Certificate of Analysis

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Analytical services

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Inovační 122
252 41 Zlatníky-Hodkovice
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Verify Results Online

Sample Identification

Sample Name	TB-500 5 mg
Batch Number	XD-BT5-PO06
Date Published	2026-09-19 09:47

Results for LYO-0882

Peptides	Result	Unit	Uncertainty	Acceptable Range
Thymosin Beta 4 (TB-500) Assay Peptide Screening 0.1% TFA	5.70	*mg*	[± 0.03]	
Thymosin Beta 4 (TB-500) Purity Peptide Screening 0.1% TFA	> 99.8	*%*		
Thymosin Beta 4 (TB-500) Identification by Spectrum (FTIR) Peptide Screening 0.1% TFA	980		[± 5]	
Thymosin Beta 4 (TB-500) Identification by RT Peptide Screening 0.1% TFA	0.997		[± 0.005]	

Microbiology	Result	Unit	Uncertainty	Acceptable Range
Total Aerobic Microbial Count USP <61>/Eur. Ph. 2.6.12. Plate Count Method	$3 \cdot 10^{2}$	*CFU/g*	[± 1.5e01]	0 - 1000
Total Yeast and Mold Count USP <61>/Eur. Ph. 2.6.12. Plate Count Method	0	*CFU/g*	[±]	0 - 100
Bacterial Endotoxin Chromgenic USP<85>/ Eur. Ph. 2.6.14. Bacterial Endotoxin Chromgenic Test	0.369	*EU/mg*	[± 0.007]	0 - 0.5

Elemental Impurities	Result	Unit	Uncertainty	Acceptable Range
Arsenic Elemental Impurities screening USP ⟨232⟩ / Ph. Eur. 5.20 / 2.4.20	< 0.001	*ppm*		0 - 1.5
Cadmium Elemental Impurities screening USP ⟨232⟩ / Ph. Eur. 5.20 / 2.4.20	< 0.001	*ppm*		0 - 0.5
Quicksilver Elemental Impurities screening USP ⟨232⟩ / Ph. Eur. 5.20 / 2.4.20	< 0.001	*ppm*		0 - 1.5
Lead Elemental Impurities screening USP ⟨232⟩ / Ph. Eur. 5.20 / 2.4.20	< 0.001	*ppm*		0 - 1.5

Mass Spectrometry	Result	Unit	Uncertainty	Acceptable Range
Molecular Ion Mass Identification (MS Deconvolution) Mass Spectrometry Identity	4963	*Da*	[± 1]	

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1. Content Assesment

1.1. Instrumentation

Module	Name	Serial Number
System Controller	Shimadzu CBM-40 Lite	L221226351398
Degassing Unit	Shimadzu DGU-403	NA
Pump	Shimadzu LC-40B XR	L22146350580
Autosampler	Shimadzu SIL-40C XR	L22216351622
Colum Thermostat	Shimadzu CTO-40S	L22236351602
PDA Detector	Shimadzu SPD-M40	L22276352808
SQ MS Detector	Shimadzu LCMS-2050	O12476200760

1.2. Chromatographic conditions

Chromatographic conditions	
Eluent A	0.1% DFA in Water (HPLC, Gradient Grade)
Eluent B	0.1% DFAin Acetonitrile (HPLC, Gradient Grade)
Flow rate	0.8 mL/min
Program	Gradient elution
Injection volume	2 µL
Colum Temperature	60°C
Column	Waters XSelect CSH C18, 100x2.1mm 2.5µm
Detection wavelength	225nm

Gradient Program		
Time [min]	A [%]	B [%]
1	98	2
13.99	35	65
14	5	95
15	5	95
15.01	98	2
19	end	

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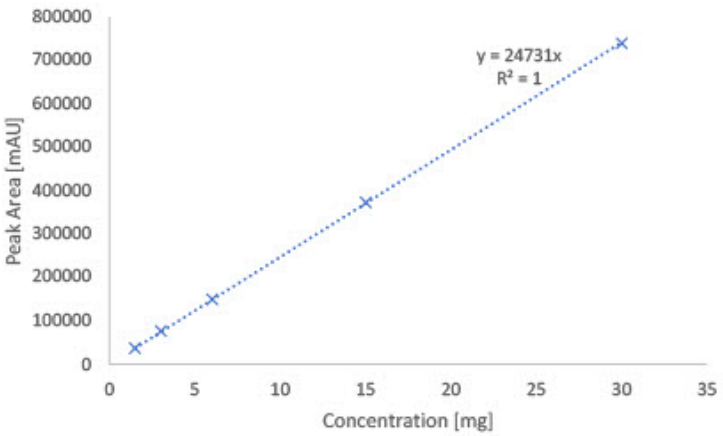
1.3. Sample preparation

Whole amount of container was dissolved in 2mL of water (LCMS Grade). 100 µL of sample was transferred to HPLC vial and diluted by 900 µL water (LCMS Grade) and submitted for analysis.

1.4. Calibration curve

Calibration curve detail	
Quantitative method	External Standard
Calibration Type	Linear
Number of calibration points	5
Force through Zero	Enabled
Weighting Method	None



2

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2. Purity assessment

2.1 Instrumentation

Module	Name	Serial Number
System Controller	Shimadzu CBM-40 Lite	L221226351398
Degassing Unit	Shimadzu DGU-403	NA
Pump	Shimadzu LC-40B XR	L22146350580
Autosampler	Shimadzu SIL-40C XR	L22216351622
Colum Thermostat	Shimadzu CTO-40S	L22236351602
PDA Detector	Shimadzu SPD-M40	L22276352808
SQ MS Detector	Shimadzu LCMS-2050	O12476200760

2.2 Chromatographic conditions

Chromatographic conditions	
Eluent A	0.1% DFA in Water (HPLC, Gradient Grade)
Eluent B	0.1% DFAin Acetonitrile (HPLC, Gradient Grade)
Flow rate	0.8 mL/min
Program	Gradient elution
Injection volume	2 µL
Colum Temperature	60°C
Column	Waters XSelect CSH C18, 100x2.1mm 2.5µm
Detection wavelength	225nm

Gradient Program		
Time [min]	A [%]	B [%]
1	98	2
13.99	35	65
14	5	95
15	5	95
15.01	98	2
19	end	

2.3 Purity assesment

Purity of compound assesed by area normalization method, comparing area of each peak to sum of area of all peaks detected at wavelenght of 225 nm.

3

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3. Identity Assessment

3.1 Instrumentation

Module	Name	Serial Number
System Controller	Shimadzu CBM-40 Lite	L221226351398
Degassing Unit	Shimadzu DGU-403	NA
Pump	Shimadzu LC-40B XR	L22146350580
Autosampler	Shimadzu SIL-40C XR	L22216351622
Colum Thermostat	Shimadzu CTO-40S	L22236351602
PDA Detector	Shimadzu SPD-M40	L22276352808
SQ MS Detector	Shimadzu LCMS-2050	O12476200760

3.2 Chromatographic conditions

Chromatographic conditions	
Eluent A	0.1% DFA in Water (HPLC, Gradient Grade)
Eluent B	0.1% DFAin Acetonitrile (HPLC, Gradient Grade)
Flow rate	0.8 mL/min
Program	Gradient elution
Injection volume	2 µL
Colum Temperature	60°C
Column	Waters XSelect CSH C18, 100x2.1mm 2.5µm
Detection	MS 220-2000 Da

Gradient Program		
Time [min]	A [%]	B [%]
1	98	2
13.99	35	65
14	5	95
15	5	95
15.01	98	2
19	end	

3.3 Molecular Ion Mass evaluation

Molecular ion mass was determined by deconvolution of multiply charged ESI-MS spectra to calculate the average neutral (zero-charge) molecular mass by equation:

$$M(neutral) = (z_i((mz_i) - H)) - ME$$

Where:
mz_i - Measured mass of charged particle
z_i - charge
H - proton mass (1.0076 Da)
ME – mass error

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Analysis Report

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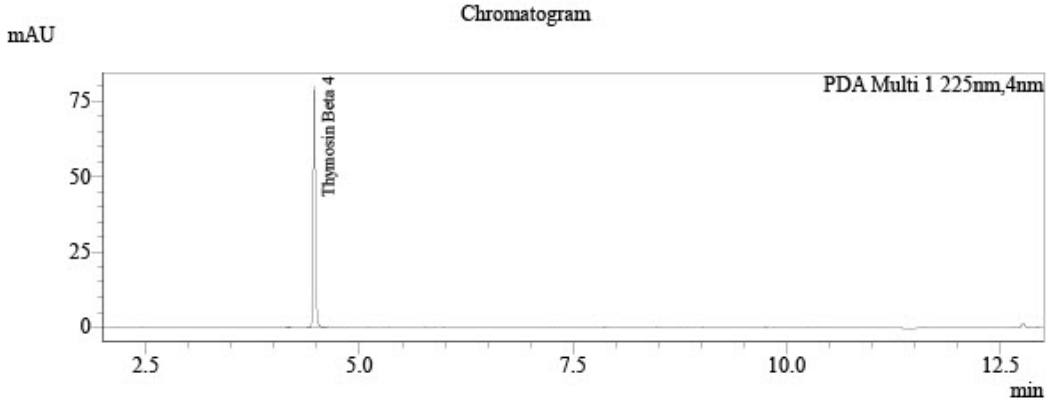
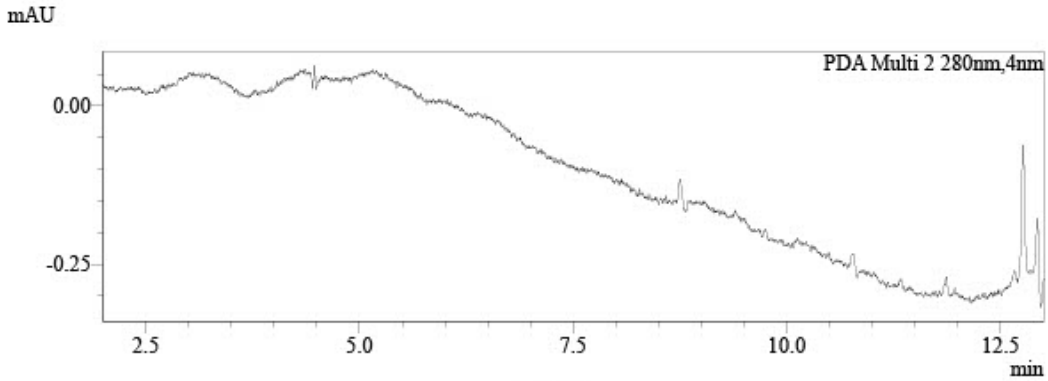
Analysis of quantity of active ingredient by UHPLC with MS detection

Sample Information

Injection Volume	: 2
Data File	: LYO-0882__003.lcd
Method File	: Peptide Screening_DFA_Group C.lcm
Date Acquired	: 9/14/2026 5:43:18 PM

Chromatogram





Peak Table

PDA Ch1 225nm

Peak#	Name	Ret. Time	Area	Area%	Conc.	Unit
1		4.158	185	0.129	0.000	
2	Thymosin Beta 4	4.469	142763	99.871	5.699	mg
Total			142947	100.000		

Peak Table

PDA Ch2 280nm

Peak#	Name	Ret. Time	Area	Area%	Conc.	Unit
1		14.225	2087	100.000	0.000	
Total			2087	100.000		

1 / 2

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Analysis Report

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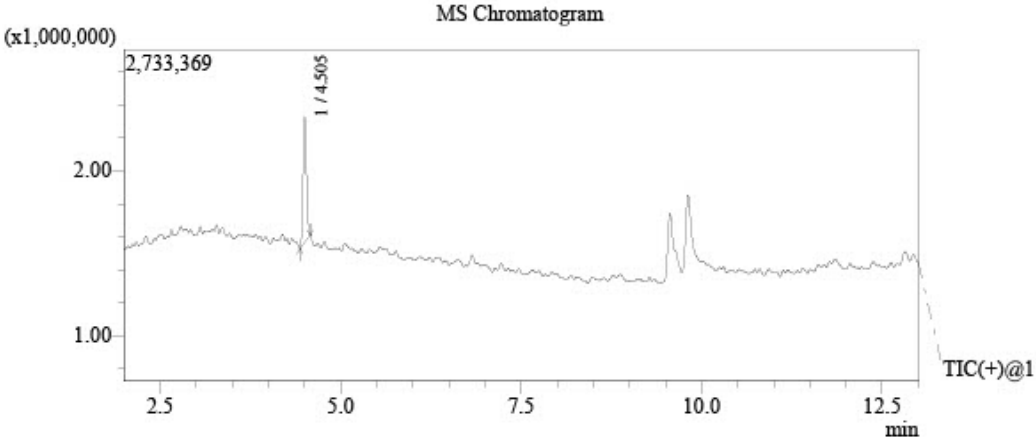
Identification of active ingredient by UHPLC Mass Spectrometr

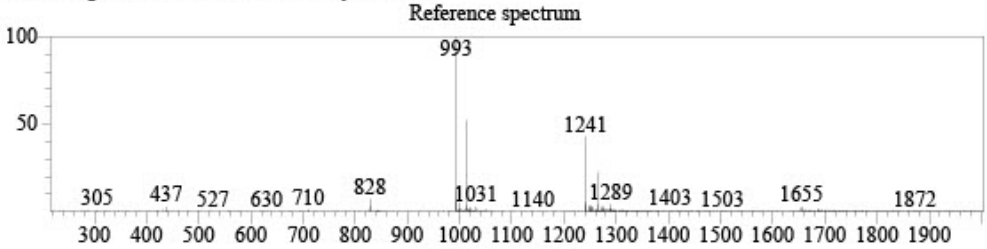
MS Chromatogram



Library Search

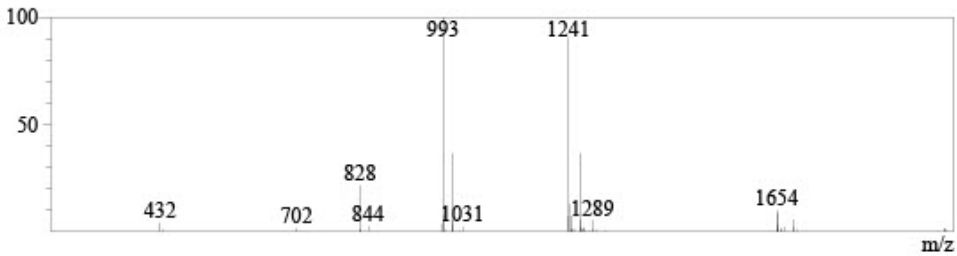
Base Peak: 993(251319)
Scan Range: 220.00 - 2000.00 Porality: Positive

Reference spectrum



Detected spectrum

Library: Peptide Screening_DFA.lib
Formula: C212H350N56O78S CAS: 77591-33-4 Mol.Weight: 4963(0!=)
Compound Name: Thymosin Beta 4



2 / 2

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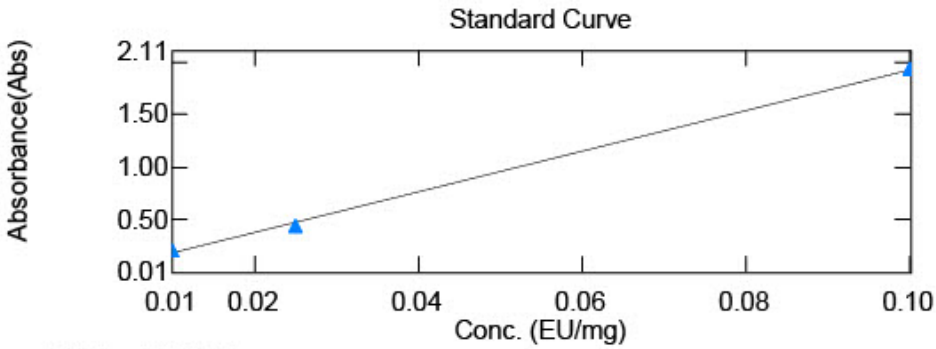
Endotoxin Determination Report

File Information
Filename: C:\UVVis-Data\Data\File_260914_161437.vqud
Date/Time: 09/14/2026 04:43:55 PM

Instrument Information
Instrument Type: UV-1900 Series
Model (S/N): UV-1900i (A12536253123)



y = 19.3540 x - 0.0100786
r2 = 0.99851

	Sample Name	Conc	Raw_WL545.0	Dilution Rate
1	LYO-0866	0.111	0.0975	20.00
2	LYO-0867	0.171	0.1556	20.00
3	LYO-0868	0.106	0.0930	20.00
4	LYO-0869	0.226	0.2084	20.00
5	LIQ-0072	0.006	0.0969	1.00
6	LIQ-0073	0.108	1.0311	2.00
7	LYO-0882	2.106	1.0090	40.00
8	LYO-0883	2.838	1.3630	40.00
9	LYO-0884	0.124	0.1101	20.00
10	LYO-0885	0.184	0.1683	20.00
11	LYO-0886	0.091	0.0782	20.00
12	LYO-0888	0.380	0.3574	20.00
13	LYO-0865	0.522	0.0909	100.00
14	LYO-0870	0.474	0.0817	100.00
15	LYO-0871	0.503	0.0872	100.00
16	LYO-0872	0.659	0.1174	100.00

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1. Instrumentation and chemicals

1.1. Instruments used

- Sterile Syringe 2mL Luer
- Sterile needles
- Ready made PCA Plate ROTI Aquatest
- Ready made Sab4 Plate ROTI Aquatest

1.2. Chemicals

Sterile physiological solution (0.9% NaCl)

2. Sample preparation and innoculation

2.1 Sample preparation

1. Fresh sterile needle and syringe was used for measuring exactly 2 mL of sterile physiological solution.
2. Needle was changed and by new needle rubber top of peptide containter was penetrated and 2 mL of sterile physiological solution was dispensed.
3. Content of container was completely dissolved and left for 5 minutes to settle potentially created bubbles.
4. This procedure is repeated for two vials.

2.2 Total Aerobic microbial count innoculation and cultivation

1. By sterile needle 1 mL of solution was filled into the sterile syringe.
2. Needle was placed above the flame for few seconds to sterilze.
3. Consequently 1 mL of solution was poured into the ready to use sterile petri dish filled with PCA agar and petri dish was closed.
4. Proces was repeated for two petri dishes.
5. With sterile needle, 1 mL of sterile physiological solution was filled into the sterile needle and was innoculated onto one sterile petri dish filled with PCA agar as negative control sample.
6. Samples and negative control sample were placed in incubator at temperature 37°C for 120h.

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2.3 Total Yeast and Mold count innoculation and cultivation

1. By sterile needle 1 mL of solution was filled into the sterile syringe.
2. Needle was placed above the flame for few seconds to sterilze.
3. Consequently 1 mL of solution was poured into the ready to use sterile petri dish filled with Sab4 agar and petri dish was closed.
4. Proces was repeated for two petri dishes.
5. With sterile needle, 1 mL of sterile physiological solution was filled into the sterile needle and was innoculated onto one sterile petri dish filled with Sab4 agar as negative control sample.
6. Samples and negative control sample were placed in incubator at temperature 25°C for 72h.

3. Evaluation of results

After incubation time, colonies are counted as cfu (colonies forming units) and result per 1g of sample is determined as:

$$CFU_{avg} = \frac{\sum CFU_n}{n}$$

$$CFU_{avg} = average\ CFU\ counted\ form\ n\ innoculations$$
$$CFU_n = CFU\ counted\ per\ innoculation$$
$$n = number\ of\ innoculations$$

$$CFU\ per\ gram = \frac{CFU_{avg}}{m_s} * DF$$
$$CFU_{avg} = Average\ CFU\ counted\ from\ n\ innoculations$$
$$m_s = mass\ of\ sample\ (mg)$$
$$DF = Dilution\ factor$$

If negative control sample is evaluated as positive, process have to be repeated due to possible contamination in the process of innoculation or incubation.

2

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Responsibles

Mr. Ján Galbavý
CEO

Analysis results relate only to the samples tested.
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