CERTIFICATI E TEST

ISO 10993-5:2009	" Tests for in vitro cytotoxicity"	nr. 4779-20en
ISO 10993-12: 2012	" Sample preparation and reference materials"	nr. 4779-20en
UNI EN ISO 10993 10:2013	" Test irritation and skin sensitization"	nr. 20/3988

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IN VITRO CYTOTOXICITY TEST

TEST ITEM NAME
Mascherina Salva Collega

TEST REPORT N° 4779-20 en

SPONSOR/CUSTOMER

Microna S.r.l.
V.le Enrico Thovez, 26
10131 Torino
Italy

On behalf of

Cm3 S.r.l.
Zona Industriale, 11
10090 Trana (TO)
Italy

Test carried out by

Lab4LIFE S.R.L.
Via dei Fornaciai, 21 g/h
40129 Bologna
(Italy)

Lab4LIFE S.R.L. P.IVA e C.F: 03096401207 Capitale Sociale € 45.000 i.v. N. REA: BO – 491522 - Sede legale Via dei Fornaciai 21 g/h 40129 Bologna Tel. 051323039 FAX. 0514170410

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Test item description[1]
Test item Name: Mascherina Salva Collega
Code: n/a
Lot: n/a
Weight of the sample: 15,79 g
Appearance of the sample: Transparent mask
Category of the body contact: Intact skin
Duration of the body contact: ≤ 24h
Sterilization: No
Manufacturing date: n/a
Expiration date: n/a
Manufactured by: Cm3 S.r.l., Zona Industriale 11 – 10090 – Trana (TO)
Sampling carried out by: Sponsor
Notes: //

Timing and analysis details
Number of tested samples: 1
Receipt Number: 200720-58
Receipt date: 20/07/2020
Test started on: 21/07/2020
Test completed on: 24/07/2020
Test carried out by: Elisabetta Longo
Notes: //

Test Method and Normative References

- ISO 10993-5:2009 "Tests for in vitro cytotoxicity"
- ISO 10993-12: 2012 "Sample preparation and reference materials"

Summary of practice
Cell cultures are grown to a near-confluent monolayer in cultures dishes. Three dishes for each sample are prepared. Moreover, three dishes are prepared for the Negative control, for the Positive control and for the Extraction solvent control. In the dishes to be treated with the sample, the medium is aspirated and replaced with test extract. Cell cultures are examined microscopically after 24 and 48h-contact to assess the presence or absence of cytotoxic effects due to the test extract.

Target cells: BSCL 56/L929 (mice connective tissue)

Extraction conditions: a representative part of the sample having a total external surface of 48 cm^2 was extracted with 16 ml of Cell Culture Medium MEM at 37°C for 24 hours under agitation at 150 RPM. Extraction ratio 3 cm^2/ml as per ISO 10993-12. The extract was used immediately after preparation.

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Reagents: MEM with Earle's salts added with foetal bovine serum, L-glutamine and some antibiotics.
Positive control: Latex gloves Pic Indolor, lot 1007097322 (internal code K⁺ cito 0212) treated as the sample
Negative control: Riblene MR10 (internal code: K⁻ cito 0712) treated as the sample

Incubation: The dishes treated with the Test extract, with the Positive and Negative controls and with the Extraction solvent control are incubated for 48 h at (37±1)°C in a 5% CO_2 atmosphere.

Apparatus

- Incubator, which maintains the cultures at 37°C, 5% CO_2;
- Microscope, with inverted phase contrast optics;
- Water Bath;
- Laminar Flow Cabinet;
- Sterile Disposable;
- Tissue Culture Dishes;
- Stirrer.

Interpretation of Results: The determination of the cytotoxicity is performed after a 24 and 48 h incubation period examining the cells under the microscope to assess general morphology, vacuolation, detachment, cell lysis, membrane integrity. The change from normal morphology of the Negative control is rated on a reactivity grade from 0 to 4 (see Grading system). Moreover, for the dishes treated with the Test extract the confluence of the monolayer is evaluated and the colour of test medium is compared to the negative control

Grading system

Grade	Reactivity	Reactivity description
0	None	Discrete intracytoplasmic granules; no cell lysis.
1	Slight	Not more than 20% of the cells are round, loosely attached and without intracytoplasmic granules; occasional lysed cells are present
2	Mild	Not more than 50% of the cells are round and devoid of intracytoplasmic granules; no extensive cell lysis and empty areas between cells
3	Moderate	Not more than 70% of the cell layers contain rounded cells or are lysed
4	Severe	Nearly complete destruction of the cell layers

The achievement of a numerical grade greater than 2, based on the reactivity table, is considered a cytotoxic effect

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RESULTS AFTER 24H INCUBATION	SCORE
Positive control	4
Positive control	4
Positive control	4
Negative control	0
Negative control	0
Negative control	0
MEM control	0
MEM control	0
MEM control	0
Extract of the test item	0
Extract of the test item	0
Extract of the test item	0
Confluency of the monolayer	Confluent
Colour of test medium	Comparable to the negative control

RESULTS AFTER 48H INCUBATION	SCORE
Positive control	4
Positive control	4
Positive control	4
Negative control	0
Negative control	0
Negative control	0
MEM control	0
MEM control	0
MEM control	0
Extract of the test item	0
Extract of the test item	0
Extract of the test item	0
Confluency of the monolayer	Confluent
Colour of test medium	Comparable to the negative control

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MatTek Corporation

EpiDerm QC (EPI-200)

LOT 30882
TESTED Post Refrigerated Storage
COMMENTS NO
TESTING DATE 29-Jul-2020

Dosed with: 1.0% Triton X-100 (100uL)

Exposure Time (hr)	Well	OD	MTT (OD)	Std Dev (OD)	Viability %	Std Dev (%)
4	1	1.0423	1.113	0.112	60.5	6.1
	1	1.2419				
	1	1.0535				
6	1	0.8301	0.796	0.044	43.3	2.4
	1	0.7458				
	1	0.8108				
8	1	0.0888	0.067	0.025	3.7	1.4
	1	0.0729				
	1	0.0401				
10	1	0.0292	0.034	0.004	1.8	0.2
	1	0.0369				
	1	0.0352				
H20 (4 hr)	1	1.6884	1.838	0.140	100.0	7.6
	1	1.966				
	1	1.8584				

Avg. cv (%): 14.4 8.5 Exp. Cv (%): 11.4

ET-50 (hr): 5.13

EPIDERM (EPI-200) Acceptance Criteria
1996 EpiDerm Database (n=184)

	TR (hr)	SDS (hr)	H2O (OD)	H2O CV	Exp CV
avg	6.74	0.92	1.47	4.6	9.6
s.d.	0.99	0.23	0.13		
c.v.	14.60	24.60	8.80		
avg +2*sd	8.72	1.38	1.73		
avg - 2*sd	4.77	0.47	1.21		

QC Evaluation: PASS
Initials: MS
Date: 7/29/2020

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CONCLUSIONS

The cells treated with the Test extract after 24 hours and 48 hours of incubation do not show any changes from normal morphology of the Negative control. The Test extract does not show any reactivity.

The sample can be considered not cytotoxic

Notes:
[1]Informations provided by the Customer. The Laboratory declines all responsibility for the data provided by the Customer which may influence the validity of the results.
This Test Report refers exclusively to the sample examined as received.
This Test Report cannot be partially reproduced, unless approved by Lab4LIFE S.r.l.

Issued: Bologna, 24/07/2020

Issue authorized by:
Head of the Laboratory
(Lorenzo Autore, Dr.)

Elisabetta Longo, Dr.
National Order of Biologists n° AA_077435

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Certificate of Analysis

MatTek IN VITRO LIFE SCIENCE LABORATORIES

Product: EpiDerm™ Reconstructed Human Epidermis

Lot Number: 30882

Part#: EPI-200, EPI-212, EPI-218
Description: Reconstructed human epidermis tissue containing normal human keratinocytes. This product is for research use only. Not for use in animals, humans or diagnostic purposes.

I. Cell source

All cells used to produce EpiDerm™ are purchased or derived from tissue obtained by MatTek Corporation from accredited institutions. In all cases, consent was obtained by these institutions from the donor or the donor's legal next of kin, for use of the tissues or derivatives of the tissue for research purposes.

Keratinocyte Strain: 00267

II. Analysis for potential biological contaminants

The cells used to produce EpiDerm™ tissue are screened for potential biological contaminants. Tests for each potential biological contaminant listed below were performed according to the test method given. Results of "Not detected" indicate that testing for the potential biological contaminant was not observed as determined by the stated test method.

HIV-1 virus - Oligonucleotide-directed amplification	Not detected
Hepatitis B virus - Oligonucleotide- directed amplification	Not detected
Hepatitis C virus - Oligonucleotide- directed amplification	Not detected
Bacteria, yeast, and other fungi - long term antibiotic, antimycotic free culture	Not detected

III. Analysis for tissue functionality and quality

Test	Specification	Acceptance criteria	Result and QA Statement	
Tissue viability	MTT QC assay, 4 hours, n=3	OD (540-570 nm) [1.0-3.0]	1.838 ± 0.14	Pass
Barrier function	ET-50 assay, 100 µL 1% Triton X-100, 4 time-points, n=3, MTT assay	ET-50 [4.77-8.72 hrs]	5.13 hrs	Pass
Sterility	Long term antibiotic and antimycotic free culture	No contamination	Sterile	Pass

Tissue viability and the barrier function test are within the acceptable ranges and indicate appropriate formation of the epidermal barrier, the presence of a functional stratum corneum, a viable basal cell layer, and intermediate spinous and granular layers. Results obtained with this lot conform to the requirements of the OECD TG 431 and 439.

Initials: SL
Date: 29.07.2020

Nelson Rivas
Quality Assurance Associate

July 29, 2020
Date

CAUTION: Whereas all information herein is believed to be correct, no absolute guarantee that human derived material is non-infectious can be made or is implied by this certificate of analysis. All tissues should be treated as potential pathogens. The use of protective clothing and eyeware and appropriate disposal procedures are strongly recommended.

MatTek In Vitro Life Science Laboratories
Mlynské Nivy 73, Bratislava - Slovakia
+421-2-3260-7401
www.mattek.com
information@mattek.com