TESTS FOR IN VITRO CYTOTOXICITY

Test Substance

FITT Pure Tek

Test Report N° 23-0315-01

Test performed for

pH s.r.l.
Via Sangallo, 29
50028 BARBERINO TAVARNELLE FI

by

BIOCHEM S.r.l.
Via Benini, 13
40069 ZOLA PREDOSA BO

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QUALITY ASSURANCE

Quality Assurance Manager: Alessandra Marchesi, PhD

GENERAL MANAGER

Giovanni Bassini, Ch.Eng.

TIME SCHEDULE OF TEST

The test was started on 12/05/2023 and was completed on 16/05/2023.

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Sample description

Denomination: FITT Pure Tek
Code: 23-MD00151
Lot: 1012222S2285
Sterilization: No
Receipt number: 27859
Receipt date: 29/03/2023
Sampling carried out by pH s.r.l.

Part of the sample to be tested: the internal part of the sample.
Pretreatment: /

Test Method

USP-NF 2022 Issue 2 par 87
ISO 10993-12: 2021

Other references

Cytotoxicity Test Protocol - /

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 48h-contact to assess the presence or absence of cytotoxic effects due to the test extract.

Target cells: BSCL 59 /L 929 (Mouse connective tissue)

Culture medium: Minimum Essential Medium (MEM) with Earle's salts added with 5 % of foetal bovine serum, 1 % of L-glutamine, 0,6 % of penicillin/streptomycin and 0,3 % of fungizone (complete MEM).

Extraction conditions: 57,75 cm^2 were extracted with 17 ml of 9 g/l sterile solution of sodium chloride (ratio 3 cm^2 / 1 ml) at 70°C for 24 hours. (Ref. ISO 10993-12)

Positive control: Polyurethane Film Lot A-223K was extracted with 10 ml of sterile solution of sodium chloride (ratio 6 cm^2 / 1 ml) at 70°C for 24 hours.

Negative control: USP High-Density Polyethylene RS Lot C-221 was extracted with 10 ml of 9 g/l sterile solution of sodium chloride (ratio 3 cm^2 / 1 ml) at 70°C for 24 hours.

Extraction vehicle control: 9 g/l sterile solution of sodium chloride was extracted with 9 g/l sterile solution of sodium chloride at 70°C for 24 hours.

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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% CO2 atmosphere.

Apparatus
Incubator, which maintains the cultures at 37°C, 5% CO2;
Microscope, with inverted phase contrast optics;
Water Bath;
Laminar Flow Cabinet;
Sterile Disposable;
Tissue Culture Dishes.

Interpretation of Results: The determination of the cytotoxicity is performed after a 48h 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 color 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	Less than or equal to 20% of the cells are round, loosely attached, and without intracytoplasmic granules; occasional lysed cells are present
2	Mild	Greater than 20% to less than or equal to 50% of the cells are round and devoid of intracytoplasmic granules; no extensive cell lysis and empty areas between cells
3	Moderate	Greater than 50% to less than 70% of the cell layers contain rounded cells or are lysed
4	Severe	Nearly complete destruction of the cell layers

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Results after 48 h 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 material	0
Extract of the test material	0
Extract of the test material	0
Confluency of the monolayer	Confluent
Color of test medium	Comparable to the negative control

OPINIONS AND INTERPRETATIONS – Not included in ACCREDIA accreditation.

The cells treated with the Test extract after 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 present test report exclusively refers to the referenced test sample.
If the sample has been sampled by the Customer, the results are referred to the sample as received.
The present test report may not be partially reproduced without Biochem authorization.
The present test report cancels and replaces the Test Report N°23-0283-01. Reason: wrong normative description.
Request: customer email to commerciale@biochem-bcm.com dated 03-05-2023.
Changes in the document are shown in italics.

(#) Data provided by the Customer. The laboratory declines responsibility for such data.

Test verified by: Buriani Giampaolo, PhD.

Issue authorized by:
Location Manager, Giampaolo Buriani, PhD.

Zola Predosa, 16/05/2023

END OF TEST REPORT

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