

ANALYTICAL CERTIFICATE

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Sample name	Thymosin Alpha 1
Batch No.	2025234
Sample No.	01
Sequence	Ac-Ser-Asp-Ala-Ala-Val-Asp-Thr-Ser-Ser-Glu-Ile-Thr-Thr-Lys-Asp-Leu-Lys-Glu-Lys-Lys-Glu-Val-Val-Glu-Glu-Ala-Glu-Asn-OH
Manufacturing date	NA
Submitter of analytical request	UAB Nauja oda eshop, Lithuania

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 μ C4-BIO 300 A, 4.6 x 100 mm
Serial No 221258

1.3 Sample preparation:

The whole amount of Thymosin Alfa 1 (5 mg) was dissolved in 1 mL of water:MeCN (70:30, pH 8.0).

Injection: 1 μ L

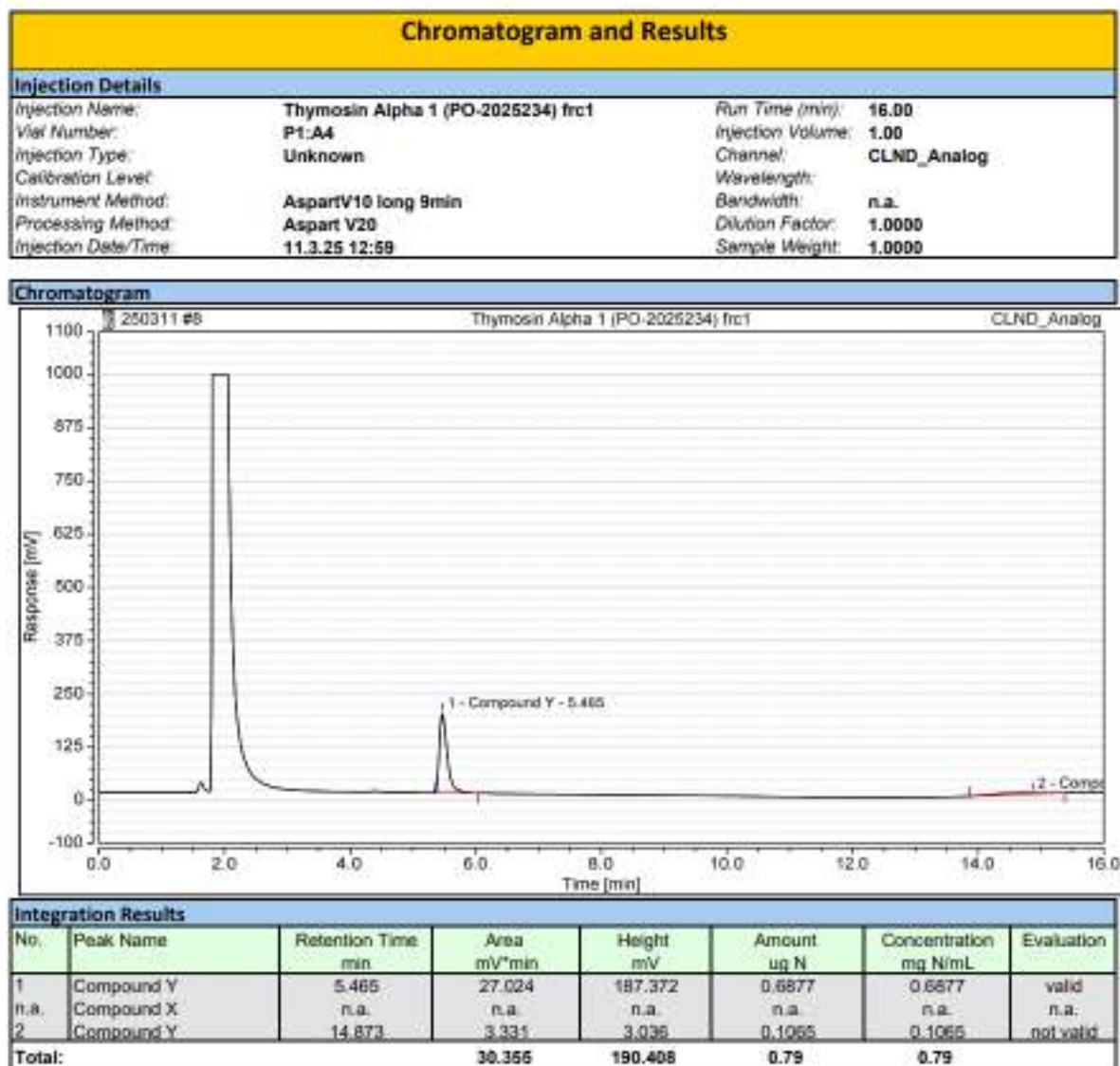
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1.4 Chromatograms and calibration curve:

Instrument: CLND-2 Sequence: 250311

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Instrument: CLND-7 Sequence: 250311

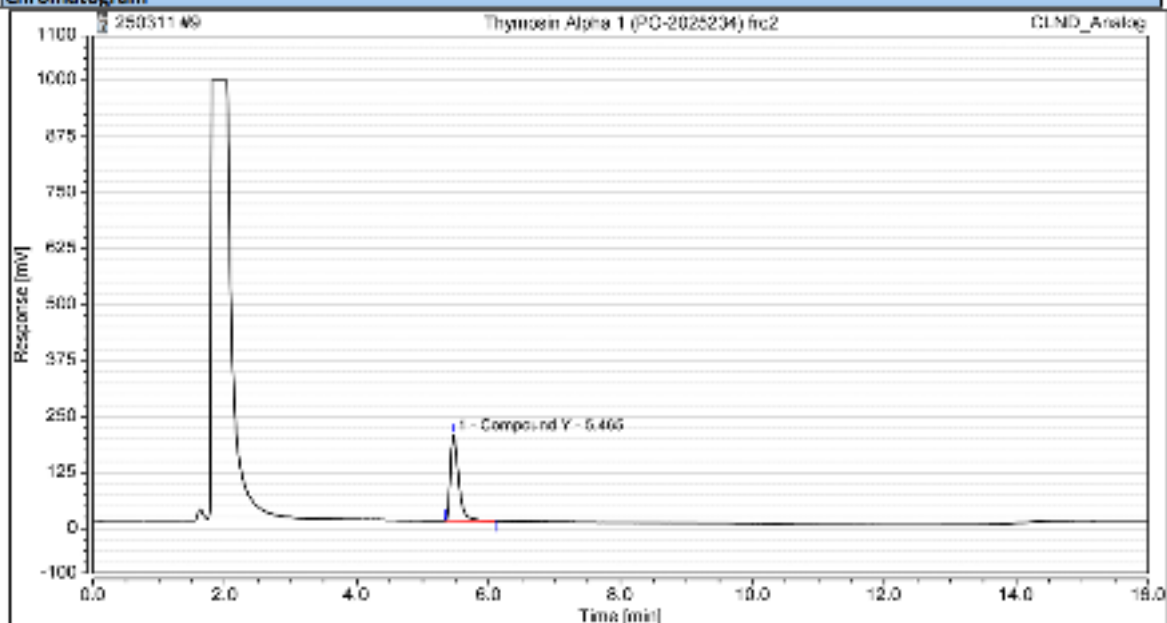
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Chromatogram and Results

Injection Details

Injection Name:	Thymosin Alpha 1 (PO-2025234) fr2	Run Time (min):	16.00
Vial Number:	P1-A4	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:	11.3.25 13:16	Sample Weight:	1.0000

Chromatogram



Integration Results

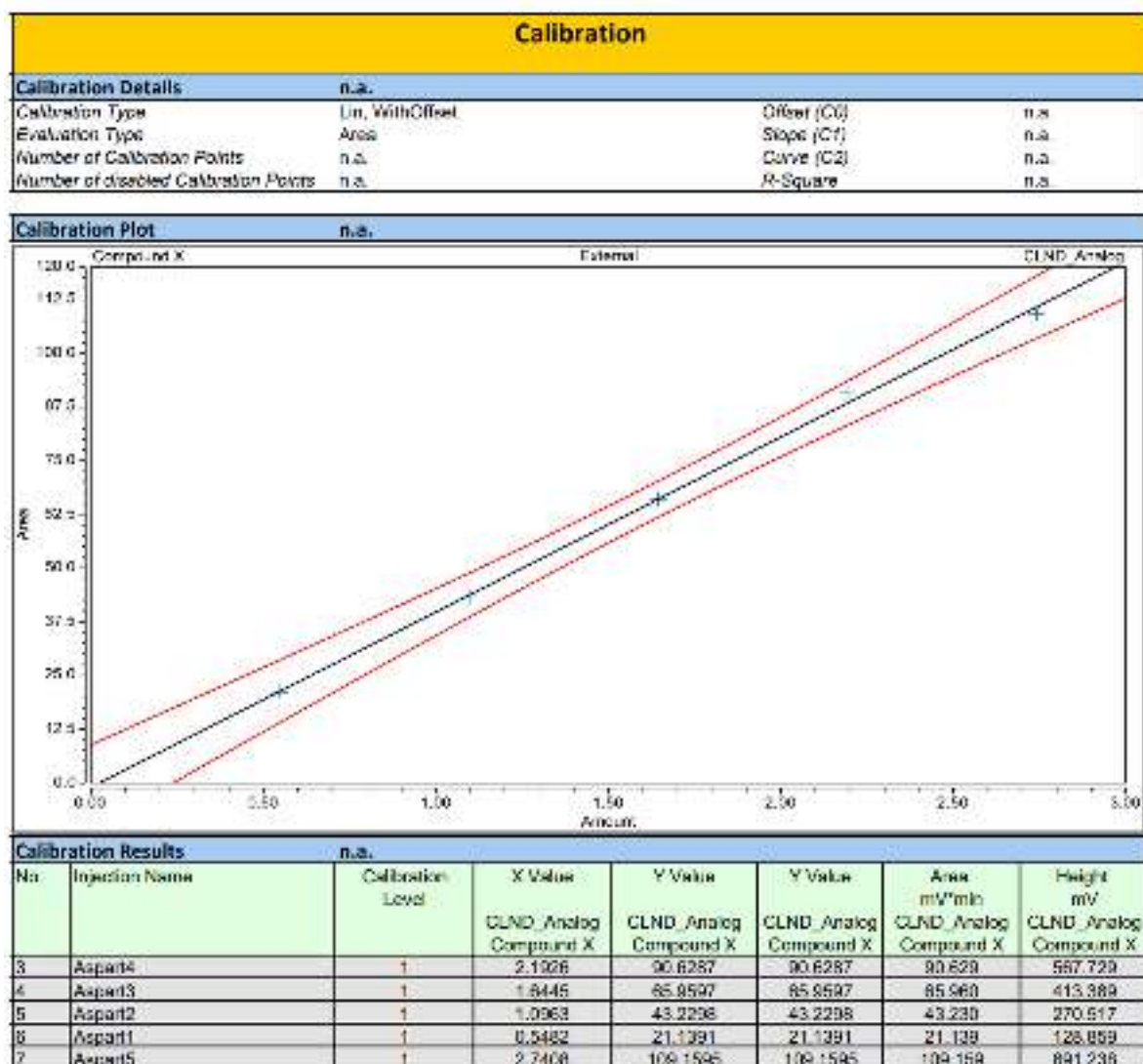
No.	Peak Name	Retention Time min	Area mV*min	Height mV	Amount ug N	Concentration mg N/ml	Evaluation
1	Compound Y	5.465	27.427	190.827	0.6976	0.6976	valid
n.a.	Compound X	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Total:			27.427	190.827	0.70	0.70	

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Instrument CLND-2 Sequence:250811

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1.4 Results:

NNC: Thymosin Alpha 1 (PO-;		Salt:	0
MW (calculated) g/mol	N content (calculated) %	N conc. (measured) mg × N/ml	
3108,32	14,87	0,6927	
Theoretical Volume ml		Lyophilizate amount mg	
1,00		5,00	
Peptide concentration mg/ml		Quantified amount mg	
4,66	1499	4,7	1 499
Peptide content assay %			
93,2			

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 Alpha 1	5	NA	4.7	NA	93,2 %

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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 Thymosin Alfa 1 (1 mg) was dissolved in 1 mL of water:MeCN
(70:30, pH 8.0).

Injection: 1,6 µL

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2.4 Chromatogram of Thymosin Alpha 1 (PO-2025234)

Sample Information

UPLC5

Channel Description PDA Ch1 214nm@4.8nm

Vial: 1-C1 Vol.: 1.60 ul

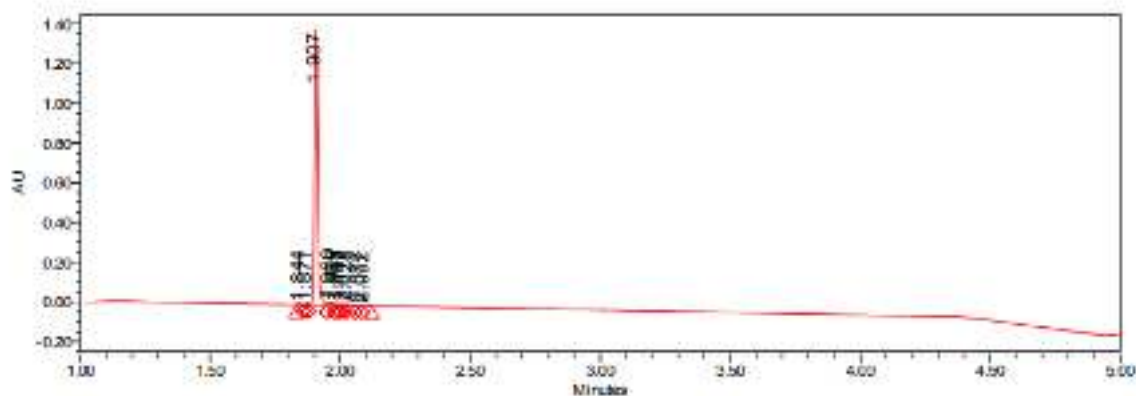
Sample: Thymosin Alpha 1 (PO-2025234)

Date Acquired 3/18/2025 4:12:33 PM CET

Date Processed 3/19/2025 2:13:19 PM CET

Acq Method Set:

Gr5_60_4mi_40C_0_45ml_K2_met_s



	RT	Area	Height (pM)	% Area
1	1.844	2949	4199	0.23
2	1.871	9953	4950	0.29
3	1.907	1229702	1285006	96.43
4	1.960	12050	9403	0.94
5	1.979	6105	5940	0.48
6	1.989	2707	4670	0.21
7	2.012	5317	5547	0.42
8	2.028	7170	4591	0.56
9	2.077	2804	2206	0.22
10	2.082	3720	2461	0.29

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 oven temp. = 40 °C

2.5 Result of purity assessment

The overall purity is 96.43 % at 214 nm.

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3. Endotoxin test:

3.1 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

3.2 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).

3.3 Result:

NEGATIVE (-)

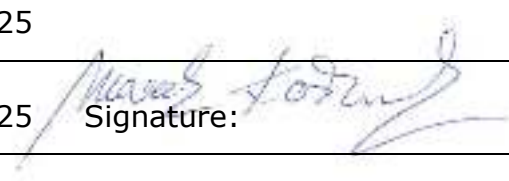
CONCLUSION:

The sample Thymosin Alpha 1 (Batch No. 2025234) was analyzed for peptide content, UV purity and endotoxins.

Peptide content is 93.2 % (4.7 mg in 5 mg)

Purity is 96.43 % (UPLC at 214 nm).

Endotoxin test (sensitivity level 5 EU/mg) - NEGATIVE.

ANALYSIS COMPLETED:	Date: 19.03.2025
Issued by QC:	Date: 26.03.2025  Signature:

Analytical report AR-25-KT-009257-03



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:

UAB Nauja oda eshop
 A. Goštauto g. 8-215
 LT-01108 Vilnius
 LITHUANIA

Date of Sample Receipt: 17.03.2025 Date of Testing: 17.03.2025 - 20.03.2025

Issue date: 27.03.2025

Information about Sampling:

Sampler: customer

Sample information: 104-2025-00009986

Sample description: Thymosin Alpha 1 (PO-2025234)

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	LS-PP-CH-85	-	TR A
Cadmium (Cd)	mg/kg	-	<0,20	-	ICP-MS	LS-PP-CH-85	-	TR A
Lead (Pb)	mg/kg	-	<0,50	-	ICP-MS	LS-PP-CH-85	-	TR A
Mercury (Hg)	mg/kg	-	<0,30	-	ICP-MS	LS-PP-CH-85	-	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.

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Andrea Podušelová
Technician worker

Worked out by: Zuzana Kubisová

Validity check of document



Test Certificate approved by

Andrea Podušelová
Technician worker