosin Beta-4 (Batch No. 2025238) was analyzed for peptide content, UV purity, identity and endotoxins.

Peptide content is 104.0 % (10,4 mg in 10 mg)

Purity is 97.74 % (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: 19.03.2025
Issued by QC:	Date: 27.03.2025 Signature: 

Analytical report AR-25-KT-009249-03

**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: 14.03.2025 **Date of Testing:** 14.03.2025 - 20.03.2025

Issue date: 27.03.2025

Sample information:

104-2025-00009806

Sample description: Thymosin Beta-4 (PO-2025238)
 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	-	<1,50	-	ICP-MS	-	TR	A
Cadmium (Cd)	mg/kg	-	<0,20	-	ICP-MS	-	TR	A
Lead (Pb)	mg/kg	-	<0,50	-	ICP-MS	-	TR	A
Mercury (Hg)	mg/kg	-	<0,30	-	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:

Andrea Podušelová
Technician worker

Worked out by: Zuzana Kubisová

Validity check of document

**Test Certificate approved by**Andrea Podušelová
Technician worker