

Certificate Of Analysis



Client:

Particle Peptides

Viktor Mužila
Kolonáda 4490/18
98401 Lučenec
Slovakia
muzila@particlepeptides.com
+421 917 381 743

Laboratory:

Liquilabs s.r.o.

Ohradní 24B
14000 Michle
Czechia
www.liquilabs.cz

Sample Identification

| Sample Name | Thymosin beta- 4 10 mg | Batch Number | 2025255 | Date Published | 2025-08-15 14:58 |
|-------------|------------------------|--------------|---------|----------------|------------------|
|-------------|------------------------|--------------|---------|----------------|------------------|

Results for Lyo-0067

| Analysis of Peptide Identity, Content and Purity | Result | Unit | Uncertainty | Reporting Limit |
|--|--------|------|-------------|-----------------|
| Thymosin Beta 4 (TB-500) Assay Peptide Screening | 10.18 | mg | [± 0.05] | |
| Thymosin Beta 4 (TB-500) Identification by RT Peptide Screening | 0.989 | | [± 0.005] | |
| Thymosin Beta 4 (TB-500) Identification by spectrum Peptide Screening | 995 | | [± 10] | |
| Thymosin Beta 4 (TB-500) Purity Peptide Screening | > 99.8 | % | | |


| Bioburden | Result | Unit | Uncertainty | Reporting Limit |
|--|--------------|-------|-------------|-----------------|
| Total Aerobic Microbial Count USP <61> Plate Count Method | Not detected | CFU/g | | >= 1000 |
| Total Yeast and Mold Count USP <61> Plate Count Method | Not detected | CFU/g | | >= 100 |

| Endotoxin Analysis | Result | Unit | Uncertainty | Reporting Limit |
|---|---------|-------|-------------|-----------------|
| Bacterial Endotoxin USP<85> Bacterial Endotoxin Chromogenic Test | < 0.001 | EU/mg | | > 0.5 |

| Heavy Metals | Result | Unit | Uncertainty | Reporting Limit |
|---|--------------|------|-------------|-----------------|
| Arsenic Elemental Impurities Screening | Not detected | ppm | | >= 1.5 |
| Cadmium Elemental Impurities Screening | Not detected | ppm | | >= 0.5 |

| Heavy Metals | Result | Unit | Uncertainty | Reporting Limit | |
|---|--------------|------|-------------|-----------------|---|
| Cobalt Elemental Impurities Screening | Not detected | ppm | | >= 25 | △ |
| Lead Elemental Impurities Screening | Not detected | ppm | | >= 1.5 | △ |
| Nickel Elemental Impurities Screening | Not detected | ppm | | >= 25 | △ |
| Quicksilver Elemental Impurities Screening | Not detected | ppm | | >= 1.5 | △ |
| Vanadium Elemental Impurities Screening | Not detected | ppm | | >= 25 | △ |

Attachments for Lyo-0067

| | | | |
|---|---------------------------------|-----------------------------|--|
|  | Method Specification | | |
| Determination of identity, content and purity of TB-500 | | | |
| <i>Document number</i> TB4_002_2025 | <i>Superseded document</i> - | <i>Number of pages</i> 3 | |

1. Content Assesment

1.1. Instrumentation

| Module | Name | Serial Number |
|-------------------|---------------------|---------------|
| System Controller | Shimadzu SCL-10ADvp | C21014112659 |
| Degassing Unit | Shimadzu DGU-14A | NA |
| LPGE valve | Shimadzu FCV-10Avp | NA |
| Pump | Shimadzu LC-10ADvp | C20964130094 |
| Autosampler | Shimadzu SIL-10ADvp | C21054109114 |
| Colum Thermostat | Shimadzu CTO-10ACvp | C21033770144 |
| Detector | Shimadzu SPD-10ADvp | C20994233588 |

1.2. Chromatographic conditions

| Chromatographic conditions | |
|----------------------------|---|
| Eluent A | 0.1% TFA in Water (HPLC, Gradient Grade) |
| Eluent B | 0.1% TFA in Acetonitrile (HPLC, Gradient Grade) |
| Flow rate | 0.7 mL/min |
| Program | Gradient elution |
| Injection volume | 0.5 µL |
| Colum Temperature | 65°C |
| Column | Phenomenex Biozen Peptide Polar C18 150x2.1mm 3µm |
| Detection wavelenght | 214nm |

| Gradient Program | | |
|------------------|-------|-------|
| Time [min] | A [%] | B [%] |
| 2 | 99 | 1 |
| 10 | 70 | 30 |
| 14 | 70 | 30 |
| 32 | 5 | 95 |
| 35 | 5 | 95 |
| 35.01 | 99 | 1 |
| 40 | end | |

1

Attachment for Lyo-0067

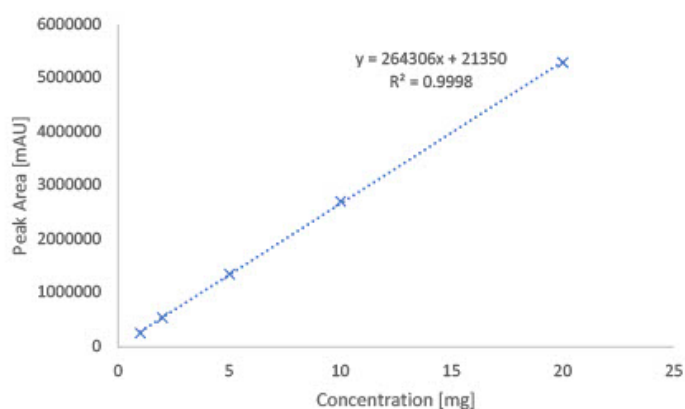
Filename: 1755268899050-87c3444f-58c0-42bf-9b8b-52a77e595524_1.jpg

1.3. Sample preparation

Whole amount of container was dissolved in 2mL of water (HPLC, Gradient Grade). Aliquote part of 1 mL was dispensed into HPLC vial for analysis.

1.4. Calibration curve

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



2. Purity assessment

2.1 Instrumentation

| Module | Name | Serial Number |
|-------------------|---------------------|---------------|
| System Controller | Shimadzu SCL-10ADvp | C21014112659 |
| Degassing Unit | Shimadzu DGU-14A | NA |
| LPGE valve | Shimadzu FCV-10Avp | NA |
| Pump | Shimadzu LC-10ADvp | C20964130094 |
| Autosampler | Shimadzu SIL-10ADvp | C21054109114 |
| Column Thermostat | Shimadzu CTO-10ACvp | C21033770144 |
| Detector | Shimadzu SPD-10ADvp | C20994233588 |

2.2 Chromatographic conditions

| Chromatographic conditions | |
|----------------------------|---|
| Eluent A | 0.1% TFA in Water (HPLC, Gradient Grade) |
| Eluent B | 0.1% TFA in Acetonitrile (HPLC, Gradient Grade) |
| Flow rate | 0.7 mL/min |
| Program | Gradient elution |
| Injection volume | 0.5 µL |
| Column Temperature | 65°C |
| Column | Phenomenex Biozen Peptide Polar C18 150x2.1mm 3µm |
| Detection wavelength | 214nm |

| Gradient Program | | |
|------------------|-------|-------|
| Time [min] | A [%] | B [%] |
| 2 | 99 | 1 |
| 10 | 70 | 30 |
| 14 | 70 | 30 |
| 32 | 5 | 95 |
| 35 | 5 | 95 |
| 35.01 | 99 | 1 |
| 40 | end | |

1.5. Sample preparation

Whole amount of container was dissolved in 2mL of water (HPLC, Gradient Grade). Aliquote part of 1 mL was dispensed into HPLC vial for analysis.

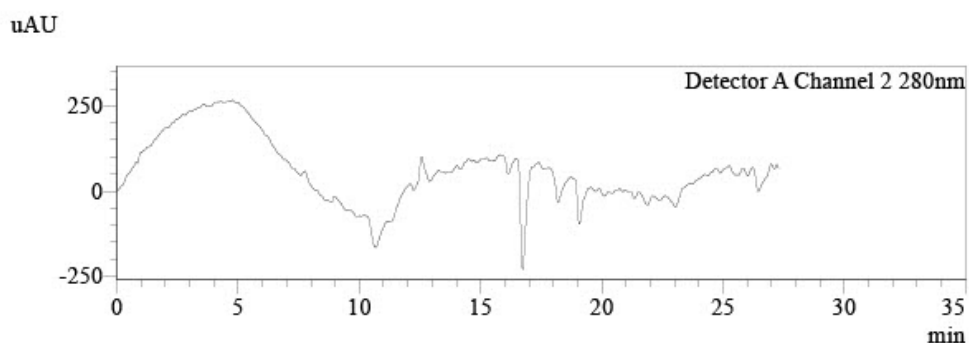
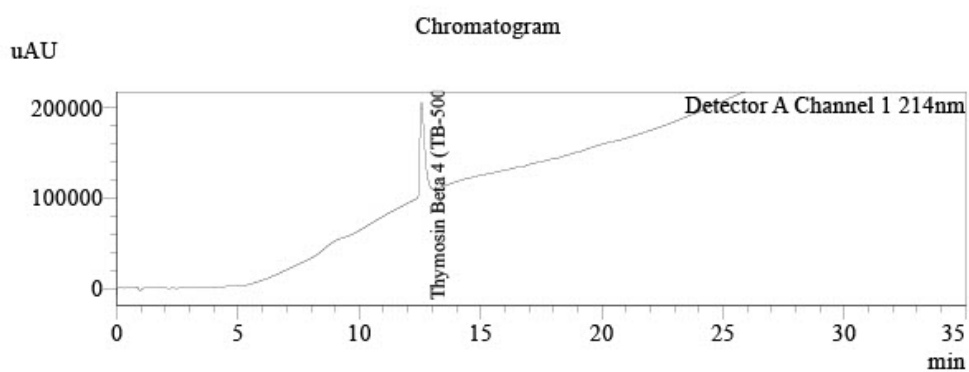
1.6. Purity assesment

Purity of compound assesed by area normalization method, comparing area of each peak to sum of area of all peaks detected at wavelenght of 214 nm.

Analysis Report



Sample Information
Injection Volume : 0,5
Data File : LYO-0067-P01_001.lcd
Method File : Peptide screening_V5_calib.lcm
Date Acquired : 07.08.2025 23:25:20




Peak Table

| Detector A Channel 1 214nm | | | | | |
|----------------------------|--------------------------|-----------|--------|------|---------|
| Peak# | Name | Ret. Time | Conc. | Unit | Area% |
| 1 | Thymosin Beta 4 (TB-500) | 12.565 | 10.180 | mg | 100.000 |
| Total | | | | | 100.000 |

Peak Table

| Detector A Channel 2 280nm | | | | |
|----------------------------|------|-----------|-------|------|
| Peak# | Name | Ret. Time | Conc. | Unit |
| Total | | | | |

Attachment for Lyo-0067
Filename: 1755269181830-dcdad62e-17d0-431c-8232-d059b908cecc_1.jpg

| | | | |
|---|-----------------------------|----------------------|--|
|  | Method Specification | | |
| Determination of bioburden of lyophilized samples | | | |
| Document number MIC_001_2025 | Superseded document - | Number of pages 2 | |

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 inoculation

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 container 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 inoculation 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 sterilize.
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 inoculated 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.

1

Attachment for Lyo-0067
Filename: Bioburden-images-0.jpg

2.3 Total Yeast and Mold count inoculation 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 sterilize.
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 inoculated 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 inoculations

CFU_n = CFU counted per inoculation

n = number of inoculations


$$CFU \text{ per gram} = \frac{CFU_{avg}}{m_s} * DF$$

CFU_{avg} = Average CFU counted from n inoculations

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 inoculation or incubation.

| | | |
|---|---|------------------------------------|
|  | Method Specification | |
| Determination of bacterial endotoxin content of lyophilized samples | | |
| <i>Document number</i> ENDOTOX_002_2025 | <i>Superseded document</i> ENDOTOX_001_2025 | <i>Number of pages</i> 2 |

1. Chromgenic LAL Assay Determination of Bacterial Endotoxin content of sample

1.1. Instrumentation

- Pipette set 1-1000 µL
- Thermostatically controlled water bath
- UV VIS spectrometer (Shimadzu UV-1601)
- GenScript ToxinSensor Chromgenic LAL Endotoxin Assay kit

1.2. Chemicals

- LAL Reagent water (endotoxin free)
- Limulus Amoebocyte Lysate
- LAL Substrate
- Color Stabilizer #1
- Color Stabilizer #2
- Color Stabilizer #3
- 35% HCl (p.a.)

1.3. Sample preparation

1. Sample container was weighed prior to dissolution and measured weight was marked.
2. Sample was completely dissolved in its container by 2 mL of LAL Reagent water.
3. 100 µL of the sample was aliquoted for analysis.
4. After analysis container was emptied and dried.
5. Dry mass of container was measured and exact weight of dissolved content was determined as:

$$m_{dc} = m_{sample} - m_{container}$$

1.4. Toxin sensor Chromgenic LAL Endotoxin Assay kit preparation

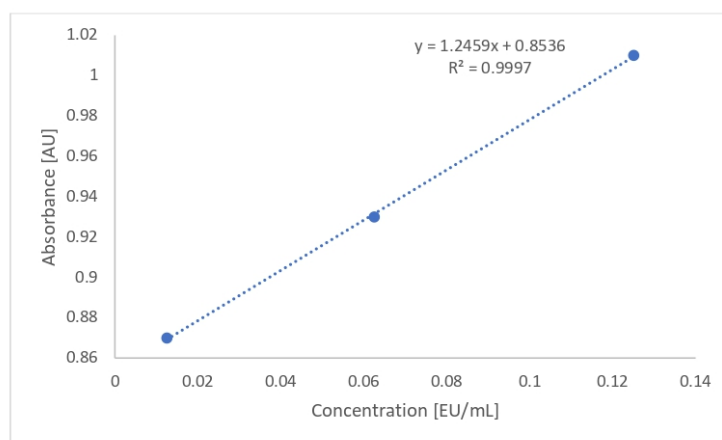
Procedures regarding preparation of reaction solutions possible to find in:

https://www.genscript.com/site2/document/5292_20080806231827.PDF

1.5. Measurement procedure

| | Standards | Samples | Blank |
|---|-----------|---------|-------|
| Standards (mL) | 0.1 | - | - |
| Samples (mL) | - | 0.1 | - |
| LAL Reagent Water (mL) | - | - | 0.1 |
| LAL Solution (mL) | 0.1 | 0.1 | 0.1 |
| Mix well and incubate at 37°C for 27 min | | | |
| Substrate solution (mL) | 0.1 | 0.1 | 0.1 |
| Mix well and incubate at 37°C for 6 min | | | |
| Color Stabilizer #1 solution | 0.5 | 0.5 | 0.5 |
| Color Stabilizer #2 solution | 0.5 | 0.5 | 0.5 |
| Color Stabilizer #3 solution | 0.5 | 0.5 | 0.5 |
| Mix well and read the absorbance at 545nm | | | |

1.6. Calibration curve



1.7. Calculation of endotoxin content

Endotoxin content of the sample was calculated from the calibration curve as:

$$Endotox[EU/mg] = \frac{\left(\frac{ABS_{sample}}{S_{calib}}\right) * 20}{m_{sample}}$$

ABS_{sample} = Measured absorbance of sample

S_{calib} = Slope of calibration curve

m_{sample} = real measured mass of sample

20 = dilution factor of measured sample



Mr. Ján Galbavý
Founder/Manager

Analysis results relate only to the samples tested.

This document shall not be reproduced except in full, without the written approval of Liquilabs s.r.o.