# Certificate Of Analysis

LIQUILABS
Analytical services

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## Sample Identification

| **Sample Name** | MOTS-C 10 mg | **Batch Number** | 2025284 | **Date Published** | 2025-10-16 10:06 |
|---|---|---|---|---|---|

## Results for Lyo-0141

| Analysis of Peptide Identity, Content and Purity | Result | Unit | Uncertainty | Reporting Limit |
|---|---|---|---|---|
| MOTS-C Assay<br>Peptide Screening | 10.16 | *mg* | [± 0.05] | |
| MOTS-C Identification by RT<br>Peptide Screening | 0.989 | | [± 0.005] | |
| MOTS-C Identification by spectrum<br>Peptide Screening | 993 | | [± 10] | |
| MOTS-C Purity<br>Peptide Screening | 97.6 | *%* | [± 0.5] | |

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

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

| Heavy Metals | Result | Unit | Uncertainty | Reporting Limit | |
|---|---|---|---|---|---|
| Arsenic<br>Elemental Impurities Screening | Not detected | *ppm* | | >= 1.5 | ⚠ |
| Cadmium<br>Elemental Impurities Screening | Not detected | *ppm* | | >= 0.5 | ⚠ |
| Cobalt<br>Elemental Impurities Screening | Not detected | *ppm* | | >= 25 | ⚠ |
| Lead<br>Elemental Impurities Screening | Not detected | *ppm* | | >= 1.5 | ⚠ |

| Heavy Metals | Result | Unit | Uncertainty | Reporting Limit | |
|---|---|---|---|---|---|
| Nickel<br>Elemental Impurities Screening | Not detected | *ppm* | | >= 25 | ⚠ |
| Quicksilver<br>Elemental Impurities Screening | Not detected | *ppm* | | >= 1.5 | ⚠ |
| Vanadium<br>Elemental Impurities Screening | Not detected | *ppm* | | >= 25 | ⚠ |

# Attachments for Lyo-0141

| LIQUILABS Analytical services | Method Specification | |
|---|---|---|
| Determination of identity, content and purity of MOTS-C | | |
| *Document number* MOTS_010_2025 | *Superseded document* - | *Number of pages* 3 |

## 1. Content Assesment

### 1.1. Instrumentation

| Module | Name | Serial Number |
|---|---|---|
| System Controller | Shimadzu SCL-10ADvp | C21014112659 |
| Degassing Unit | Shimadzu DGU-14A | NA |
| Pump A | Shimadzu LC-10ADvp | C20964130075 |
| Pump B | Shimadzu LC-10ADvp | C20953770781 |
| 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 | 0.4 mL/min |
| Program | Gradient elution |
| Injection volume | 0.5 µL |
| Colum Temperature | 60°C |
| Column | Phenomenex Biozen Peptide Polar C18, 150x2.1mm 3µm |
| Detection wavelenght | 280nm |

| Gradient Program | | |
|---|---|---|
| Time [min] | A [%] | B [%] |
| 1 | 95 | 5 |
| 19 | 40 | 60 |
| 20 | 5 | 95 |
| 24 | 5 | 95 |
| 25 | 95 | 5 |
| 33 | end | |

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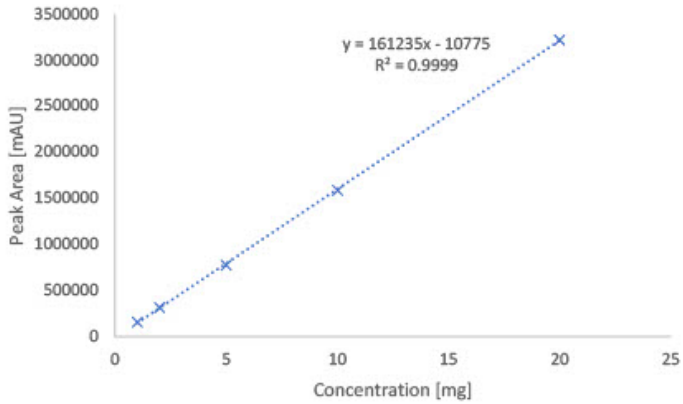
Attachment for Lyo-0141
Filename: 1760608511983-156649ac-3c0a-4f11-977f-de8532d836f0_1.jpg

### 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 |

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Attachment for Lyo-0141
Filename: 1760608511983-156649ac-3c0a-4f11-977f-de8532d836f0_2.jpg

# 2. Purity assessment

## 2.1 Instrumentation

| Module | Name | Serial Number |
|---|---|---|
| System Controller | Shimadzu SCL-10ADvp | C21014112659 |
| Degassing Unit | Shimadzu DGU-14A | NA |
| Pump A | Shimadzu LC-10ADvp | C20964130075 |
| Pump B | Shimadzu LC-10ADvp | C20953770781 |
| Autosampler | Shimadzu SIL-10ADvp | C21054109114 |
| Colum 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 | 0.4 mL/min |
| Program | Gradient elution |
| Injection volume | 0.5 µL |
| Colum Temperature | 60°C |
| Column | Phenomenex Biozen Peptide Polar C18, 150x2.1mm 3µm |
| Detection wavelenght | 214nm |

| Gradient Program | | |
|---|---|---|
| Time [min] | A [%] | B [%] |
| 1 | 95 | 5 |
| 19 | 40 | 60 |
| 20 | 5 | 95 |
| 24 | 5 | 95 |
| 25 | 95 | 5 |
| 33 | 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.

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Attachment for Lyo-0141
Filename: 1760608511983-156649ac-3c0a-4f11-977f-de8532d836f0_3.jpg

# Analysis Report

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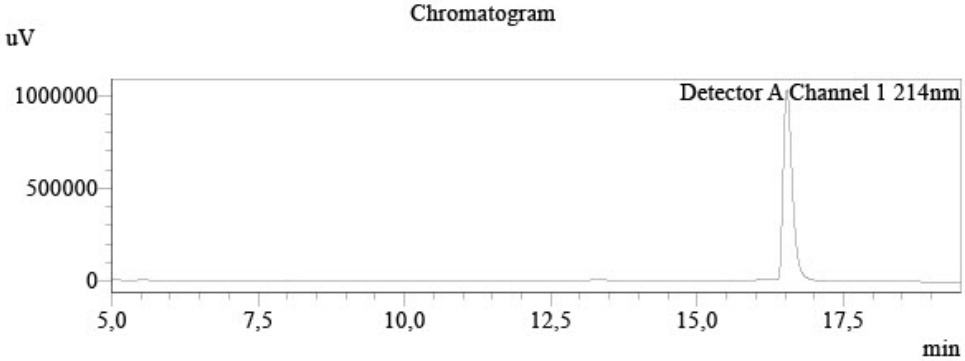
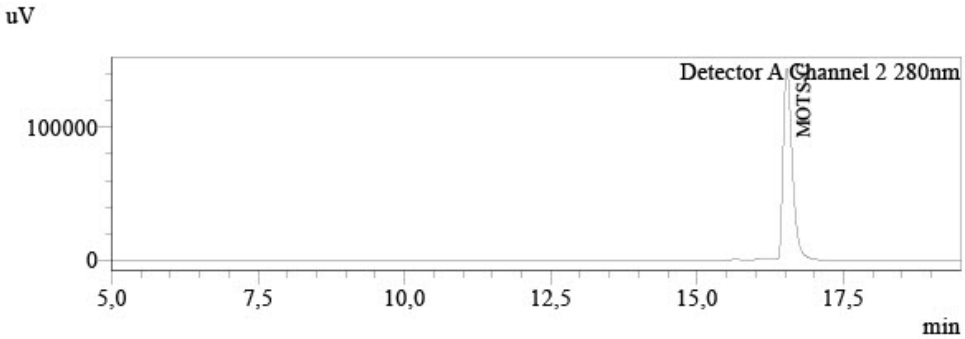
Sample Information

| | |
|---|---|
| Injection Volume | : 0,5 |
| Data File | : LYO-0141-P01__002.lcd |
| Method File | : Peptide screening_V10_Group B.lcm |
| Date Acquired | : 07.10.2025 11:56:52 |

Chromatogram




Peak Table

Detector A Channel 1 214nm

| Peak# | Name | Ret. Time | Conc. | Unit | Area% |
|---|---|---|---|---|---|
| 1 | | 15,638 | 0,000 | | 0,710 |
| 2 | | 16,118 | 0,000 | | 1,678 |
| 3 | | 16,532 | 0,000 | | 97,613 |
| Total | | | | | 100,000 |

Peak Table

Detector A Channel 2 280nm

| Peak# | Name | Ret. Time | Conc. | Unit |
|---|---|---|---|---|
| 1 | | 16,117 | 0,000 | |
| 2 | MOTS-C | 16,531 | 10,158 | mg |
| Total | | | | |

Attachment for Lyo-0141
Filename: LYO-141.jpg

# 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.



Attachment for Lyo-0141
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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.



Attachment for Lyo-0141
Filename: Bioburden-images-1.jpg

| LIQUILABS Analytical services | Method Specification | |
|---|---|---|
| Determination of bacterial endotoxin content of lyophilized samples | | |
| *Document number* *ENDOTOX_002_2025* | *Superseded document* ENDOTOX_001_2025 | *Number of pages* *2* |

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

## 1.1. Instrumentation

- Pipette set 1-1000 µL
- Termostatically 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

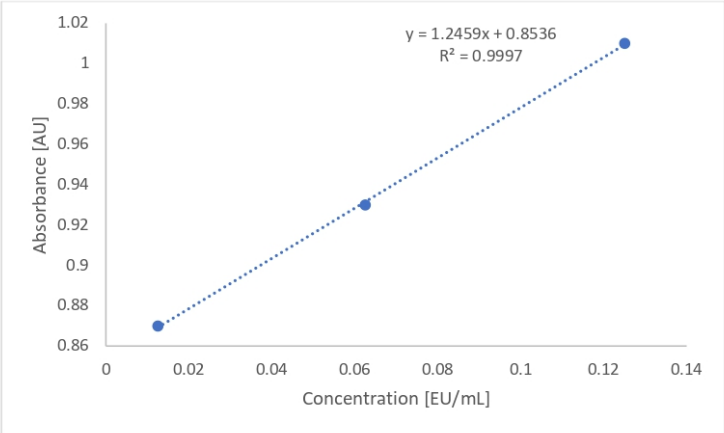


Attachment for Lyo-0141
Filename: Endotoxin_072025_1_page-0001.jpg

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:

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

$$ABS_{sample} = Measured\ absorbance\ of\ sample$$
$$S_{calib} = Slope\ of\ calibration\ curve$$
$$m_{sample} = real\ measured\ mass\ of\ sample$$
$$20 = dilution\ factor\ of\ measured\ sample$$

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Attachment for Lyo-0141
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## Responsibles

**Mr. Ján Galbavý**
Founder/Manager

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