pH srl
Via Sangallo, 29
I-50028 Barberino Tavarnelle
Italy

Sample ID: 23-MD00149
ID report: 23-MD00149_test report

Subject: Test Report – Determination of cytotoxicity

Customer (1)	
Name	Fitt S.p.A
Address	Via Piave, 8 – 36066 Sandrigo – Vicenza, Italy
Declaration of conformity	Not Required

Test items	
Arrival Date in Lab	07/03/2023
Sample's description/Name (1)	FITT Pure Tek
Reference (customer ID) (1)	n/a
Batch/Code/Lot ID (1)	n/a
Intended clinical use (if applicable) (1)	Used as part of a medical device
Type of material (1)	Thermoplastic Elastomer based on SEBS
Physicochemical properties (1)	Solid

(1) Info provided by customer

Sample Preparation	
Number of tested items:	1
Measures:	Total inner surface Area: 173,6 cm^2 Thickness: > 0.5 mm

Sample analyzed (picture)

Description: TPE Flexible Hose

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Method	
Regulatory background	ISO 10993-5:2009 ISO 10993-12:2021 ISO/IEC 17025:2018
Internal Procedure/SOP	Current version of PP.ISO.089
Cell Lines (Name and Supplier details, Lot)	L929 (ATCC Lot. 70026472) according to ISO 10993-5:2009
Cell Culture Medium (Name and Supplier details, Lot)	MEM Eagle (Sigma; Lot. RNBL0674)
Medium supplemented with (in Lab)	1% penicillin/streptomycin (Pan Biotech), 1% L-glutamine (Pan Biotech), 10% FBS (Pan Biotech)
Pre-extraction manipulation of Test Item (if applicable	n/a
Pre-Treatment manipulation of the extract (if applicable):	n/a
Test Incubation conditions (1)	(24±2)h; (37±1)°C; (5±1)% CO_2
Extraction conditions (if applicable) (1)	(72±2)h; (37±1)°C; under orbital agitation (80 rpm)
Extraction vehicle (if applicable) (1)	Cell culture medium with supplements, 10% FBS
Extraction ratio (1)	3 cm^2/mL
Application of Test Item (1)	Extract obtained through filling of the sample
Extraction dilutions (if applicable) (1)	100%
Sterility status (1)	Not sterile
Assay (1)	MTT
Experimental procedure	L929 cells are seeded into 96-well plates and maintained in culture medium for 24 h to form a semi-confluent monolayer. The extract of the test sample is prepared and applied to the pre-plated cells. The test is performed in triplicate. After 24h of treatment, the quantitative evaluation of the cytotoxic potential is assessed via a colorimetric test. The yellow water-soluble MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromid) is metabolically reduced in viable cells to blue-violet insoluble formazan. The number of viable cells correlated to the color intensity is determined by photometric measurements after dissolving the formazan in alcohol.
Positive Control	Natural rubber latex (Icoguanti s.p.a. lot. 204096767LLZA)
Negative Control	High density polyethylene (Hatano Research Institute, lot. C-211)
Test Control	Extraction vehicle with same procedure as used for test items, w/o test items
Test Start Date	17th March 2023
Test End Date	22nd March 2023
Test performed by (technical analyst)	Adele Contini
Test performed at Lab	0_A (Via Sangallo, Barberino Tavarnelle, Italy)

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Controls:
A test meets acceptance criteria if:

- the ratio between the standard deviation and the average of OD's of the triplicates is ≤ 20% (CV ≤ 20%);
- the positive control is accepted when the viability is ≤ 25%; the negative control is accepted when the viability is ≥ 80%.

		Average OD 570 nm	Viability (%)
Controls	Positive Control	0.101	1.3
	Negative Control	1.327	94.6
	Test Control	1.398	100.0

Note: the results shown above are the averages of 3 replicates for positive and negative controls; for the test control, the average is obtained from both 3 replicates of the left and right side of the 96-well plate.

Abbreviations	
PO	Purchase Order
SOP	Standard Operating Procedure
OD	Optical density
PE	Polyethylene
n/a	Not Applicable
CV	Coefficient of variation

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Results

Qualitative Evaluation (if applicable): n/a

Quantitative Evaluation:

	Sample	Average OD 570nm	CV (%) (1)	Acceptance Criteria (1)	Viability (%) (2)	Evaluation (2)
Test Item	23-MD00149 100%	1.368	2.0	Passed	97.7	Not-Cytotoxic

Note: Results are referred to sample as received
The results shown above are the averages of 3 replicates for each sample.
(1) the ratio between the standard deviation and the average of OD's of the triplicates must be ≤ 20% (CV ≤ 20%).
(2) The test item is considered to have a potential cytotoxic effect if viability is < 70%;
(3) The 23-MD00149 100% corresponds to the pure extract in cell culture medium with serum.

Additional info: n/a

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Final Statement

The test item is not considered to have a potential cytotoxic effect for the intended clinical use/for the described method, under the applied conditions.
Note: the laboratory adopts as its decision rule the direct comparison with the acceptance criteria of ISO 10993-5:2009.

General Notes

- The laboratory declines all responsibility for the indications/instructions provided by the customer.
- The test item is sampled by the customer: the laboratory declines all responsibility for sampling. Samples are stored for 30 days after the report submission unless otherwise agreed.
- Technical records are kept for 10 years.
- If a Declaration of Conformity is present, the Laboratory adopts as a decision-making rule the direct comparison of the result with the applied limit without taking into account the measurement uncertainty.

Signature

Test Report approved by: Dr.ssa Elena Ciofi
as per Technical Manager

Date: 22/03/2023

Signature

Barberino Tavarnelle, 23/03/2023
(Note: The above date represents the date of writing of this test report. The date of issue of the test report shall be the date of digital signature).

--- End of Test Report ---

P.H. s.r.l. Single-member company subject to the control and coordination of TÜV SÜD AG e-mail: info@phsrl.it www.tuv.it/ph
- Headquarters and Food Laboratory: Via Sangallo, 29 50028 Barberino Tavarnelle (FI) phone: +39 055 80961 fax: +39 055 8071099
- Offices and Environmental Laboratory: Via Bramante, 10/12 50028 Barberino Tavarnelle (FI) phone: +39 055 80677 fax: +39 055 8067850
- Environmental Laboratory: Z.I. Tito Scalo 85050 Tito (PZ) phone: +39 0971 485795 fax: +39 0971 485795

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