Certificate of Analysis

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Analytical services

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Verify Results Online

Sample Identification

Sample Name	TB-500 20 mg
Batch Number	GF-TB50020-B242
Date Published	2026-11-10 11:53

Results for LYO-1175

Peptides	Result	Unit	Uncertainty	Acceptable Range
Thymosin Beta 4 (TB-500) Assay Peptide Screening 0.1% TFA	21.3	*mg*	[± 0.1]	
Thymosin Beta 4 (TB-500) Purity Peptide Screening 0.1% TFA	98.4	*%*	[± 0.5]	
Thymosin Beta 4 (TB-500) Identification by Spectrum (FTIR) Peptide Screening 0.1% TFA	981		[± 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	0	*CFU/g*	[±]	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	0.008	*EU/mg*	[± 0]	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
Nickel Elemental Impurities screening USP ⟨232⟩ / Ph. Eur. 5.20 / 2.4.20	< 0.001	*ppm*		0 - 25
Vanadium Elemental Impurities screening USP ⟨232⟩ / Ph. Eur. 5.20 / 2.4.20	< 0.001	*ppm*		0 - 25
Cobalt Elemental Impurities screening USP ⟨232⟩ / Ph. Eur. 5.20 / 2.4.20	< 0.001	*ppm*		0 - 25

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

Counter Ion Analysis	Result	Unit	Uncertainty	Acceptable Range
Acetate Counter Ion Analysis	3.94	*ppm*	[± 0.08]	
Bromide Counter Ion Analysis	0.51	*ppm*	[± 0.01]	
Chloride Counter Ion Analysis	0.16	*ppm*	[± 0]	
Fluoride Counter Ion Analysis	0.00	*ppm*	[±]	
Nitrate Counter Ion Analysis	0.08	*ppm*	[± 0]	
Phosphate Counter Ion Analysis	0.00	*ppm*	[±]	
Sulphate Counter Ion Analysis	0.00	*ppm*	[±]	
TFA Counter Ion Analysis	0.00	*ppm*	[±]	

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Excipients	Result	Unit	Uncertainty	Acceptable Range
Mannitol	82.75	*mg*	[± 1.66]	

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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% DFA in Acetonitrile (HPLC, Gradient Grade)
Flow rate	0.4 mL/min
Program	Gradient elution
Injection volume	1 µL
Colum Temperature	60°C
Column	Waters ACQUITY CSH C18, 100x2.1mm 1.7µm
Detection wavelength	225nm

Gradient Program		
Time [min]	A [%]	B [%]
2	98	2
10	35	65
10.01	5	95
11	5	95
11.01	98	2
15	end	

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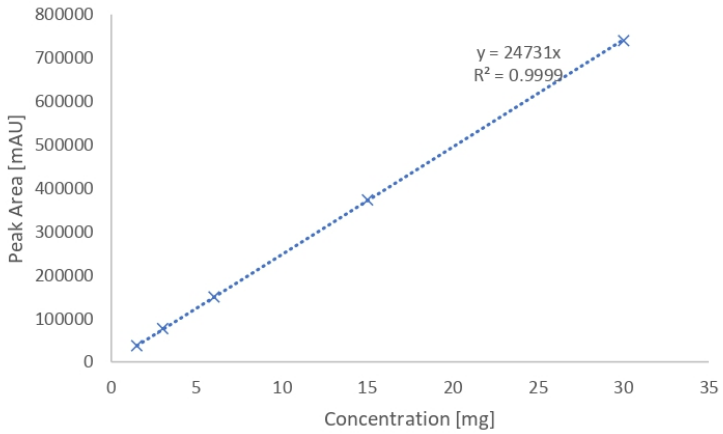
1.3. Sample preparation

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



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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% DFA in Acetonitrile (HPLC, Gradient Grade)
Flow rate	0.4 mL/min
Program	Gradient elution
Injection volume	1 µL
Colum Temperature	60°C
Column	Waters ACQUITY CSH C18, 100x2.1mm 1.7µm
Detection wavelength	225nm

Gradient Program		
Time [min]	A [%]	B [%]
2	98	2
10	35	65
10.01	5	95
11	5	95
11.01	98	2
15	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.

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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% DFA in Acetonitrile (HPLC, Gradient Grade)
Flow rate	0.4 mL/min
Program	Gradient elution
Injection volume	1 µL
Colum Temperature	60ºC
Column	Waters ACQUITY CSH C18, 100x2.1mm 1.7µm
Detection	MS 220-2000 Da

Gradient Program		
Time [min]	A [%]	B [%]
2	98	2
10	35	65
10.01	5	95
11	5	95
11.01	98	2
15	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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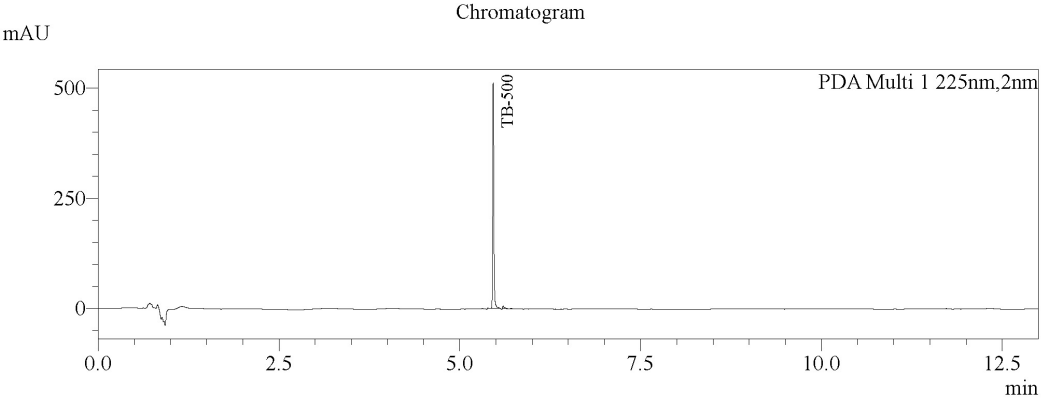
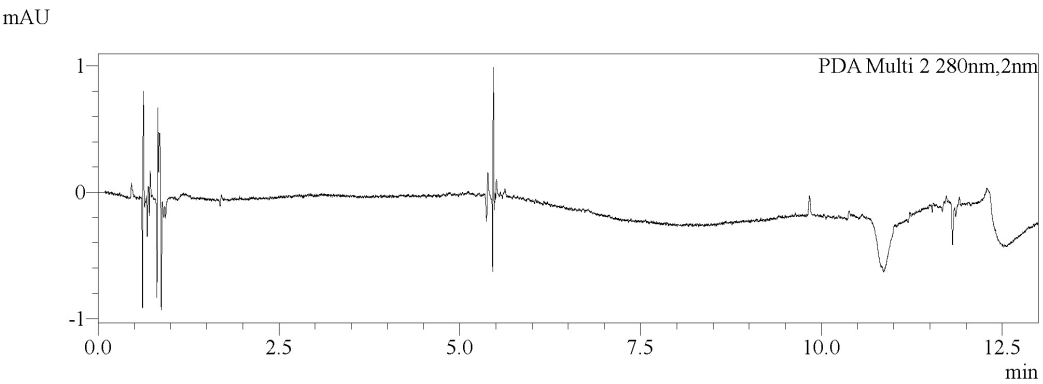
Analysis of quantity of active ingredient by UHPLC with MS detection

Sample Information

Injection Volume	: 1
Data File	: LYO-1175__005.lcd
Method File	: Peptide Screening_DFA_V2_C.lcm
Date Acquired	: 11/2/2026 6:40:17 PM

Chromatogram





Peak Table

PDA Ch1 225nm

Peak#	Name	Ret. Time	Area	Area%	Conc.	Unit
1	TB-500	5.462	577679	98.381	21.288	mg
2		5.601	6649	1.132	0.000	
3		5.629	1557	0.265	0.000	
4		5.711	1301	0.222	0.000	
Total			587187	100.000		

Peak Table

PDA Ch2 280nm

Peak#	Name	Ret. Time	Area	Area%	Conc.	Unit
Total						

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Analysis Report

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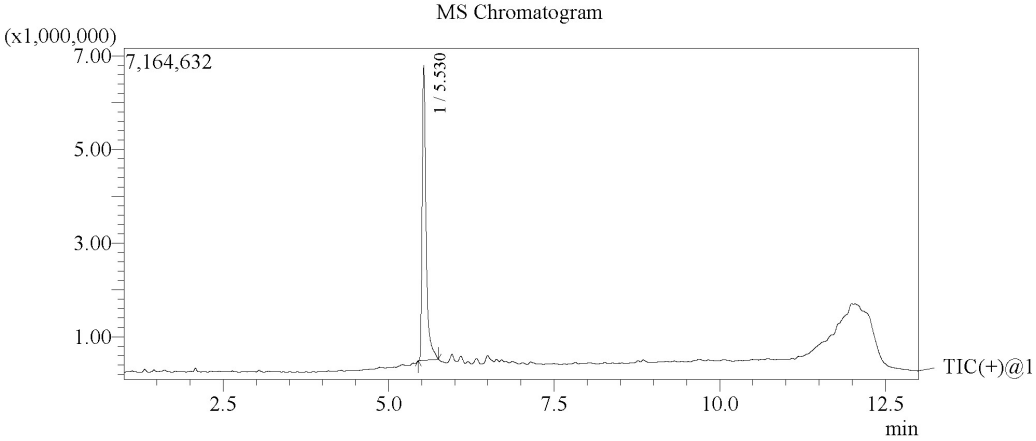
Identification of active ingredient by UHPLC Mass Spectrometr

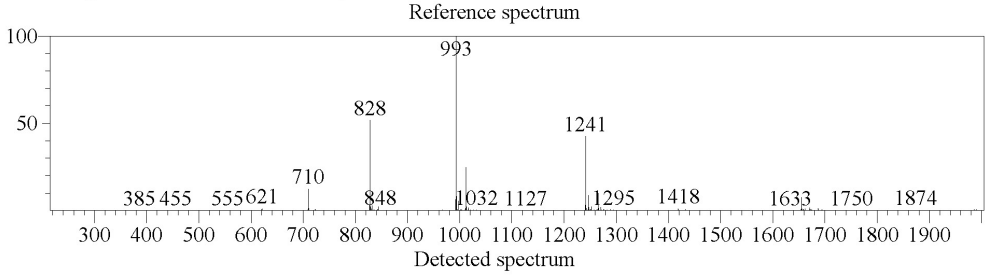
MS Chromatogram



Library Search

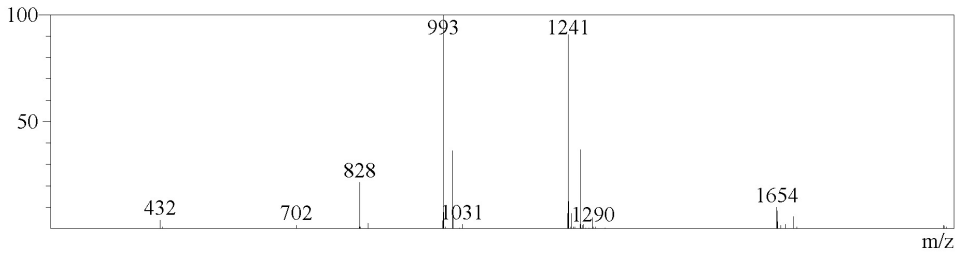
Base Peak: 993(1767747)
Scan Range: 220.00 - 2000.00 Porality: Positive

Reference spectrum



Detected spectrum

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



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Analysis Report

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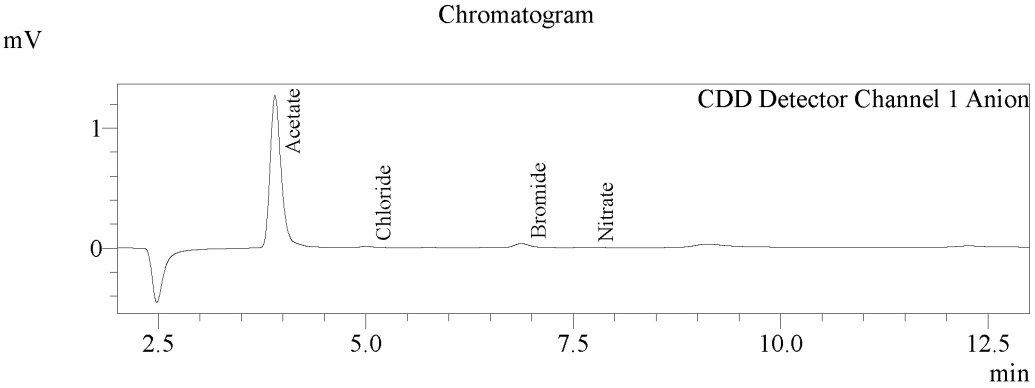
Determination of anions and cations in peptide sample

Sample Information

Injection Volume	: 10
Data File	: LYO-1175__004.lcd
Method File	: Anions+Cations.lcm
Date Acquired	: 11/3/2026 2:19:26 PM

Chromatogram



Peak Table

CDD Detector Channel 1 Anion

Name	Ret. Time	Area	Conc.	Unit
Acetate	3.903	12013	3.943	ppm
Chloride	5.000	95	0.160	ppm
Bromide	6.870	422	0.505	ppm
Nitrate	7.683	32	0.079	ppm
		12563		

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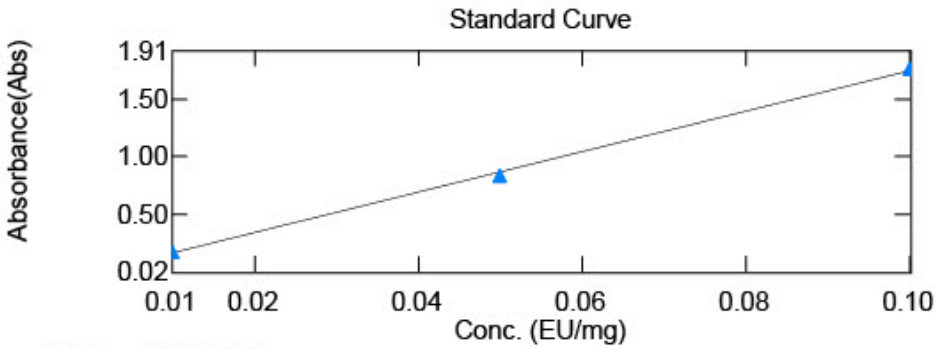
Endotoxin Determination Report

File Information
Filename: C:\UVVis-Data\Data\File_261102_102508.vqud
Date/Time: 11/02/2026 11:13:25 AM

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



y = 17.4393 x - 5.62824e-004
r2 = 0.99905

	Sample Name	Conc	Raw_WL545.0	Dilution Rate
1	LYO-1170	3.258	1.4198	40.00
2	LYO-1171	0.098	0.0845	20.00
3	LYO-1174	0.492	0.4285	20.00
4	LYO-1175	0.162	0.1403	20.00
5	LYO-1176	0.093	0.0806	20.00
6	LYO-1177	0.108	0.0934	20.00
7	LYO-1178	0.114	0.0987	20.00
8	LYO-1179	0.116	0.1004	20.00
9	LYO-1180	0.101	0.0876	20.00
10	LYO-1181	0.441	0.3839	20.00
11	LYO-1182	0.105	0.0907	20.00
12	LYO-1183	0.147	0.1279	20.00
13	LYO-1169	0.095	0.0823	20.00
14	RAW-0013	0.127	0.1098	20.00
15	RAW-0014	0.167	0.1452	20.00
16	LIQ-0101	0.004	0.0759	1.00
17	LIQ-0102	0.014	0.2467	1.00
18	LIQ-0103	0.009	0.1590	1.00
19	LIQ-0104	0.006	0.1026	1.00
20	LIQ-0105	0.006	0.1044	1.00

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Mannitol content determination

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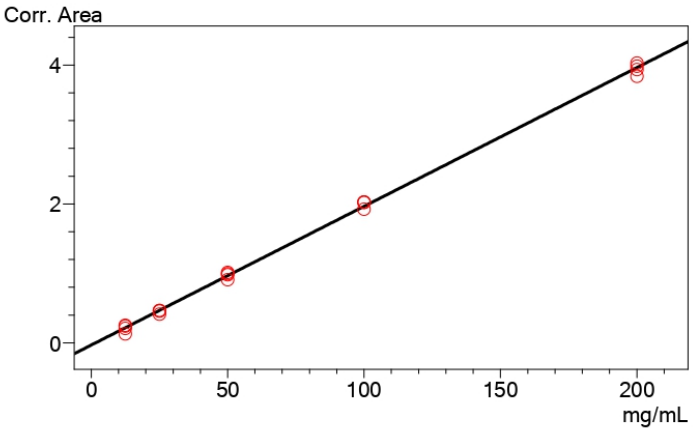
	Parameters	Value
1	Compornent	Mannitol
2	Unit	mg/mL
3	MSC	OFF
4	Preprocessings	Smoothing, Points=43
5	Quantitation method	Peak area
6	Name	Area01
7	Equation	PeakArea(967.328,1150.000,BC:ON)
8	Calibration parameters	Multi point method
9	Order	1st
10	Origin	Ignore
11	Correlation coefficient	r2



Spectrum name	Concentration	Value	Date	Time	Comment
LYO-11741 minus blank1	38.904	0.745	11/3/2026	10:18:31 AM	
LYO-1175 minus blank1	41.378	0.795	11/3/2026	10:21:51 AM	
LYO-1176 minus blank1	42.625	0.820	11/3/2026	10:24:44 AM	
LYO-1177 minus blank1	44.739	0.862	11/3/2026	10:28:26 AM	
LYO-1178 minus blank1	40.543	0.778	11/3/2026	10:31:18 AM	
LYO-1179 minus blank1	43.370	0.835	11/3/2026	10:34:12 AM	
LYO-1180 minus blank1	66.071	1.288	11/3/2026	10:37:11 AM	
LYO-1181 minus blank1	59.347	1.154	11/3/2026	10:40:13 AM	
LYO-1182 minus blank1	90.333	1.773	11/3/2026	10:42:40 AM	
LYO-1183 minus blank1	60.412	1.175	11/3/2026	10:45:31 AM	

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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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Responsibles



Mr. Ján Galbavý
CEO

Analysis results relate only to the samples tested.
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