MEDICERT
TESTING LABORATORY
ISO/IEC 17025:2017
IAKS-TL-1018

ISO 10993-5:2010
In vitro Cytotoxicity Test

IAKS ACCREDITED

Company Name Firma Adı	**AVAS KOZMETİK SANAYİ VE TİCARET LİMİTED ŞİRKETİ**
Company Address Firma Adresi	**GÖKEVLER MAH. 2331 SK. PASİAD NO. 1 D ESENYURT - İSTANBUL / TÜRKİYE**
Test Name Testin Adı	**In vitro Cytotoxicity Test**
Test Standard Test Standardı	**TS EN ISO 10993-5:2010**
Commercial Brand (If You Have) Ticari Marka (Varsa)	**Felix Filler**
Description of the Sample Numunenin Adı ve Tarifi	**1 cc Felix Filler Dolgu**
Lot Number Lot Numarası	**AVAS012023-1**
Sample Registration Number Numune Kayıt Numarası	**FLXFHA/202302**
Sample Acceptance Date Numune Kabul Tarihi	**27.02.2023**
Report Number Rapor Numarası	**2023-03/BIYO/1532HA-VDS**
Date of Report Rapor Tarihi	**09.03.2023**
Date of Test Deney Tarihi	**01.03.2023 - 08.03.2023**
Report Total Page Raporun Sayfa Sayısı	**9 Page /** ***Sayfa***

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INDEX

SUMMARY

Biocompatibility testing according to TS EN ISO 10993-5 Biological evulation of medical devices - Part 5 : Extracorporeal cytotoxicity tests was performed on 1 cc Felix Filler Dolgu sample number AVAS012023-1. Samples are prepared in accordance with TS EN ISO 10993-12 Biological evaluation of medical devices - Part 12: Sample preparation and extraction according to the reference materials standard. L929 cells were seeded in 96-well plates and kept in culture for 24 hours to farm a semi-confluent monolayer. After 24 hours of exposure, the formation of the forest was determined for each treatment concentration and compared with the results determined in the control cultures. Growth inhibition percentage was calculated for each treatment. **There was no evidence of cytotoxic effect on the cells** of the sample extract under these working conditions.

1. INTRODUCTION

Purpose : This test was performed to evaluate the viability of the cells by mitochondrial dehydrogenases in the case of indirect contact of the sample described below with mammalian cells.

Test Guide: This study was conducted according to the requirements of the International Organization for Standardization. 10993: Biological Assessment of Medical Devices, Part 5: Extracorporeal cytotoxicity assays.

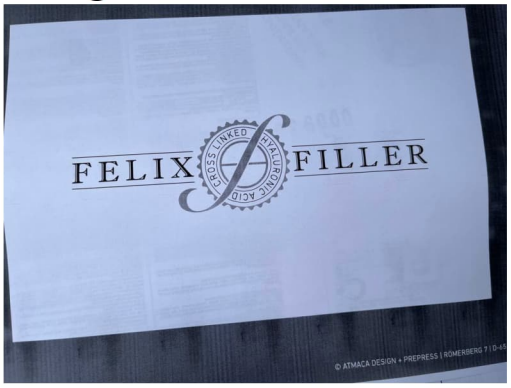
Dates

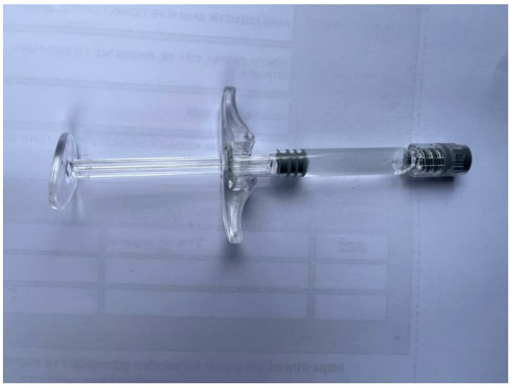
Sample Acceptance Date : 27.02.2023
Test Date : 01.03.2023
Observation Date : 01.03.2023 - 08.03.2023

2. SAMPLE INFORMATION

Company Name :	Avas Kozmetik
Date of the Sample Acceptance :	27.02.2023 13.05
Sample Record Number :	FLXFHA/202302
Sample Lot Number :	AVAS012023-1
Number of Sample :	4
Packaging Infırmation :	CLOSED PACKED
Sample Delivery Method :	CARGO
Expiration Date of the Sample :	17.01.2028
Production Date of the Sample :	17.01.2023
Description of the Sample :	Bir şırınga 1 cc Felix Filler dolgu maddesi içermektedir.
Characteristics of the Sample Use/Application :	Cross linked hyaluronic acid

Sample Image :





3. TEST SYSTEM

Cell Line Used in the Test Line :	The L2929 (Mouse Fibroblast Cell) cell line was used.
Culture Medium :	DMEM+L-Glutamine, Fetal Bovine Serum, Penicillin-Streptomycin
Blank :	Sterile cell culture medium
Negative Control :	Poliethylene Cryo Tube + Cells
Positive Control :	Lanolin + Cells

4. EXTRACTION METHOD

Extraction Standard :	Extraction of the sample is carried out as specified in TS EN ISO 10993-12 standard.
Extraction Procedure :	Samples taken from the sample are kept in a water bath that oscillates at a speed of 50 rpm at 37℃ for 24 hours in 10% serum cell medium at the dimensions specified in the standard, extraction is terminated and used within 24 hours.

5. MATERIALS

Devices and Raw Materials :	-20°C deep freezer, -80°C deep freezer, Laminar Air Cabinet, Centrifuge, Micropipette, Invert Microscope Liquid Nitrogen Tank, Carbon Dioxide Incubator, Vortex (Stirrer)
Chemicals, Standard Materials and Reagents :	MEM, DMEM, TOX2 in vitro toxicology assay kit, XTT based, L-glutamine, Penicillin-streptomycin, Trypsin/EDTA, DMSO (Dimethyl Sulfoxide), FBS (Fetal Bovine Serum), Phosphate Buffer Saline, Hemocytometer slide, Sodium Hydrogen Bicarbonate

6. METHOD

6.1. Qualitative Evaluation

★ Cells were seeded in 96-well plates and allowed to become confluent.

★ In the next stage; Cells were exposed to control + control and sample extracts in a 37℃ 5% CO_2 incubator for 24 hours.

★ After incubation, microscopic examination was performed and evaluated according to TS EN ISO 10993-5 / XTT Cytotoxicity Test Standard.

6.2. Quantitative Evaluation

★ According to TS EN ISO 10993-5 / XTT Cytotoxicity Test Standard, 96/ well plates were counted as 100 / well and incubated for 24 hours to ensure 80% confluency.

★ In the next step, the sample was exposed to 1/1 - dilutions of the extract for 4 hours.

★ At the end of the process, 1 mg / mL XTT was added to the wells and plates were incubated for 3 hours in a 37℃ 5% CO_2 incubator.

★ The experiment was terminated by adding isopropyl alcohol to the wells.

★ Color change in plates was measured by spectrophotometer (570-650 nm) and % viability values were calculated.

7. EVALUATION

7.1. Qualitative Evaluation

Qualitative evaluation was made by taking into consideration Table 1 which was prepared considering TS EN ISO 10993-5 / XTT Cytotoxicity Test Standard.

Table 1. Qualitative morphological grading of cytotoxicity of extracts

DEGREE	REACTION	CULTURE STATE
0	-	★ Separate intracellular granules ★ No cell destruction ★ No reduction in cell proliferation
1	Very Little	★ More than 20% of the cells are non-round, poorly adherent, rarely contain cells that do not contain intracellular granules or show changes in morphology ★ Only mild growth inhibition is observed
2	Mild	★ The number of round cells is less than 50% ★ No intracellular granules ★ No excessive cell destruction ★ Observable cell inhibition not more than 50%
3	Moderate	★ The number of rounded and destroyed cells is not more than 70% of the cell layer ★ Cell layers are not completely fragmented ★ Observable cell inhibition more than 50%
4	Severe	★ All or almost all cell layers are destroyed

Table 2. - control, + control and sample reaction and qualitative evaluation

TEST MATERIAL	REACTION SCORE	QUALITATIVE CULTURES EVALUATION
NEGATIVE CONTROL	0	★ Separate intracellular granules ★ No cell destruction ★ No reduction in cell proliferation
POSITIVE CONTROL	4	★ All or almost all cell layers are destroyed
SAMPLE	0	★ Separate intracellular granules ★ No cell destruction ★ No reduction in cell proliferation

7.2. Quantitative Assessment

Table 3. XTT Test Results

DILUTION RATE		%100	%75	%50	%25
1 cc Felix Filler Dolgu	1st TRIAL	1,108	1,002	1,003	1,330
	2nd TRIAL	1,023	1,071	1,171	1,298
	3rd TRIAL	1,100	1,074	1,017	1,258
	MEAN	1,077	1,049	1,063	1,295
POSITIVE CONTROL	1st TRIAL	0,146	0,176	0,222	0,280
	2nd TRIAL	0,128	0,134	0,244	0,295
	3rd TRIAL	0,136	0,112	0,265	0,213
	MEAN	0,136	0,140	0,243	0,262
NEGATIVE CONTROL (%100 EKSTRACT)	1st TRIAL	1,138	-	-	-
	2nd TRIAL	1,155	-	-	-
	3rd TRIAL	1,156	-	-	-
	MEAN	1,149			

$$\text{Viab. \%} = 100 \times \frac{OD_{450e}}{OD_{450b}}$$

OD_{450e} : %100 optical density average value of the sample extract

OD_{450b} : Optical density average value of blanks

Test Sample Viab.% = %93

Positive Control Viab.% = %11

Negative Control Viab.% = %108

8. RESULT

Qualitative evaluation result; Based on the protocol and evaluation criteria specified in the standard TS EN ISO 10993-5 biological evaluation of medical devices, part 5, extracorporeal cytotoxicity assays; As indicated in Table 2, the negative control had no toxic effect on cells (0), the positive control had a high toxic effect (4), and the sample extract had no toxic effect. When the scoring indicated in Table 1 prepared in accordance with the criteria specified in the standard is taken into consideration, it has been determined that the test specimen **"1 cc Felix Filler Dolgu" does not have cytotoxic effect**.

Quantitative evaluation results; Based on the protocol and evaluation criteria specified in the standard TS EN ISO 10993-5 biological evaluation of medical devices, part 5, extracorporeal cytotoxicity assays; As stated in Table 3, when the negative control and positive control results are taken into consideration, it is seen that test validity criteria are met. In the experiment, the effects of 1/1 dilutions of the sample extract on the cells were examined and the viability was determined as 93% by the full dilution (1/1) of the sample extract. In line with the criteria specified in the standard; viability ratio was determined to be over 70% of the value obtained from the blind, so **the test specimen "1 cc Felix Filler Dolgu" was found to have no cytotoxic effect.**

9. RECORD

All raw data and a copy of the final report are stored in the Medicert archive files.

10. REFERENCES

- Guide for The Care and Use of Laboratory Animals Eighth Edition National Research Council of The National Academies
- TS EN ISO 10993-1 Biological evaluation of Medical Devices - Chapter 1: Evaluation and experiment in a risk management process
- TS EN ISO 10993-5 Biological evaluation of Medical Devices – Chapter 5: Extra-body cytotoxicity assays
- TS EN ISO 10993-12 Biological evaluation of Medical Devices Chapter 12: Sample preparation and reference materials

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