NATIONAL INSTITUTE OF PUBLIC HEALTH
Šrobárova 49/48
Praha 10
100 00
Czech Republic

YOUR REFERENCE:
DATE: November 23, 2022
OUR REFERENCE: **SZÚ/15973/2022; EX 221436 3/22/118**

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Date: December 14, 2022

Prusa Polymers a.s.
Partyzánská 188/7A
170 00 Praha 7

Czech Republic

Subject: EXPERT OPINION on the assessment of the in vitro cytotoxicity.

SUBJECT OF APPLICATION:
Regarding your application of 23-November-2022 for evaluation of the in vitro cytotoxicity of the test material, we hereby report:

SUBMITTED SAMPLE:
TM 3/22/118: Prusament Resin Biobased60 Natural Yellow – post cured 10 min

Manufacturer:
Prusa Research a.s.
Partyzánská 188/7A
170 00 Praha 7
Czech Republic

SUBMITTED DOCUMENTATION:
Not submitted.

PERFORMED TESTS:
The test was carried out in compliance with: SOP 1/3 Tests for in vitro cytotoxicity (EN ISO 10993-5: Biological evaluation of medical devices – Part 5, Articles 1, 2, 3, 4, 5, 6, 7, 8.1, 8.2, 8.3, 8.5, 9, 10, Annex A).

EXPERT OPINION:
The test was performed by the Centre for Laboratory Testing, accredited by the Czech Accreditation Institute (Accredited Laboratory No.1206), Centre of Toxicology and Health Safety.

CONCLUSION:
The result of the test for cytotoxicity is summarized in the attached test report.

Under the test conditions (test on extract) the sample TM3/22/118 does not induce cytotoxic effects.

National Institute of Public Health
Centre of Toxicology and Health Safety
Šrobárova 49/48, 100 00 Praha 10
Czech Republic

Dagmar Jírová, M.D., Ph.D.
Head of
Centre of Toxicology and Health Safety

ANNEX:
Test Report No. 3/22/118 – TEST REPORT CYTOTOXICITY IN VITRO

National Institute of Public Health
Centre for Laboratory Testing

Laboratories of Toxicology
Šrobárova 49/48, 100 00 Prague 10
Tel.: +420 267082439 E–mail: hana.bendova@szu.cz

L 1206

Laboratory No.1206, accredited by Czech Accreditation Institute
according to EN ISO/IEC 17025:2017

Test Report No.3/22/118

Customer: Prusa Polymers a.s.

Address: Partyzánská 188/7A, 170 00 Praha 7, Czech Republic

Reference No.: SZÚ/15973/2022

Test Material

Identification:

TM 3/22/118: Prusament Resin Biobased60 Natural Yellow - post cured 10 min

Laboratory Tests

SOP 1/3 Tests for in vitro cytotoxicity (EN ISO 10993–5: 2009 Biological evaluation of medical devices – Part 5, Articles 1, 2, 3, 4, 5, 6, 7, 8.1, 8.2, 8.3, 8.5, 9, 10, Annex A)

Sample reception date: 28.11.2022

Date of study: 30.11. - 7.12.2022

Date of issue: 13.12.2022

Number of pages: 5

Authorized by Technical Manager: Hana Bendová, M.Sc., Ph.D.

The tests were performed at the address of the laboratory. The test results refer only to the sample as submitted by the sponsor and to the objectives of the study. This test report does not substitute for any other document or certification of the product. Without written approval of the testing laboratory this report should not be reproduced in other form than as a whole.

TEST REPORT
CYTOTOXICITY IN VITRO

Testing facility: Laboratories of Toxicology (Centre for Laboratory Testing, National Institute of Public Health, Šrobárova 49/48, 100 00 Prague 10, Czech Republic).

The test was carried out in compliance with: SOP 1/3 Tests for in vitro cytotoxicity. (EN ISO 10993-5: Biological evaluation of medical devices – Part 5, Articles 1, 2, 3, 4, 5, 6, 7, 8.1, 8.2, 8.3, 8.5, 9, 10, Annex A).

Aim of the study: Assessment of the in vitro cytotoxicity of the test material.

MATERIALS AND METHODS

TEST MATERIAL (TM):

TM 3/22/118: Prusament Resin Biobased60 Natural Yellow - post cured 10 min

Customer: Prusa Polymers a.s.

Partyzánská 188/7A

170 00 Praha 7

Czech Republic

CELL LINE

Balb/c 3T3 mouse fibroblasts (3T3-L1), European Collection of Cell Cultures, Great Britain, ECACC No.86052701, passage 86-88, free from mycoplasma (qPCR).

CULTURE MEDIUM

DMEM (Dulbecco´s Minimum Essential Medium, LONZA, Lot 1018146) supplemented with antibiotics (PNC 100 IU/ml, STM 100 μg/ml, LONZA, Lot 21MB 041) and 10% of inactivated calf serum (GIBCO, Lot 2199647), pH 7.2, freshly prepared, stored no longer than 1 week.

CONTROLS

Positive control (PC) – material which provides a reproducible cytotoxic response: Dodecylsulphate sodium salt SDS (SIGMA), final concentration 1, 10, 20 μg/ml DMEM without serum.

Negative control (NC) – material which does not produce a cytotoxic response: Hydron (poly[(2-hydroxyethyl) methacrylate], Institute of Macromolecular Chemistry, Academy of Sciences of the Czech Republic, Prague). The material was extracted according to ISO 10993-12 in the ratio of 3 cm^2 of the material per 1 ml of extraction vehicle (DMEM with serum).

Reagent control (RC) – extraction vehicle without test material subjected to extraction conditions and test procedures: culture medium with serum.

Cell control (C/C without serum) - culture medium with/without serum.

- **TEST PROCEDURE (test on extract)**

The cells were seeded into individual wells of 96-well microtitre tissue culture plates. The prepared cell suspension of 1 x 10^5 cells/ml DMEM was inoculated in the volume of 0.1 ml (1 x 10^4 cells) per well. After 24 h preincubation (37°C, 7.5% CO_2) the medium was removed and replaced by 0.2 ml of liquid extracts of the test material and controls. The extracts were diluted in culture medium with serum and all the test samples and controls were run in quadruplicates. At the end of the 24 h treatment (37°C, 7.5% CO_2) the medium was removed and the cells were stained by Neutral Red dye according to INVITTOX Protocol No. 46 (0.2 ml Neutral Red solution per well, 3 h incubation, Neutral Red desorb solution – ethanol/acetic acid). The Neutral Red uptake was measured fluorimetrically.

PREPARATION OF LIQUID EXTRACT

The test material was extracted according to ISO 10993-12 in the ratio of 3 cm^2 of the material per 1 ml of extraction vehicle (DMEM with serum), in chemically inert closed containers by using aseptic techniques. Extraction time: 24 h, extraction temperature: 37°C. The extract was used immediately after preparation. The concentrated (100%) extract was further diluted in DMEM with serum.

- **EVALUATION OF CYTOTOXICITY**

The degree of cytotoxicity (i.e. the decrease of cell viability) was quantitatively determined as the Neutral Red uptake measured by fluorescence-luminiscence reader FLX800TBI (BioTek). The evaluation of cytotoxicity by means of cold light fluorimetry is based on the incorporation of a vital dye (Neutral Red) into living cells (Neutral Red Uptake) and detection of fluorescence in the system of excitation (530 nm) and emission (590 nm) filters. The results (Fluorescence Units, FSU) obtained for wells treated with the test material were compared to untreated control wells (culture medium, 100% viability) and converted to a percentage value (Ref.: Rat, P. et al. (1994). New in vitro fluorimetric microtitration assays for toxicological screening of drugs. Cell Biology and Toxicology, 10, 329-337).

Calculation:
The mean FSU value of eight blank wells (containing only Neutral Red desorb solution) was subtracted from the mean FSU value of four treated wells (treated with the test material, positive control or culture medium).

The viability of cells was calculated as

$$\text{viability (\%)} = \frac{\text{mean FSU of test wells} - \text{mean FSU of blanks}}{\text{mean FSU of cell control wells} - \text{mean FSU of blanks}}$$

Cytotoxicity scale:
viability 70% and higher..... no cytotoxicity
viability higher or equal to 50% and lower than 70%.....mild cytotoxicity
viability higher or equal to 30% and lower than 50%.....moderate cytotoxicity
viability lower than 30%.....severe cytotoxicity

RESULTS

- **CYTOTOXICITY - FLUORIMETRIC EVALUATION**

Test No. 1

Test material extract dilution	**Fluorescence (FSU) mean value**	**Viability % of cell control**
TM 3/22/118		
5%	4240,5	**79,1**
10%	4291,0	**80,0**
25%	4212,3	**78,5**
50%	4036,3	**75,3**
100%	4514,5	**84,2**
C	5363,5	100,0
PC – SDS		
1 μg/ml	5094,6	95,3
10 μg/ml	857,9	16,1
20 μg/ml	275,5	5,2
C without serum	5344,8	100,0
NC	5739,1	99,7
RC	6225,6	108,1
C	5757,9	100,0

Test No. 2

Test material extract dilution	Fluorescence (FSU) mean value	Viability % of cell control
TM 3/22/118		
5%	3744,0	**88,6**
10%	3762,9	**89,1**
25%	3707,6	**87,8**
50%	3682,1	**87,2**
100%	3350,4	**79,3**
C	4224,6	100,0
PC – SDS		
1 µg/ml	3409,1	89,2
10 µg/ml	395,9	10,4
20 µg/ml	373,3	9,8
C without serum	3821,8	100,0
NC	4425,1	97,2
RC	4300,9	94,5
C	4550,9	100,0

Test carried out by: K. Kejlová, M.Sc., Ph.D., J. Losová

Principal investigator: K. Kejlová, M.Sc., Ph.D.

----------end of report----------