Certificate Of Analysis

LIQUILABS
Analytical services

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Sample Identification

Sample Name	LL-37 5 mg	**Batch Number**	2025254	**Date Published**	2025-07-25 18:24

Results for Lyo-0057

Analysis of Peptide Identity, Content and Purity	Result	Unit	Uncertainty	Reporting Limit
LL-37 Assay Peptide Screening	5.13	*mg*	[± 0.03]	
LL-37 Identification by RT Peptide Screening	0.999		[± 0.005]	
LL-37 Identification by spectrum Peptide Screening	1.00		[± 0.01]	
LL-37 Purity Peptide Screening	> 99.8	*%*		

Bioburden	Result	Unit	Uncertainty	Reporting Limit	
Total Aerobic Microbial Count USP <61> Plate Count Method	150	*CFU/g*	[± 15]	>= 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 Chromgenic Test	0.0100	*EU/mg*	[± 0.0002]	> 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	⚠
Cobalt Elemental Impurities Screening	Not detected	*ppm*		>= 25	⚠
Lead Elemental Impurities Screening	Not detected	*ppm*		>= 1.5	⚠

Heavy Metals	Result	Unit	Uncertainty	Reporting Limit	
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-0057

LIQUILABS	Method Specification	
Determination of identity, content and purity of LL-37		
Document number: LL37_001_2025	Superseded document: -	Number of pages: 2

1. Content and Purity Assesment

1.1. Instrumentation

Module	Name	Serial Number
System Controller	Shimadzu SCL-10AVp	[illegible]
Degassing Unit	Shimadzu DGU-14A	NA
LPGE valve	Shimadzu FCV-10ALvp	NA
Pump	Shimadzu LC-10ADvp	[illegible]
Autosampler	Shimadzu SIL-10ADvp	[illegible]
Colum Thermostat	Shimadzu CTO-10ADvp	[illegible]
Detector	Shimadzu SPD-10AVp	[illegible]

1.2. Chromatographic conditions

Chromatographic conditions	
Eluent A	1% TFA in Water (HPLC, Gradient Grade)
Eluent B	1% TFA in Acetonitrile (HPLC, Gradient Grade)
Flow rate	1mL/min
Program	Gradient elution
Injection volume	2 µL
Colum Temperature	25°C
Column	Avantor ACE UltraCore C18, 150x3,0 mm, 3,5µm
Detection wavelength	214nm

Gradient Program		
Time [min]	A [%]	B [%]
0	99	1
25	5	95
28	5	95
28.01	99	1
32	end	

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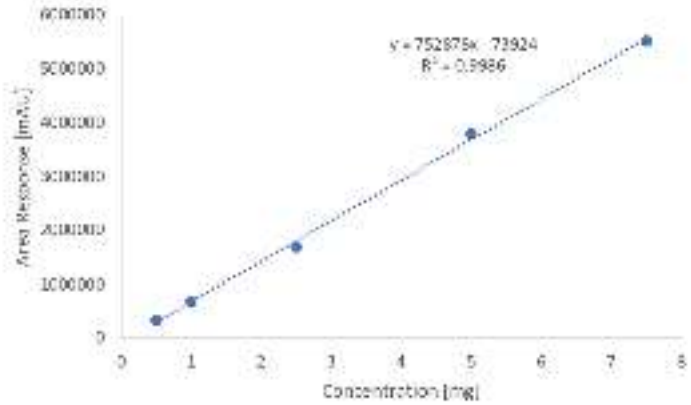
Attachment for Lyo-0057
Filename: LL-37_page-0001.jpg

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

1.5. Calculation of purity of peptide

Purity of T.T.-37 in sample was determined by area normalization method as ratio of T.T.-37 peak and sum of all peaks detected at wavelength 214 nm.

$$Purity(\%) = \frac{Peak\ Area_{T.T.-37}}{\sum Peak\ Area}$$

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Attachment for Lyo-0057
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Analysis Report

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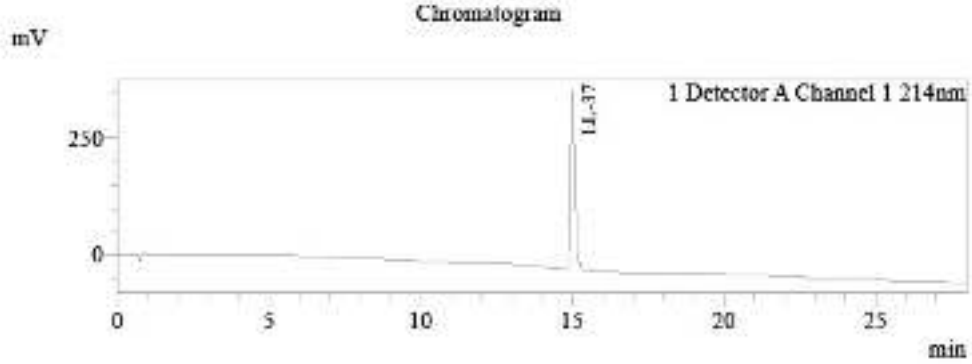
Sample Information

Injection Volume	: 2
Data File	: LYO-0057-P04_002_008.lcd
Method File	: Peptide screening.lcm
Date Acquired	: 30.06.2025 19:19:56

Chromatogram



Peak Table

Detector A Channel 1 214nm

Peak#	Name	Ret. Time	Conc.	Unit	Area%
1	LL-37	15,006	5,130	mg	100,000
Total			5,130		100,000

Attachment for Lyo-0057
Filename: LL-37.jpg

LIQUILABS	**Method Specification**	
Determination of bacterial endotoxin content of lyophilized samples		
Document number ENDOTOX_001_2015	*Superseded document* -	*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 Chromogenic LAL Endotoxin Assay kit

1.2. Chemicals

- LAL Reagent water (endotoxin free)
- Limulus Amebocyte 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/site?/document/5293_20080605231827.PDF

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Attachment for Lyo-0057
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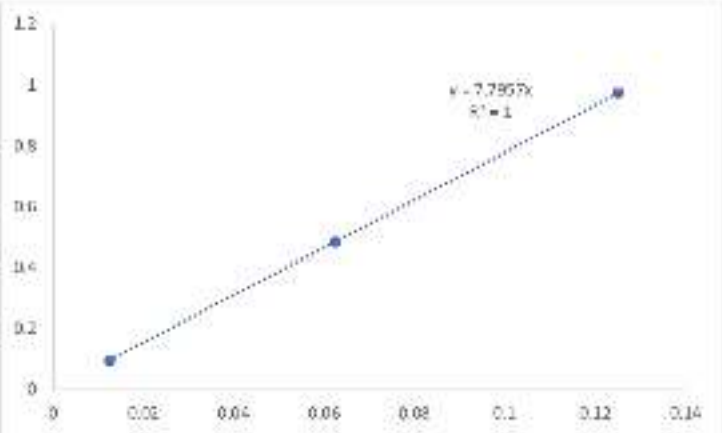
1.5. Measurement procedure

	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	0.6	0.6	0.6
Color Stabilizer #2 solution	0.5	0.5	0.5
Color Stabilizer #3 solution	0.6	0.6	0.6
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:

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

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-0057
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LIQUILABS	Method Specification	
Determination of bioburden of lyophilized samples		
[illegible] MS_[illegible]_2025	[illegible] -	[illegible] 2

1. Instrumentation and chemicals

1.1 Instruments used

- Sterile Syringe 2ml Luer
- Sterile needles
- Ready made PCA Plate ROTI Aquatest
- Ready made Sab 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 sterile 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 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 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 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 [illegible] for [illegible]

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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 5 seconds to sterilize.
3. Consequently 1 mL of solution was poured into the ready to use sterile petri dish filled with SAB agar and petri dish was closed.
4. Process 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 SAB 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 and [illegible] and result per 1g of sample is determined as:

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

CFU_{avg} = average CFU counted from n inoculations
CFU_n = CFU counted per inoculation
n = number of inoculations

$$CFU\ per\ g\ of\ sample = \frac{CFU_{c.u.}}{m_s} \times DF$$

$CFU_{c.u.}$ = Average CFU counted from n inoculations
m_s = mass of sample (mg)
DF = Dilution factor

The negative control sample is [illegible] process have to be repeated due to possible contamination in the process of inoculation or incubation.

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Attachment for Lyo-0057
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Responsibles

Mr. Ján Galbavý
Founder/Manager

Analysis results relate only to the samples tested.
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