

## ANALYTICAL CERTIFICATE

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|                           |                        |
|---------------------------|------------------------|
| <b>Sample name</b>        | <b>Thymosin Alfa 1</b> |
| <b>Batch No.</b>          | <b>2023165</b>         |
| <b>Sample No.</b>         | <b>01</b>              |
| <b>Specification</b>      | <b>NA</b>              |
| <b>Manufacturing date</b> | <b>NA</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 Thymosin Alfa 1 (5 mg) was dissolved in 1 mL of water:MeCN (70:30, pH 8.0).

Injection: 1  $\mu$ L

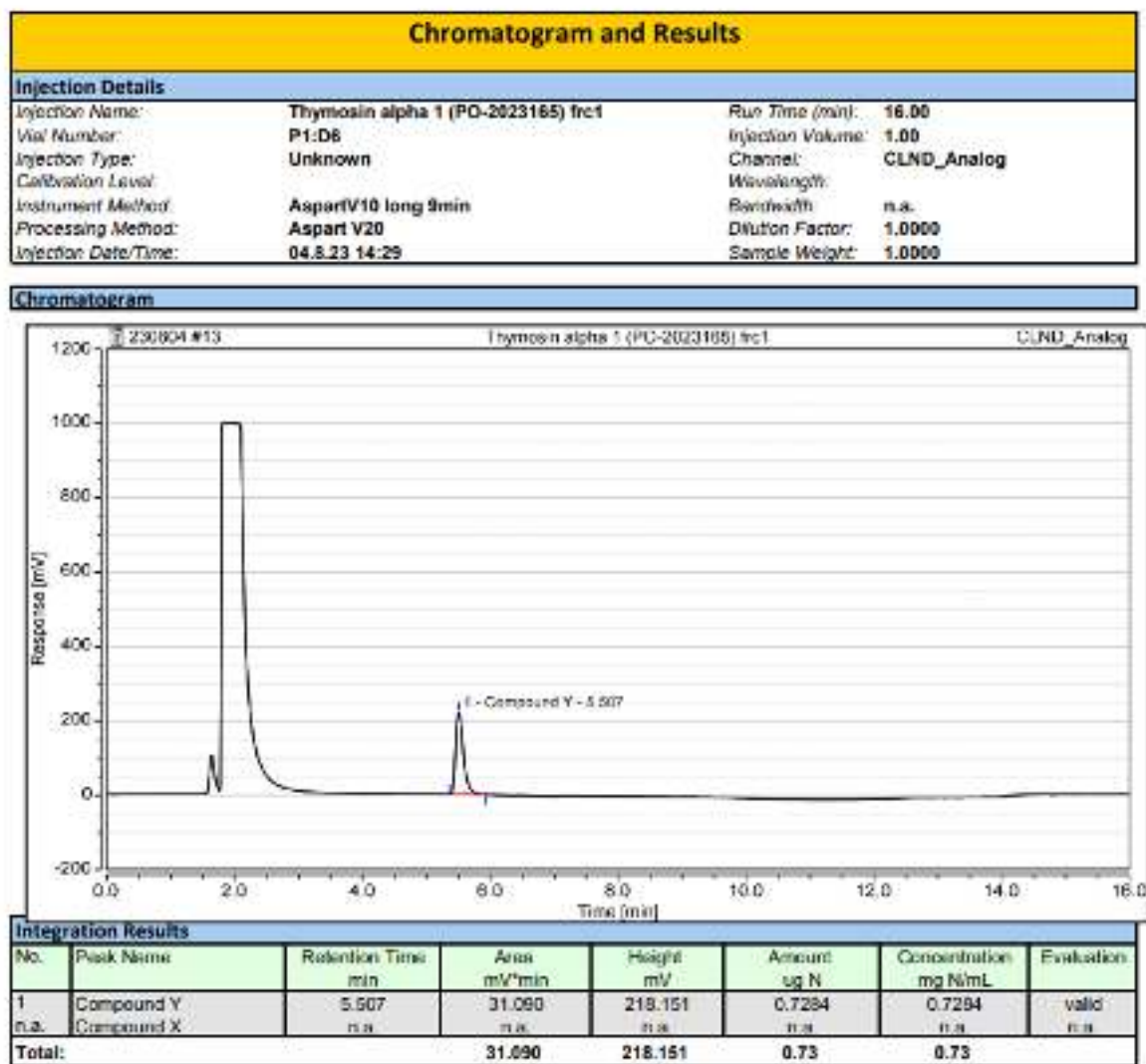
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## 1.4 Chromatograms and calibration curve:

Instrument: CLND-2 Sequence: 230804

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Instrument: CLND-2 Sequence: 230804

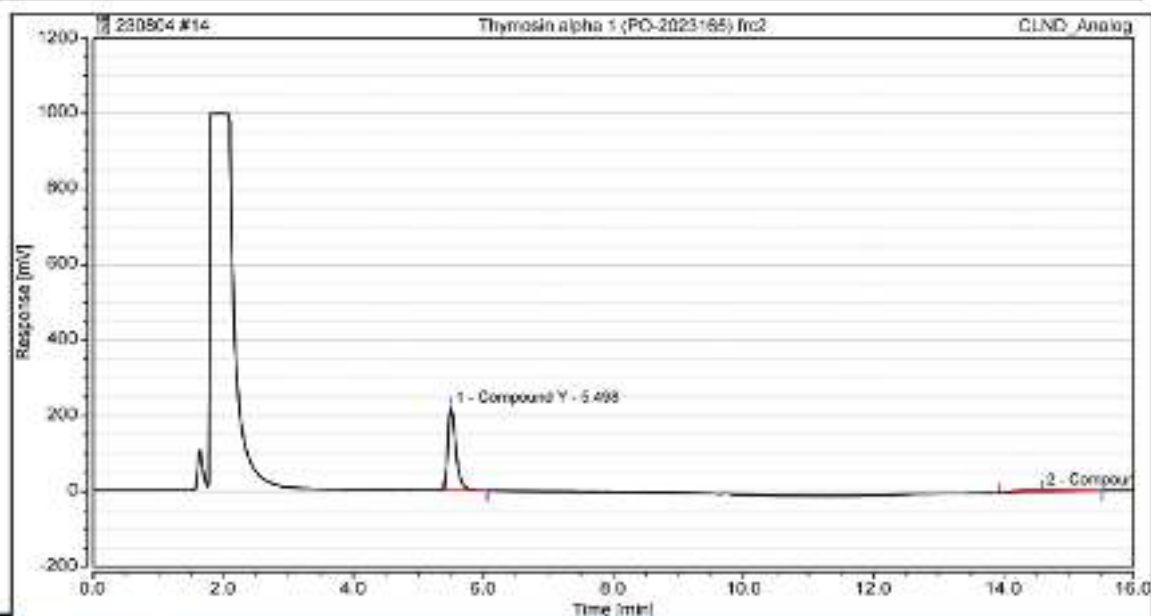
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## Chromatogram and Results

### Injection Details

|                      |                                    |                   |             |
|----------------------|------------------------------------|-------------------|-------------|
| Injection Name:      | Thymosin alpha 1 (PO-2023165) frc2 | Run Time (min):   | 16.00       |
| Vial Number:         | P1:D6                              | 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: | 04.8.23 14:45                      | Sample Weight:    | 1.0000      |

### Chromatogram



### Integration Results

| No.    | Peak Name  | Retention Time<br>min | Area<br>mV*min | Height<br>mV | Amount<br>ug N | Concentration<br>mg Ni/mL | Evaluation |
|--------|------------|-----------------------|----------------|--------------|----------------|---------------------------|------------|
| 1      | Compound Y | 5.498                 | 31.574         | 218.928      | 0.7378         | 0.7378                    | valid      |
| n.a.   | Compound X | n.a.                  | n.a.           | n.a.         | n.a.           | n.a.                      | n.a.       |
| 2      | Compound Y | 14.592                | 4.692          | 5.001        | 0.2111         | 0.2111                    | not valid  |
| Total: |            |                       | 36.266         | 224.019      | 0.95           | 0.95                      |            |

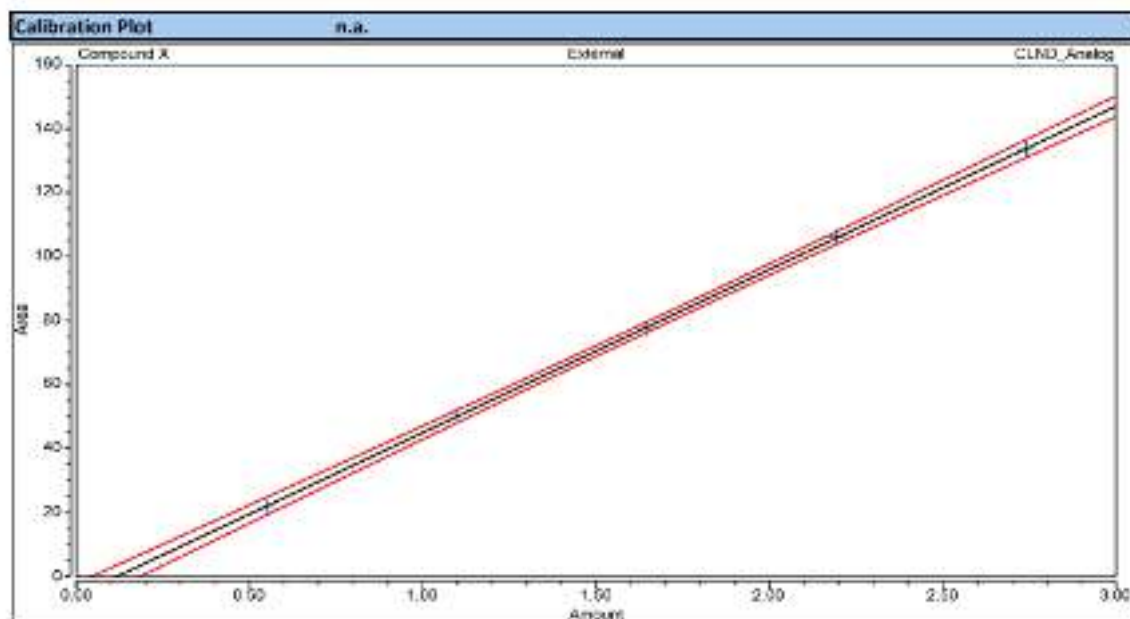
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Instrument: CLND-2 Sequence: 230804

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| Calibration                           |                 |             |      |
|---------------------------------------|-----------------|-------------|------|
| <b>Calibration Details</b>            |                 |             |      |
| Calibration Type                      | n.a.            | Offset (C0) | n.a. |
| Evaluation Type                       | Lin. WithOffset | 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<br>Compound X | CLND_Analog<br>Compound X | CLND_Analog<br>Compound X | mV*min<br>CLND_Analog<br>Compound X | mV<br>CLND_Analog<br>Compound X |
| 2                   | Aspart6        | 1                 | 2.7408                    | 133.5080                  | 133.5080                  | 133.500                             | 833.653                         |
| 3                   | Aspart4        | 1                 | 2.1028                    | 106.2189                  | 106.2189                  | 106.219                             | 872.482                         |
| 4                   | Aspart3        | 1                 | 1.6446                    | 77.4350                   | 77.4350                   | 77.436                              | 480.636                         |
| 5                   | Aspart2        | 1                 | 1.0963                    | 50.5943                   | 50.5943                   | 50.594                              | 318.098                         |
| 6                   | Aspart1        | 1                 | 0.5482                    | 21.4489                   | 21.4489                   | 21.450                              | 136.841                         |

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### 1.4 Results:

| NNC: Thymosin Alpha 1 (PO-;                             |                                | Salt:                                                 | 0     |
|---------------------------------------------------------|--------------------------------|-------------------------------------------------------|-------|
|                                                         |                                |                                                       |       |
| MW<br>(calculated)<br>g/mol                             | N content<br>(calculated)<br>% | N conc.<br>(measured)<br>mg × N/ml                    |       |
| 3108,32                                                 | 14,87                          | 0,7331                                                |       |
|                                                         |                                |                                                       |       |
| Theoretical Volume<br>ml                                |                                | Lyophilizate amount<br>mg                             |       |
| 1,00                                                    |                                | 5,00                                                  |       |
|                                                         |                                |                                                       |       |
| Peptide concentration<br>mg/ml                  nmol/ml |                                | Quantified amount<br>mg                          nmol |       |
| 4,93                                                    | 1586                           | 4,9                                                   | 1 586 |
|                                                         |                                |                                                       |       |
| Peptide content assay<br>%                              |                                |                                                       |       |
| 98,6                                                    |                                |                                                       |       |

### Summary table:

| Peptide         | Aliquoting<br>(mg) | Total weight of<br>sample (mg) | Content of the<br>peptide by CLND<br>(mg) | Content of the peptide<br>in the sample (%) | Content of the peptide against<br>the amount on label. |
|-----------------|--------------------|--------------------------------|-------------------------------------------|---------------------------------------------|--------------------------------------------------------|
| Thymosin Alfa 1 | 5                  | NA                             | 4.93                                      | <b>NA</b>                                   | 98,6%                                                  |

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

Injection:               0,2 µL

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## 2.4 Chromatogram of Thymosin Alfa 1 (PO-20223165)

### Sample information

#### UPLC2

Channel Description ACQUITY TUV ChA 214nm

Vial: 1:8,6 Vol.: 0,20 ul

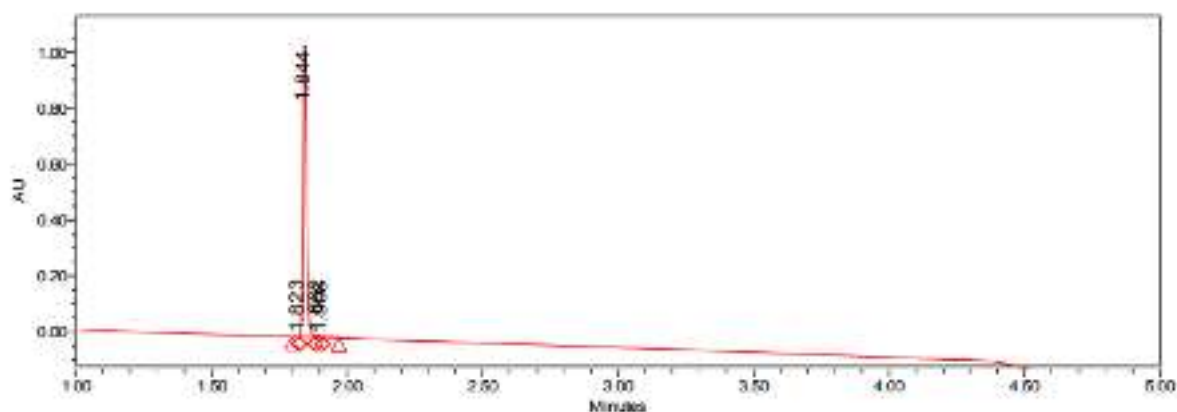
**Sample: Thymosin alpha 1 (PO-20223165)**

Date Acquired 8/4/2023 2:31:31 PM CEST

Date Processed 8/4/2023 2:51:00 PM CEST

Acq Method Set:

Gr\_5\_60\_4ml\_40C\_0\_45\_K2\_met.s



|   | RT    | Area   | Height<br>(µV) | % Area |
|---|-------|--------|----------------|--------|
| 1 | 1.823 | 1470   | 2106           | 0.76   |
| 2 | 1.844 | 306786 | 1043667        | 98.44  |
| 3 | 1.862 | 7530   | 6509           | 0.80   |
| 4 | 1.898 | 6648   | 3231           | 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 oven temp. = 40 °C

## 2.5 Result of purity assessment

The overall purity is 98.44 % at 214 nm.

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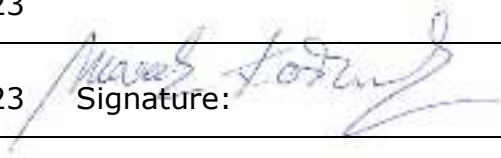
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**CONCLUSION:**

**The sample Thymosin Alfa 1 (Batch No. 2023165) was analyzed for peptide content and UV purity.**

**Peptide content is 98.6 % (4.93 mg in 5 mg)**

**Purity is 98.44 % (UPLC at 214 nm).**

|                            |                                                                                                                  |
|----------------------------|------------------------------------------------------------------------------------------------------------------|
|                            |                                                                                                                  |
| <b>ANALYSIS COMPLETED:</b> | Date: 04.08.2023                                                                                                 |
| <b>Issued by QC:</b>       | Date: 09.08.2023 Signature:  |

# Analytical report AR-24-KT-043642-02



## 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:

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

Date of Sample Receipt: 22.11.2024 Date of Testing: 22.11.2024 - 26.11.2024

Issue date: 03.12.2024

## Information about Sampling:

Sampler: customer

Sample information: 104-2024-00048109

# Sample description: Thymosin alpha 1 (PO-2023165)

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         | LS-PP-CH-85 | -  | TR A |
| Cadmium (Cd) | mg/kg | -             | <0,2           | -                                  | ICP-MS         | LS-PP-CH-85 | -  | TR A |
| Lead (Pb)    | mg/kg | -             | <0,5           | -                                  | ICP-MS         | LS-PP-CH-85 | -  | TR A |
| Mercury (Hg) | mg/kg | -             | <0,3           | -                                  | 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.

Responsible for correctness:

Michaela Ruttkayová  
Specialist worker

Worked out by: Andrea Podušelová

Validity check of document



**Test Certificate approved by**

Michaela Ruttkayová  
Specialist worker

