eurofins | BioPharma Product Testing

Test Report

Analytical Report: AAM70831
Eurofins Sample Number: LV21AA1034-1
Version: 2

LAB N° 0032 L

XXXXXXXXXXXXXXXX
xxxxxxxxxxxxxxxxx
xxxxxxxxxxxxxxxxx
IT

Client Account Number: A00963855DQX
Eurofins Quote Number: PO9YPH21001801

Eurofins Sample Number LV21AA1034-1	
Original Received Date:	20-Jan-2021
Description:	§ BMP30-NWF-SB + PhotoACTIVE® TEX
Lot Number:	§ 1

Analysis	Result	Unit
# Citotossicità in vitro/In vitro cytotoxicity		
RESULT:	Not cytotoxic	----
Test item:	30mm²	----
Vehicle:	Routine medium	----
Positive control:	30mm² of Latex	----
Negative control:	30mm² of HDPE	----
Notes:	N/A	----

Addendum #1: Qualitative and quantitative evaluation

Method: ISO 10993-5:2009
Analysis Date: 01-Feb-2021 to 03-Feb-2021

LAB N° 0032 L

Supplemental Information

Analytical Report Version 2 Comment: The present test report LV21AA1034-1 Vers 2 supersedes the test report LV21AA1034-1 Vers 1 in order to modify the name of the sample, as requested by the Sponsor.

Sample description: white cloth

Storage: room temperature

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Contracted Company: Eurofins Biolab Srl (Vimodrone)

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Reviewed and electronically signed for Technical Supervisor Approval by
Elena Bizzotto, Employee
for Eurofins Biolab Srl, on 18-Feb-2021 15:47:57 UTC+01:00

eurofins	In vitro cytotoxicity ISO10993-5:2009	1-P-QM-TEM-9070229
	Test by Direct Contact - NRU	Addendum N. 1

Study ID: //

Sample ID: LV21AA1034-1

Test start: 01/02/2021

Test end: 03/02/2021

Cell line	Manufacturer
Mammal fibroblasts BALB/3T3 clone A31 (ATCC® CCL163™)	American Type Culture Collection (ATCC)

Reagent	Manufacturer	Batch
Dulbecco's Modified Eagle Medium (DMEM)	Lonza	0000920735
Fetal Bovine Serum (FBS)	Sigma-Aldrich	20C567
Penicillin/Streptomycin solution	Sigma-Aldrich	0000088375

QUALITATIVE EVALUATION

	Contact time: 24 h			
	PLATE 1			
	Blank	Vehicle control	Negative control	Test Sample
Replicate 1		0	0	0
Replicate 2		0	0	0
Replicate 3		0	0	0

	Contact time: 24 h			
	PLATE 2			
	Blank	Vehicle control	Positive control	Test Sample
Replicate 1		0	4	0
Replicate 2		0	4	0
Replicate 3		0	4	0

INTERPRETATION OF RESULT

Grade	Reactivity	Conditions of all Cultures
0	None	No detectable zone around or under specimen
1	Slight	Some malformed or degenerated cells under specimen
2	Mild	Zone limited to area under specimen
3	Moderate	Zone extending specimen size up to 1.0 cm
4	Severe	Zone extending farther than 1.0 cm beyond specimen

ACCEPTANCE CRITERIA

Negative control	
Grade ≤1	VALID

Positive control	
Grade ≥3	VALID

RESULTS

	Reactivity grade
Test Sample	0

eurofins	In vitro cytotoxicity ISO10993-5:2009	1-P-QM-TEM-9070229
	Test by Direct Contact - NRU	Addendum N. 1

Study ID: // Sample ID: LV21AA1034-1

Test start: 01/02/2021 Test end: 03/02/2021

QUANTITATIVE EVALUATION - 540 nm

	Contact time: 24 h			
	PLATE 1			
	Blank	Vehicle control	Negative control	Test Sample
Replicate 1	0,051	1,131	1,117	1,100
Replicate 2	0,052	1,116	1,096	1,103
Replicate 3	0,045	1,154	1,164	1,195

	Contact time: 24 h			
	PLATE 2			
	Blank	Vehicle control	Positive control	Test Sample
Replicate 1	0,049	1,100	0,046	1,107
Replicate 2	0,051	1,110	0,049	1,170
Replicate 3	0,050	1,153	0,044	1,174

MEAN, STANDARD DEVIATION, CV% AND VIABILITY

	Mean OD	Standard Deviation	CV %	Mean OD - Mean OD Blanks	Viability %
Blanks	0,050				
Vehicle control	1,127	0,023	2,007	1,078	100
Negative control	1,126	0,035	3,093	1,076	100
Positive control	0,046	0,003	5,432	-0,003	0
Test Sample	1,142	0,043	3,743	1,092	101

ACCEPTANCE CRITERIA

	Vehicle ≥ 0,3
Mean OD	VALID

	Negative control ≥ 70%	Positive Control < 70%
Quantitative Evaluation	VALID	VALID

	Vehicle control	Negative control	Positive control	Test Sample
CV between replicates ≤ 15%	VALID	VALID	VALID	VALID

$$\%\,viability = \frac{OD_{\underset{sample}{mean}} - OD_{\underset{blank}{mean}}}{OD_{\underset{vehicle}{mean}} - OD_{\underset{blank}{mean}}} \times 100$$

INTERPRETATION OF RESULTS

Reduction of Viability	Result
≤ 30%	Not Cytotoxic
> 30%	Cytotoxic

RESULTS

	Reduction Viability %	
Test Sample	0	NOT CYTOTOXIC

*0 (% viability test item ≥ vehicle); 100 (% viability test item ≤ Blank)

Finished on: 03/02/21 Technician signature: [signature]

Date: 04/02/21 Approved by: EP

eurofins | Medical Device Testing	Test Facility Eurofins Biolab S.r.l.	Sample ID: LV21AA1034-1 Page: 1 of 1

ADDENDUM N.2: EXPERIMENTAL REPORT

REFERENCE/GUIDELINE:	ISO 10993-5:2009 - In vitro cytotoxicity	
CELL LINE	Mammal fibroblasts BALB/3T3 clone A31 (ATCC®; CCL163™) Source: ATCC.	
MATERIALS	Fetal Bovine Serum (FBS), Neutral Red dye, Trypan Blue, Penicillin/Streptomycin solution, Dulbecco's Phosphate buffer solution (DPBS)	Sigma-Aldrich
	Dulbecco's Modification of Eagle's Medium (DMEM), Trypsin-EDTA	Lonza
	Acetic Acid, Ethanol solution	VWR
	Water for Injection	Eurospital
	High density polyethylene (HDPE, USP Reference Standard negative control)	Nova Chimica (lot.K0M357)
	Latex from laboratory gloves	Artsana (lot.1811191C170)
EQUIPMENT	Laminar flow hood, CO_2 incubator, Microplate reader Mod EL800, Chronometer, Common laboratory equipment, Water, Inverted Microscope Diavert,Orbital shaker, Refrigerator	

EXPERIMENTAL DESIGN

The experimental design included two 12-well plate containing a subconfluent cell monolayer subdivided in the following groups:

GROUPS	REPLICATES PLATE N.1			REPLICATES PLATE N.2		
	Blank	Blank	Blank	Blank	Blank	Blank
	Vehicle	Vehicle	Vehicle	Vehicle	Vehicle	Vehicle
	Negative control	Negative control	Negative control	Positive control	Positive control	Positive control
	Test sample	Test sample	Test sample	Test sample	Test sample	Test sample

BLANK	Supplemented culture medium alone (without cells).
VEHICLE	Supplemented culture medium (without test sample).
TEST SAMPLE	**30 mm²** of the test sample were placed in the middle of each well.
NEGATIVE CONTROL	The negative control was represented by **30 mm²** of HDPE placed in the middle of each well.
POSITIVE CONTROL	The positive control was represented **30 mm²** of latex placed in the middle of each well.

TREATMENT: Verified that a subconfluent monolayer was present, supplemented culture medium was replaced with 1mL of fresh supplemented culture medium and the test sample was added. The plates were incubated in a thermostat at (37 ± 1)°C in (5±1)% CO_2 atmosphere for 24 hours. This procedure was repeated for vehicle, negative and positive controls.

QUALITATIVE EVALUATION (GRADE OF CYTOTOXICITY): After 24 hours the plates were observed under an inverted microscope and biological reactions were evaluated following a 0 to 4 scale according to ISO10993-5:2009.

QUANTITATIVE EVALUATION (OPTICAL DENSITY): After microscopic observation, cells were treated with Neutral Red Medium for 3 hours at (37 ± 1)°C in (5±1)% CO_2 atmosphere. Subsequently, the Neutral Red medium was removed and each well was rinsed with DPBS. The plates were totally made dry reversing the plates, then Desorb Solution was added and the plates were incubated for at least 15 minutes at room temperature with gentle agitation to form a homogeneous solution. Optical density was measured at 540nm by Gen5 software (Biotek) using microtiter plate reader.

$$\%\ \text{of cell viability} = \frac{\text{OD test sample - OD blank}}{\text{OD vehicle - ODblank}} \cdot 100$$

ACCEPTABILITY CRITERIA	QUALITATIVE EVALUATION	Negative control ≤ 1; Positive control ≥ 3
	QUANTITATIVE EVALUATION	The OD mean of the vehicle must be ≥ 0,3. The positive control % cellular viability must be < 70%. The negative control % cellular viability must be ≥ 70%. Coefficient of variation of each group must be ≤15%.
INTERPRETATION OF RESULTS	The achievement of a numerical grade greater than 2 is considered a cytotoxic effect. A cellular viability reduction more than 30% is considered a cytotoxic effect.	
QUALITY CRITERIA	Satisfied	
QUANTITATIVE EVALUATION	Cells treated with test sample have shown a cell viability reduction of **0±0,76%.**	

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Reviewed and electronically signed for Technical Supervisor Approval by
Elena Bizzotto, Employee
for Eurofins Biolab Srl, on 18-Feb-2021 15:47:57 UTC+01:00