eurofins | BioPharma Product Testing

Certificate of Analysis

Analytical Report: AAB23933
Eurofins Sample Number: LV18AB4645-1
Version: 1

Eurofins Bactimm B.V.
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Nijmegen, 6545 CH
NL

Client Account Number: A0052261218L
Eurofins Quote Number: VZB22018067701

Eurofins Sample Number LV18AB4645-1

Original Received Date:	25-Sep-2018
Description:	Cellulose Hydrogel uncrosslinked
Lot Number:	35

Analysis	Result	Unit
Cytotoxicity, Elution test - ISO 10993-5:2009		
RESULT:	Not cytotoxic	----
Extraction ratio:	0.2 g/mL	----
Extraction time:	24	hour
Extraction conditions:	Dynamic	----
Extraction temperature:	37	°C
Negative control:	HDPE	----
Positive control:	Latex	----
Vehicle:	Supplemented culture medium	----
Notes:	N/A	----

Addendum #1: Qualitative and Quantitative Evaluation

Addendum #2: Test for in vitro cytotoxicity

Method: EN ISO 10993-5
Analysis Date: 01-Oct-2018 to 03-Oct-2018

Contracted Company: Eurofins Biolab Srl (Vimodrone)

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Questions about this report should be directed to your project manager or the general email listed above.

Reviewed and electronically signed for Technical Supervisor Approval by
Robert Narloch, Employee
for Eurofins Biolab Srl, on 05-Oct-2018 17:29:28 UTC+02:00

eurofins	In vitro cytotoxicity ISO10993-5:2009	1-P-PR-TEM-9005812
	Test on extracts-NRU	Addendum N. 1

ID study	//	ID sample	LV18AB4645-1
Test product	Cellulose Hydrogel uncrosslinked	Batch	35
Receiving	//	Date	25/09/2018
Test starting on	01/10/2018	Test finished on	03/10/2018

QUALITATIVE EVALUATION

1- Qualitative morphological grading of cytotoxicity of

	Contact time:24 hours					
	Replica 1	*Replica 2*	*Replica 3*	*Replica 4*	*Replica 5*	*Replica 6*
Vehicol control	0	0	0	0	0	0
Negative control	0	0	0	0	0	0
Positive control	4	4	4	4	4	4
Test product	0	0	0	0	0	0

2- Interpretation of results

Grade	Reactivity	Conditions of all Cultures
0	**None**	Discrete intracytoplasmic granules; no cell lysis; no reducgion of cell growth
1	**Slight**	Not more than 20% of the cells are round, loosely attached and without intracytoplasmic granules or show changes in morphology; occasional lysed cells are present; only slight growth inhibition observable
2	**Mild**	Not more than 50% of the cells are round, devoid of intracytoplasmic granules; no extensive cell lysis; not more than 50% growth inhibition observable
3	**Moderate**	Not more than 70% of the cell layers contain rounded cells or are lysed; cell layers not completly destroyed but more than 50% growth inhibition observable
4	**Severe**	Nearly complete or complete destruction of the cell layers

3-Acceptability criteria

Negative control	
Grade ≤1	**VALID**

Positive control	
Grade ≥3	***VALID***

4- Results

	Reactivity grade	
Test product	**0**	**NOT CYTOTOXIC**

eurofins	In vitro cytotoxicity ISO10993-5:2009	1-P-PR-TEM-9005812
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QUANTITATIVE EVALUATION

1- Optical Density Measurements - OD Value

	Contact time:24 hours					
	Replica 1	*Replica 2*	*Replica 3*	*Replica 4*	*Replica 5*	*Replica 6*
Vehicol control	1,197	1,251	1,329	1,439	1,345	1,295
Negative control	1,164	1,252	1,242	1,286	1,315	1,278
Positive control	0,042	0,044	0,041	0,043	0,045	0,047
Test product	1,14	1,23	1,267	1,29	1,296	1,238

2- Mean, Std Dev and % CV of OD value

	Contact time:24 hours		
	Mean OD value	*Standard deviation*	*CV%*
Vehicol control	1,309	0,083	6,341
Negative control	1,256	0,052	4,140
Positive control	0,044	0,002	4,545
Test product	1,244	0,057	4,582

3-Counting of % viability

Vehicol control	Negative Control	Positive Control	Test Product
OD corresponding to 100% viability	Viability %	Viability %	Viability %
1,309	96	3	95

4-Acceptability criteria

	Vehicol control
mean OD : ≥0,3	VALID

	Negative control ≥70%	Positive control <70%
Viability	VALID	VALID

	Vehicol control	Negative control	Positive control	Test product
CV between replicates <18%	VALID	VALID	VALID	VALID

5- Interpretation of results

Reduction of Viability % measured after contact time	Result
>30% after contact time	Cytotoxic
≤30% after contact time	Not Cytotoxic

6- Results

	Reduction Viability %	
Test product	5	NOT CYTOTOXIC

Technician signature JM Date 03/10/18

Supervisor signature RO Date 05/10/18

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ADDENDUM N.2 TO THE REPORT: TEST FOR IN VITRO CYTOTOXICITY – ELUTION TEST

REFERENCES/GUIDELINES:	- ISO 10993-5:2009 - Biological evaluation of medical devices Part 5: Tests for in vitro cytotoxicity; - ISO 10993-12-2012 - Biological evaluation of medical devices Part 12: Sample preparation and reference materials.	
CELL LINE	Mammal fibroblasts BALB/3T3 clone A31 (ATCC®; CCL163™) Source: ATCC.	
	Dulbecco's Modification of Eagle's Medium (DMEM), Fetal Bovine Serum (FBS), Neutral Red dye, Ethanol solution, Trypsin-EDTA	(Sigma-Aldrich)
	Penicillin/Streptomycin solution, Dulbecco's Phosphate buffer solution (DPBS)	(Lonza)
	High density polyethylene (HDPE, USP Reference Standard negative control), Latex from laboratory gloves	/
	Water for injections, Acetic Acid	
EQUIPMENT	Laminar flow hood, CO_2 incubator, Microplate reader Mod EL800, Chronometer, Common laboratory equipment, Inverted Microscope Diavert,Orbital shaker, Refrigerator, Balance (if needed).	

EXPERIMENTAL DESIGN

The experimental design included one 24-well plate containing a subconfluent cell monolayer subdivided in the following groups:

vehicle	*vehicle*	*vehicle*	*vehicle*	*vehicle*	*vehicle*
negative control	*negative control*	*negative control*	*negative control*	*negative control*	*negative control*
positive control	*positive control*	*positive control*	*positive control*	*positive control*	*positive control*
test sample	*test sample*	*test sample*	*test sample*	*test sample*	*test sample*

VEHICLE	Supplemented culture medium (without test sample)	
TEST SAMPLE	See the Report for extraction ratio details. Prior to the testing the sample was stored in room temperature.	Extracted at 37 ± 1 °C in dynamic conditions
NEGATIVE CONTROL	Extract of HDPE, 3 cm^2/ml.	
POSITIVE CONTROL	Extract of latex, 6 cm^2/ml.	

TREATMENT : Verified that a subconfluent monolayer was present, the supernatant was removed and substituted with 0,5 ml of extracts and vehicle. The plate was incubated in an incubator at 37°C ±1°C in 5% CO_2 atmosphere for 24 hours. This procedure was repeated for positive and negative 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°C ±1°C in 5% 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 10 minutes at room temperature with gentle agitation to form an homogeneous solution. Optical density was measured at 540nm by Gen5 software (Biotek) using microtiter plate reader.

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

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 ≤ 18%.

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

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