Certificate Of Analysis

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Analytical services

Client:

V.O.F. **Buy Peptides**
Buy Peptides
16192 Coastal Hwy
Lewes, DE 19958
USA
support@buypeptides.store

Laboratory:

Liquilabs s.r.o.
Ohradní 24B
14000 Michle
Czechia
www.liquilabs.cz

Sample Identification

Sample Name	FoxO4 10mg	**Batch Number**	GF082025009	**Date Published**	2025-10-12 19:07

Results for Lyo-0127

Analysis of Peptide Identity, Content and Purity	Result	Unit	Uncertainty	Reporting Limit
FOX-04 DRI Assay Peptide Screening	10.57	*mg*	[± 0.05]	
FOX-04 DRI Identification by RT Peptide Screening	0.997		[± 0.005]	
FOX-04 DRI Identification by spectrum Peptide Screening	998		[± 20]	
FOX-04 DRI 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 Chromgenic 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
Cobalt Elemental Impurities Screening	Not detected	*ppm*		>= 25
Lead Elemental Impurities Screening	Not detected	*ppm*		>= 1.5

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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	⚠

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Attachments for Lyo-0127

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LIQUILABS Analytical services	Method Specification	
Determination of identity, content and purity of FOX-04 DRI		
Document number FOX_010_2025	*Superseded document* -	*Number of pages* 3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	60ºC
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-0127
Filename: 1760291583468-0be22149-b5be-42bd-8c5c-8906f5d24157_1.jpg

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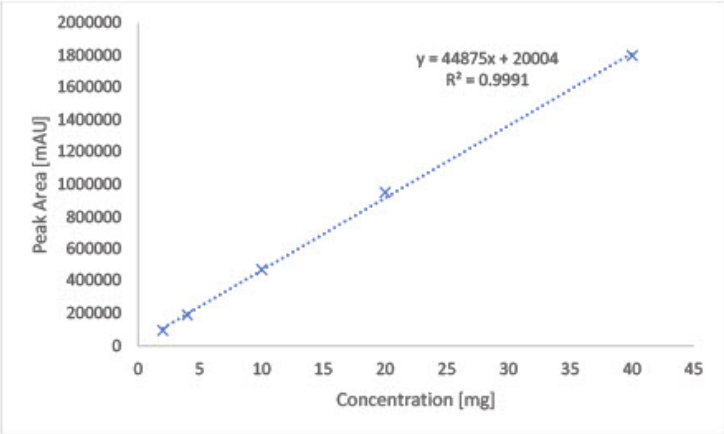
1.3. Sample preparation

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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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	

2.3 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.

2.4 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.

Attachment for Lyo-0127
Filename: 1760291583468-0be22149-b5be-42bd-8c5c-8906f5d24157_3.jpg

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Analysis Report

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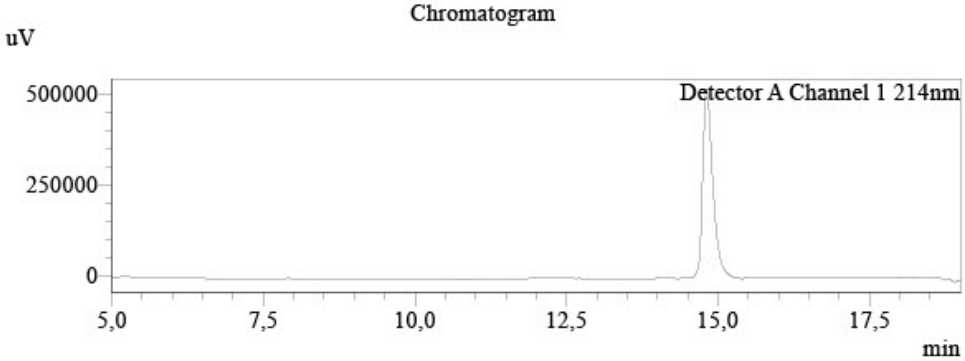
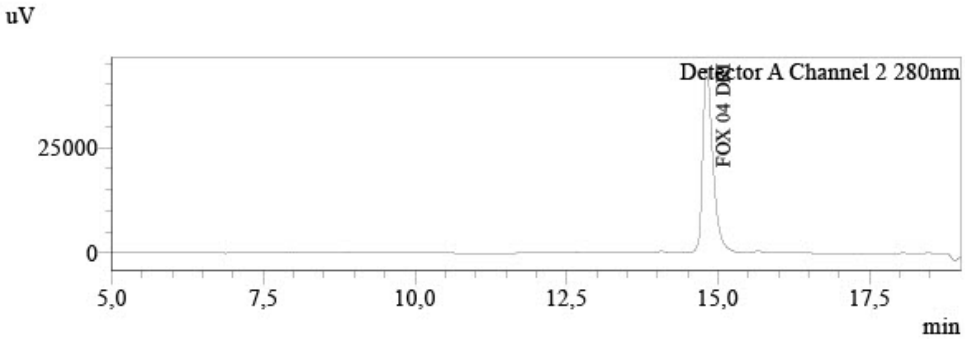
Sample Information

Injection Volume	: 0,5
Data File	: LYO-0127-P01__014.lcd
Method File	: Peptide screening_V10_Group C.lcm
Date Acquired	: 06.10.2025 7:11:48

Chromatogram





Peak Table

Detector A Channel 1 214nm

Peak#	Name	Ret. Time	Conc.	Unit	Area%
1		14,812	0,000		100,000
Total					100,000

Peak Table

Detector A Channel 2 280nm

Peak#	Name	Ret. Time	Conc.	Unit
1	FOX 04 DRI	14,813	10,570	mg
Total				

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Filename: LYO-0127.jpg

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LIQUILABS Analytical services	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 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.

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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-0127
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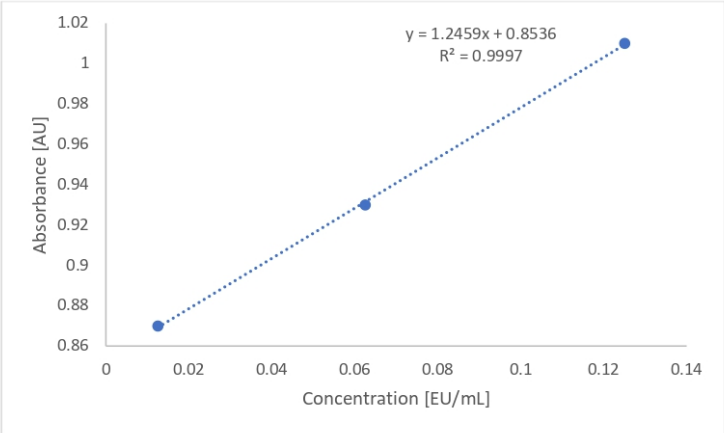
1.5. Measurement procedure

	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	0.5	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:

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

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

2

Attachment for Lyo-0127
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Mr. Ján Galbavý
Founder/Manager

Analysis results relate only to the samples tested.
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