Report

T-700 - IN VITRO CYTOTOXICITY ASSAY FOR EVALUATION OF MATERIALS AND MEDICAL DEVICES (EXTRACTION METHOD – MTT TEST)

HBI Study Number:	NAD/007/CTXe
General Study Plan Version followed:	Extraction CTX /ISO 10993/5-14
SSS version followed:	Original study specific supplement
Sponsor Name:	Nano Dimension
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Compliance:	GLP
Study Director:	Efrat Sharvit
Testing Facility:	**HBI Biotech Sciences** Mailing address: P.O.B 4019 Science Park, Ness-Ziona 7414001, Israel Einstein St. Building 13B, Weizmann Science Park, Ness-Ziona, Israel

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COMPLIANCE WITH GOOD LABORATORY PRACTICE

T-700 - IN VITRO CYTOTOXICITY ASSAY FOR EVALUATION OF MATERIALS AND MEDICAL DEVICES (EXTRACTION METHOD – MTT TEST)

With the exception/s stated below, the study described in this report was conducted in compliance with the following Good Laboratory Practice standards and I consider the data generated to be valid:

OECD Principles of Good Laboratory Practice (as revised in 1997), ENV/MC/CHEM(98)17.

These principles are compatible with Good Laboratory Practice regulations specified by regulatory authorities throughout the European Community, the United States (EPA and FDA), and Japan (MHLW, MAFF and METI), and other countries that are signatories to the OECD Mutual Acceptance of Data Agreement.

Exception from GLP was as follows

#	The exception	The justification & Impact
	No expiry date was indicated for the Test Item in the Information Sheet	The test item is a solid plastic part that does not biodegrade nor disintegrates. Thus, there were no impact on the study.

Efrat Sharvit
Study Director
HBI Biotech Sciences

01.08.24
Date

QUALITY ASSURANCE STATEMENT

T-700 - IN VITRO CYTOTOXICITY ASSAY FOR EVALUATION OF MATERIALS AND MEDICAL DEVICES (EXTRACTION METHOD – MTT TEST)

Study based activities at the Testing Facility, HBI Biotech Sciences, were audited and inspected. The details of these audits and inspections are given below.

Type of Inspection	Date(s) of Inspection	Date Reporting to Study Director, Test Facility Management
General Study Plan: In Vitro Cytotoxicity Assay: Extraction CTX /ISO 10993/5-14	12 November, 2023	12 November, 2023
Study Specific Supplement	30 June 2024	30 June, 2024
Study – based: Test Materials preparation, application of TI	15 July, 2024	22 July, 2024
Raw Data and Draft Report Audit	18-21 July, 2024	21 July, 2024
Final report audit	01 August, 2024	01 August, 2024

General facilities and activities where this study was conducted were inspected on an annual basis and results are reported to the relevant responsible person and Management.

This statement serves to confirm that the final report reflects the raw data.

Process based inspection (Procedures inspected on representative studies):

Type of Inspection	Date(s) of Inspection	Date Reporting to Study Director, Test Facility Management
TI receipt	11 July, 2024	22 July, 2024
Cell culture propagation	11 July, 2024	11 July, 2024

Julia Vilensky, D.V.M.
Quality Assurance Manager
HBI Biotech Sciences

01.08.24
Date:

1 SUMMARY

1.1 The objective of this study was to assess the cytotoxic potential of leachable substances in extracts of the Test Item: ***T-700 (Batch No.: NF2404245)*** on L929 mouse cell line. The cytotoxic potential was measured using the MTT assay.

1.2 The test was performed using the well-characterized cells L929. Test Item was subjected to extraction process in L929 Assay Medium for about 24hrs, at 37°C. The extract was tested diluted (12.5-100%). Positive & Negative Controls were extracted likewise. The Vehicle Control (L929 Assay Medium) was subjected to the same conditions and tested as is.

1.3 Cells were seeded on one 96-wells plate. Various concentrations of Test Item was assessed with replicates of the respective Controls (undiluted). Cells viabilities were evaluated using the indicator MTT, which is metabolically reduced only in viable cells. Subsequently cell lysis was induced by Isopropanol and the absorbencies were measured at 570nm wavelength (650nm reference).

1.4 All acceptance criteria for testing the Test Item ***T-700 (Batch No.: NF2404245)*** were met, supporting the validity of the test plate.

1.5 Under the conditions of this study, and according to calculated viability, the extract of Test Item, T-700 (Batch No.: NF2404245) is considered non-cytotoxic at all concentrations tested, 12.5% - 100%, with 87.31-94.86% viability.

2 INTRODUCTION AND PURPOSE

2.1 Introduction

Cytotoxicity tests represent one of the easiest methods for the analysis of detrimental effects of substances, such as new materials, devices or formulations for possible use in medical applications. Cell culture techniques allow a rapid yet sensitive diagnosis of the biological reactivity of leachable or diffusible components of these substances.

Cellular damage will inevitably result in loss of the cell ability to maintain and provide energy for metabolic cell function and growth. Metabolic activity assays, which are based on this premise, measure mitochondrial activity.

The MTT assay is based on the fact that viable cells are able to reduce in their mitochondria the water-soluble yellow-colored 3-(4,5-dimethyldiazol-2-yl)-2,5 diphenyltetrazolium bromide (MTT) to a water-insoluble purple-colored formazan product. The amount of formazan product formed, determined spectrophotometerically after dissolving the formazan crystals in Iso-Propanol, is proportional to the metabolic activity and the number of cells in the test sample.

In standard cytotoxicity extraction method, cell monolayers are grown to their logarithmic growth. The Test Item/Product is extracted using Polar and Non-Polar environment. Typically, cells growth medium is used since it contains Polar (buffer, salts, amino acids, sugars, etc.) and Non-Polar (lipids, vitamins, serum hydrophobic components, etc.) moieties, thus serves as biologically compatible medium. The obtained extract is then applied to the culture-cell monolayers, replacing their growth medium. Following incubation of the cultures with this fresh nutrient medium containing extractable derived from the Test Product/Item or from the Positive and Negative Controls, MTT is added for additional 2 hours and then dissolved with Iso-Propanol. MTT absorbance is subsequently measured in spectrophotometer at 570nm wavelength (650nm reference).

In vitro cytotoxicity test measures the effect of different doses of the Test Product/Item extract on L929 cells. To validate the test, Positive and Negative Controls extracts are tested in parallel to the Test Product/Item. The Positive Control demonstrates a cytotoxic effect, assuring the cells are sensitive to leachable materials.

2.2 Purpose

The objective of this study was to assess the cytotoxic potential of leachable substances in extracts of Test Item ***T-700 (Batch No.: NF2404245)*** in consideration of its intended use for 3D cartridge used for medical device printing. The Cytotoxic potential on L929 mouse cell line was measured using the MTT assay.

2.3 Justifications

2.3.1 Justification for Cell Line Selection

The L929 cell line was selected for this study, as it is the cells of choice specified by the respective Guidelines for use in cytotoxicity testing.

2.3.2 Justification for Test Item Sample Size

The Test Item sample sizes were determined according to the information specified in the guideline.

3 STUDY SITE/S DETAILS

Sponsor	Nano Dimension
	6 Ilan Ramon St. Ness-Ziona Science Park
Study Monitor	Yarden Gercci
	Tel: 052-8712265
	Email: yarden.gercci@nano-di.com
Testing Facility	**HBI Biotech Sciences**
	Address: 13B Einstein St., Weizmann Science Park, Ness-Ziona, Israel
Study Director	**Efrat Sharvit, PhD**
	Tel: 08-9409451 ext. 121
	Email: Efrat.sharvit@hbi-cro.com
Quality Assurance	**Julia Vilensky, D.V.M.**
	Address: same as the Testing Facility
	Tel: 972-8-9409451 (Ext. 212)
	Email: julia.vilensky@hbi-cro.com
GLP Status	GLP-Compliant

4 STUDY SCHEDULE

Experimental start date: 14 July 2024
Experimental completion date: 16 July 2024

5 REGULATORY TESTING GUIDELINES

The study was performed in compliance with the following regulations or guidelines:

- ISO 10993, published by the International Organization for Standardization: "Biological evaluation of medical devices ", Part 1: "Evaluation and testing within a risk management process", adopted 15th August 2018.

- ISO 10993, published by the International Organization for Standardization: "Biological evaluation of medical devices", Part 5: "Tests for in vitro cytotoxicity" adopted 1st June 2009.

- ISO 10993, published by the International Organization for Standardization: "Biological evaluation of medical devices ", Part 12: "Sample preparation and reference materials", Fifth edition January 2021.

6 METHODS FOR THE CONTROL OF BIAS

The following scenario/s in which the Sponsor could have possibly influence the outcome of the study were relevant to this study and the following measures were taken to prevent bias:

Scenario	Measures taken to prevent bias
The Draft Study Report or Draft Phase Report were reviewed by the Sponsor before being finalized	• Relevant correspondence between the Study Director and the Sponsor and/or draft versions of the report/s with the Sponsor's comments were retained. • The study report was re-audited by QA after the Study Director integrated changes ensue by the Sponsor, into the Draft Report.
The Sponsors played a primary role in Test Item management	• Communication between the Sponsor and the Testing Facility related to the Test Item were retained. • Since characterization data was not fully disclosed by the Sponsor to the Testing Facility, and since the latter did not perform characterization, this fact is explicitly mentioned in the Final Report and its impact on the validity and integrity of the study was evaluated.

7 TEST MATERIALS AND SUPPORTING INFORMATION

7.1 Test Items and Supporting Information

The Test item was supplied by or on behalf of the Sponsor. Information including the description, batch, purity, expiry / retest date, manufacturing date, delivery & storage conditions, safety precautions etc. were recorded in an '*Information Sheet'* form returned signed by the Sponsor and which will be retained with the rest of the study data on archive.

7.2 Test Item

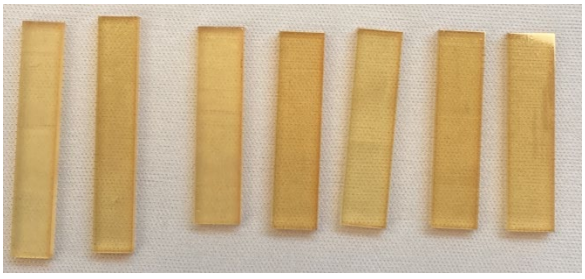
Information as provided by the Sponsor[1].

[1] Full details are in the *Information Sheet*, which was filled and signed by the Sponsor, and which will be retained with the rest of the study data.

Name of Device/Product (to be used in final report):	T-700
Clinical application of the Test Device:	The Test Item is a 3D cartridge used for medical device printing
Batch No. or Lot No.:	NF2404245
Catalog No. (if applicable):	NA
General description of the Test Device:	Polymerized acrylate (solid) for microfluidics
Courier for delivery to the Testing Facility:	Personal delivery by Nano Dimension
Storage conditions during delivery:	Ambient temperature
Is the Test Item sensitive to temperature and/or humidity?	No
Data logger in use:	No
Should the Test Item be protected from light:	No
Physical state:	Solid
Name of supplier:	Nano Dimension
Manufactured by:	Nano Dimension
Manufacturing date:	18 May 2024
Quality System of manufacturer:	NA
Type of Certification provided:	NA
Total amount of Test Device to be delivered (define units):	7 bars. A test device comprises several bars grouped together
Type of container to be delivered:	Closed Plastic Bag
Number of containers to be delivered:	1
Amount of Test Device per container:	7 bars
Expiry date:	NA (see exception from GLP)
Justification:	The Test Item is a solid plastic part that does not biodegrade nor disintegrates
Storage after receipt:	Room Temperature
Characterization Data	
Nature of Material	Synthetic Polymer
Does the Test Device absorbs liquids:	Non-Absorbing liquids
Does the Test Device biodegradable:	Non degradable
Form of Material:	3D printed polymer
Method of Manufacturing (e.g. injection molding, ect.):	3D printing
In case of a Polymer, please indicate:	Manufacturer: Nano Dimension
	Chemical Nature: Acrylate
	Glass transition temperature (°C): 100
	Melting temperature (°C): NA
	Softening Temperature (°C): NA
Thickness:	>1.0mm

Sterility (if sterile, please indicate sterilization method)	Non-Sterile
Storage after receipt:	Room Temperature
Protected from light:	No
Calculated Surface (cm²) or Weight (g):	5 shorter bars: 4.1cm^2 each 2 longer bars: 4.29cm^2 each Total: 29.08cm^2
Does the Test Device/Product have a surface coating:	No
Does the material undergo Hydrolysis:	No
Is the Test Device/Product tested in its Final Product Form and condition:	Yes
Does the Test Device/Product contain adhesives; radio frequency or solvents seals:	No
Nature of contact	
For a Surface Device - define the surface in contact with the device:	Skin
Parts of the product in contact with the Body:	The Whole Test Item
Contact Duration:	Limited (≤24h)
Method of disposal:	Standard trash
Hazards:	Non-Hazardous
Safety Precautions:	Routine hygienic procedures (gloves, gown)
Remaining Test Device	
After completion of dosing:	Remains of *Test Device* can be discarded
After completion of the study:	Remaining Test Device (unused) can be discarded by HBI

Figure 1 Illustration of the Test Item



7.3 Test Materials

Specifications of other materials used in this study (supplied by the Testing Facility) are as follows:

Name	Purpose	Manufacturer/ Supplier	Catalogue #	Batch/Lot #	Expiry Date
0.1% Zinc Diethyldithiocarbamate (ZDEC) in Polyurethane film	Positive Control Item	Hatano Research Institute	RM-A	A-222K	14 Jun 2025
Glass Vial	Negative Control Item	La-Pha-Pack, Germany	11090210	16491	NA
0.25% Trypsin/EDTA Solution	Rinsing, detaching solution	Sigma	03-052-1A	SLCM6820	30 July 2024
L929 Growth medium (with phenol red)	Trypsin/EDTA Diluent	Sartorius	01-025-1A (EMEM)	070724HB	07 August 2024
L929 Assay Medium (without phenol red)	Extraction Medium, diluent for extracts (Vehicle Control) & diluent for MTT	Gibco (for MEM)	51200 (MEM)	070724HB	07 August 2024
Thiazolyl Blue Tetrazolium Bromide (MTT)	Viability indicator	Merck	M5655	MKCS4540	September 2027
Iso-Propanol	Solubilizer of MTT crystals	Sigma-Aldrich	33539	STBK4504	September 2024

EMEM - Eagle's Minimum Essential Medium; MEM – Minimum Essential Medium; suffix HB – Represents internal batch No. after preparation.

7.4 Preparation of Vehicle Control

The Vehicle Control (Assay Medium) was subjected to the same extraction procedures as the Test Item and used undiluted.

7.5 Preparation of Test Item

The Test Item was prepared separately and placed in a sterile container. L929 Assay Medium was added according to the ratio of $3cm^2/ml$.
The container was sealed and placed in an incubator and subjected to the following extraction procedure:

- Medium: ‘L929 Assay Medium’- the use of culture medium with serum is preferred for extraction because of its ability to support cellular growth as well as extract both polar and non-polar substances.
- Time & Temperature: 24±2 hrs at 37±1°C.
- Agitation: Low
- Amounts & Volumes: see summarizing table of extraction procedure (see section below).

Following the extraction procedure, the Test Item’s extract was filtered using 0.2µm filter and pH was measured. The extract was used immediately after preparation. The test item extraction was diluted by the vehicle extract. Six different concentrations of the test item extract were prepared, and six replicates were used for each dilution.
The extraction media (assay medium) and conditions of preparation used were regarded as appropriate to the nature and use of the final product and to the purpose of the test.

7.6 Preparation of the Negative and Positive Controls

The Negative and the positive Control were placed in sterile containers containing extraction media identical to that which hold the Test Sample and subjected to the same extraction procedure.

7.7 Summarizing Table of Extraction Procedure

Treatment		Amount Taken		Extraction Ratio	Extraction Volume (ml)	Extraction Conditions		Filtration (0.22µm)	pH	Appearance of Extracts
Treatment	Name	Units used	Total Surface/ Weight			Temp (^{0}C)	Duration (hours)			
Positive Control	*ZDEC*	Cut into small pieces	9cm^2	6cm^2/ml	1.5	37	24h & 1m	Yes	8.0	Clear
Negative Control	*Glass Vial*	1	2.3g	0.2g/ml	11.5	37	24h & 1m	No	8.0	Clear
Vehicle Control	*L929 Assay Medium*	NA	NA	NA	7	37	24h & 1m	No	8.0	Clear
Test Item	*T-700*	7 units	29.08 cm^2	3cm^2/ml	9.69	37	24h & 1m	Yes	8.0	Clear

NA – Not applicable.

All the extracts were used immediately after preparations.

Positive Control and the Test Item were non-sterile thus the extracts were filtered through a 0.22µm filter to avoid contamination that may lead to false positive results.

No other process or manipulation such as centrifugation, pH adjustment etc. was done to Test extracts prior to testing.

8 TEST SYSTEM

8.1 Cell line Information

Cell line:	European Collection of Authenticated Cell Cultures (ECACC), Cat. No. 85103115, L929 (NCTC), clone of L strain, mouse connective tissue. This widely used cell line is known for its high cloning efficiency and high proliferation rate.
Batch No.:	L929-W5-110724
Maintenance:	Maintenance of cell cultures followed the recommendations set by ECACC. L929 working batches were sterile, *Mycoplasma*-free.
Growth Conditions:	Cultures were propagated at 37±1°C, humidified, 5±0.5% CO_2 /air, in plastic flasks.

8.2 Cell Media

Growth Medium	EMEM with phenol red supplemented with 10% horse serum, 4mM L Glutamine, 100U/ml penicillin and 100µg/ml streptomycin. Growth Medium was kept at 4°C
Assay Medium	EMEM without phenol red supplemented with 10% horse serum, 4mM L Glutamine, 100U/ml penicillin and 100µg/ml streptomycin. Assay Medium was kept at 4°C.

9 EXPERIMENTAL PROCEDURES

9.1 Pre-Test Procedures – Culture Seeding

Exponentially growing cultures with more than 50% confluent, were rinsed and detaches from flasks with Trypsin/EDTA solution. Trypsin activity was stopped by the addition of the L929 Growth Medium with phenol red and a single cell suspension was prepared.

L929 cells were Trypsinized and seeded on two 96-well tissue culture plates according to concentration of 1×10^5 cells/ml (= 1×10^4 cells/100µl/well). Columns 1-12 and rows A-H of each plate were filled with only L929 Growth Medium.

Plates were incubated for 24±2 hours, at 37±1°C, humidified, 5±0.5% CO_2 /air, to enable cells adherence to the wells (for further incubation periods after treatment see next section).

9.2 Treatments

Medium from columns 2-11 rows B-G were replaced with the 100µl of various treatments according to the plates plan below. In addition, columns 1 and 12 and rows A and H were replaced by 100 L929 Assay Medium.

The plate was incubated for 24±1 hours, at 37±1°C, humidified, 5±0.5% CO_2 /air.

9.3 Plates Plan:

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank											
B	Blank	VC	NC	PC	TI 100%	TI 85%	TI 75%	TI 50%	TI 25%	TI 12.5%	VC	Blank
C		VC	NC	PC	TI 100%	TI 85%	TI 75%	TI 50%	TI 25%	TI 12.5%	VC	
D		VC	NC	PC	TI 100%	TI 85%	TI 75%	TI 50%	TI 25%	TI 12.5%	VC	
E		VC	NC	PC	TI 100%	TI 85%	TI 75%	TI 50%	TI 25%	TI 12.5%	VC	
F		VC	NC	PC	TI 100%	TI 85%	TI 75%	TI 50%	TI 25%	TI 12.5%	VC	
G		VC	NC	PC	TI 100%	TI 85%	TI 75%	TI 50%	TI 25%	TI 12.5%	VC	
H	Blank											

9.4 MTT Labelling and measurement

Following the incubation period, cells were observed before MTT labeling and observation such as cell morphology were recorded.

Then, the medium was removed and replaced with freshly prepared 50µl of 1mg/ml MTT solution per well. The plate was then left for 2±0.25 hrs, at 37±1°C, humidified, 5±0.5% CO_2 /air, to enable MTT labeling.

Following the 2-hrs of labelling, the MTT solution was removed from each well and Iso-Propanol was added at a volume of 100µl/well. The plate was shaken for at least 30 minutes on a microplate shaker at room temperature and then subjected to OD measurements.

10 OBSERVATIONS AND EXAMINATIONS

10.1 Absorbance Measurements

Absorbance signal was measured in microplate spectrophotometer (Multiskan® FC; Thermo Scientific) at 570nm wavelength filter (reference 650nm).

11 DATA EVALUATION

11.1 Determination of Cytotoxicity:

Absorbance signal was measured at two wavelengths: 570nm and 650nm (reference) and automatically calculated by the instrument (microplate spectrophotometer) according to the following formula:

$$\text{Results (OD)} = OD_{570nm} - OD_{650nm}$$

Blank signals were averaged and subtracted from each treatment replicates (located at columns 2-11 in rows B-G). Sample replicates were then averaged with standard deviations (SD).

Viability were calculated according to the following formula:

$$\text{Viability (\%)} = \frac{\text{(OD Sample Average)}}{\text{(OD Vehicle Control Average)}} \times 100$$

Where:

- OD Sample Average = The average value of the 100% extracts of the Test Item, Negative Control or Positive Control.
- OD Vehicle Control Average = The average value of the Vehicle Control

The lower the Viability % value, the higher the cytotoxic potential of the Test Item.
Cytotoxicity was calculated for samples according to the following formula:

$$\text{Cytotoxicity (\%)} = 100 - \text{Sample Viability (\%)}$$

The cytotoxic potential was determined according to the following criteria:

- Non-cytotoxic: Viability ≥70% as compared to the Vehicle Control
- Cytotoxic: Viability <70% as compared to the Vehicle Control

11.2 Validity Criteria:

A test is considered acceptable if the following criteria are met:

Test Material	Criteria
Vehicle Control	1) The mean OD of the Vehicle Control wells (columns 2 & 11) is ≥0.2 2) The left and the right mean viability (%) of the Vehicle Control (columns 2 and 11) values do not differ by more than 15% from the mean of all Vehicle Controls
Positive Control	<70% viability to confirm a cytotoxic effect
Negative Control	≥70% viability to confirm Non-cytotoxic effect
Test Item	The 50% dilution of the extract Test Item should have at least the same or a higher viability than the 100% extract.

11.3 Major Computerized Systems

- Multiscan FC software version 3.1
- Ex001CTXe-1 (validated Excel Spreadsheet for calculations of Treatment and Blank OD Mean, Standard Deviation, % Viability and % Cytotoxicity. Only the manually transferred data was subjected to QC process.

12 DEVIATIONS FROM STUDY PLAN

There were no deviations from Study Plan

13 ARCHIVING

Type	Period	Fate at termination of the period
Records and documentation relating to this study (including electronic records): raw data, original hard copy of the Study Plan, original hard copy of the Final Report and deviation, relevant correspondence.	2 years from the issue of the Final Report	The Sponsor will be contacted in order to determine and arrange the final disposition of the records and/or materials. The Sponsor will be responsible for all costs associated with the retention, retrieval, onward transfer or destruction/disposal of these Materials.
Remains of Test Items	Not applicable	Discarded

Whenever records will be transferred to the Sponsor, the latter should ensure that the materials and records in support of regulatory studies will be retained and maintained under conditions that guarantee their integrity and continued access according to archiving

requirements of the principles of GLP. The Sponsor should also ensure that such materials and records will be retained for as long as required by relevant authorities.

14 RESULTS

A summary of the mean ± SD MTT signal after blank subtractions, as well as the calculated viability of the experiment, are presented at Tables 1- 3 and Figure 1. Individual and additional calculated data are presented in Appendix 1, Tables A-B.

14.1 Acceptance Criteria

Mean OD of the Vehicle Control wells:	1.35	√ Criteria met
Left and the right mean viability of the Vehicle Control values/plate:	103.78% (L) 96.22% (R)	√ Criteria met
Mean viability of all Vehicle Controls/plate:	100%	
Mean viability of Positive Control:	-1.01%	√ Criteria met
Mean viability of Negative Control:	99.20%	√ Criteria met
Mean viability of the 50% Test Item's extract concentration:	93.25%	√ Criteria met
Mean viability of the 100% Test Item's extract concentration:	88.92%	

14.1.1 Vehicle Control

Microscopic evaluation revealed normal cell morphology, no lysed cells and ~95% confluence. The Vehicle Controls were not cytotoxic, showing 100.00% viability.

14.1.2 Negative Control

Microscopic evaluation revealed normal cell morphology, no lysed cells and ~95% confluence. The Negative Controls were not cytotoxic, showing 99.20% viability.

14.1.3 Positive Control

Microscopic evaluation revealed abnormal cell morphology, ~40% confluence, all cells were lysed or rounded. The Positive Controls were cytotoxic, showing -1.01% viability.

14.1.4 Test Item: *T-700 (Batch No.: NF2404245)*

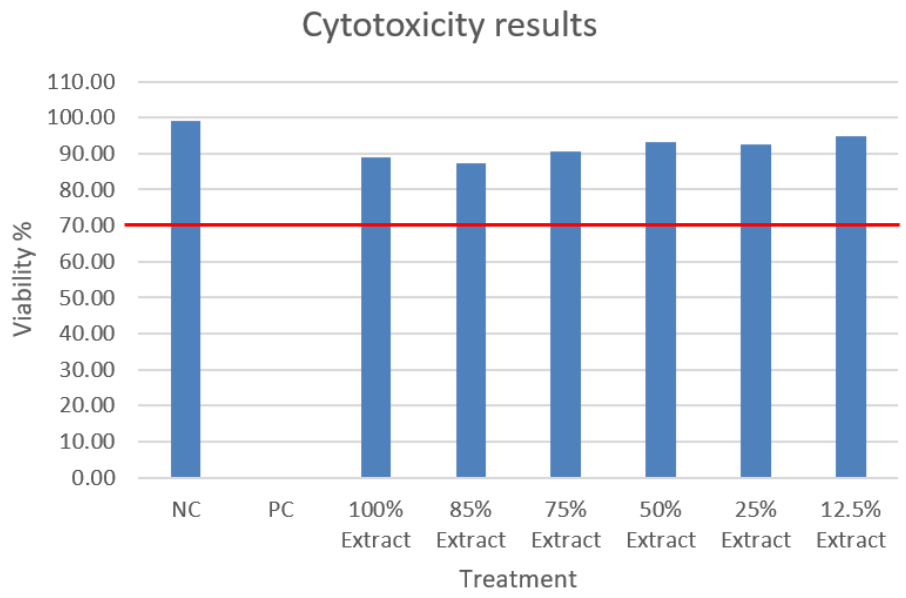
The Test Item's extract at concentrations 12.5%-100% had normal cell morphology, no lysed cells and ~95% confluence. The Test Item's extract at concentrations 12.5%-100% were non-cytotoxic with 87.31-94.86% viability**.**

15 CONCLUSION

Under the conditions of this study, and according to calculated viability, the extract of Test Item, ***T-700 (Batch No.: NF2404245)*** is considered non-cytotoxic at all concentrations tested, 12.5% - 100%, with 87.31-94.86% viability.

16 FIGURES

Figure 2 L929 Viability following Treatment with different dilutions of Test Item *T-700 (Batch No.: NF2404245)*



17 TABLES

Table 1 MTT Signal and Viability (% from Vehicle Control) of the Negative Control, Positive Control and Vehicle Control Extracts

Treatment	Extract (%)	Mean ± SD (O.D. 570-650nm)			Viability (%)
Negative Control	*100*	**1.34**	±	**0.03**	99.20
Positive Control	*100*	**-0.01**	±	**0.01**	-1.01
Vehicle Control	*100*	**1.35**	±	**0.08**	100

Table 2 The mean OD and the % viability of the left and the right Vehicle Control (columns 2 and 11) from the mean of all Vehicle Control

	Mean OD	Mean OD Column 2 & 11	Mean viability (%)	% of Mean viability
Vehicle Control column 2	1.40	***1.35***	***103.78***	± ***3.78***
Vehicle Control column 11	1.30		***96.22***	

Table 3 MTT Signal, Viability and Cytotoxicity of 12.5-100% Extract of the Test Item T-700 *(Batch No.: NF2404245)*

Extract (%)	Mean ± SD (O.D. 570-650nm)			Viability (%)	Cytotoxicity (%) (100-viability)
100%	**1.20**	±	0.07	88.92	**11.08**
85%	**1.18**	±	0.03	87.31	**12.69**
75%	**1.22**	±	0.03	90.53	**9.47**
50%	**1.26**	±	0.07	93.25	**6.75**
25%	**1.25**	±	0.06	92.63	**7.37**
12.5%	**1.28**	±	0.06	94.86	**5.14**

APPENDICES

Appendix 1 Individual Data Tables

Plates Map

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank											
B	Blank	VC	NC	PC	TI 100%	TI 85%	TI 75%	TI 50%	TI 25%	TI 12.5%	VC	Blank
C		VC	NC	PC	TI 100%	TI 85%	TI 75%	TI 50%	TI 25%	TI 12.5%	VC	
D		VC	NC	PC	TI 100%	TI 85%	TI 75%	TI 50%	TI 25%	TI 12.5%	VC	
E		VC	NC	PC	TI 100%	TI 85%	TI 75%	TI 50%	TI 25%	TI 12.5%	VC	
F		VC	NC	PC	TI 100%	TI 85%	TI 75%	TI 50%	TI 25%	TI 12.5%	VC	
G		VC	NC	PC	TI 100%	TI 85%	TI 75%	TI 50%	TI 25%	TI 12.5%	VC	
H	Blank											

Table A: MTT absorbencies at 570-650nm

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.0238	0.0218	0.0085	0.0117	0.0147	0.0123	0.0148	0.0139	0.0224	0.0231	0.0165	0.0158
B	0.0244	1.4300	1.3200	0.0161	1.1900	1.2200	1.2300	1.2700	1.2500	1.2400	1.2300	0.0345
C	0.0299	1.4000	1.3500	0.0181	1.2500	1.2100	1.2500	1.3000	1.3100	1.3500	1.3200	0.0163
D	0.0174	1.3400	1.3700	0.0073	1.1900	1.1600	1.1900	1.2400	1.2400	1.2900	1.3000	0.0210
E	0.0236	1.3900	1.3500	-0.0048	1.1900	1.1600	1.2300	1.1700	1.1800	1.2300	1.3100	0.0248
F	0.0239	1.4400	1.4100	0.0116	1.1600	1.2100	1.2800	1.3500	1.2700	1.3600	1.3600	0.0227
G	0.0264	1.5200	1.3500	0.0066	1.3400	1.2300	1.2700	1.3400	1.3700	1.3300	1.3900	0.0254
H	0.0244	0.0312	0.0408	0.0313	0.0214	0.0252	0.0266	0.0276	0.0274	0.0192	0.0261	0.0268

Table B: MTT absorbencies at 570-650nm of Test Plate following subtraction of the mean Blank signal (0.023)

	1	2	3	4	5	6	7	8	9	10	11	12
A	Not Applicable											
B	Not Applicable	1.407	1.297	-0.007	1.167	1.197	1.207	1.247	1.227	1.217	1.207	Not Applicable
C		1.377	1.327	-0.005	1.227	1.187	1.227	1.277	1.287	1.327	1.297	
D		1.317	1.347	-0.015	1.167	1.137	1.167	1.217	1.217	1.267	1.277	
E		1.367	1.327	-0.028	1.167	1.137	1.207	1.147	1.157	1.207	1.287	
F		1.417	1.387	-0.011	1.137	1.187	1.257	1.327	1.247	1.337	1.337	
G		1.497	1.327	-0.016	1.317	1.207	1.247	1.317	1.347	1.307	1.367	
H	Not Applicable											

18 ANNEXES

Annex 1 GLP Certificate

הרשות הלאומית להסמכת מעבדות
Israel Laboratory Accreditation Authority

Israel GLP Compliance Monitoring Unit

GLP Statement of Compliance No. 71

HBI Biotech Sciences Ltd.

Address: : 13 B Einstein St., Weizmann Science Park, Ness-Ziona, Israel

Valid from: 04.03.2024 **Until: 02.05.2026**

The test facility has been inspected by the Israel GLP compliance monitoring unit and has been found to be in compliance to OECD Principles of Good Laboratory Practice (GLP), as revised in 1997 and adopted on 26th November 1997 by decision of the OECD Council (C (97)186/Final) in the following areas of expertise:

- **Toxicity studies**
- **Other studies:**
  - **Pharmacodynamics studies**
  - **In Vitro testing**
  - **Biocompatibility Medical Devices**

Israel Laboratory Accreditation Authority (ISRAC) recognizes and confirms that the test facility is able to conduct the aforementioned studies in compliance with the OECD principles of GLP.

Etty Feller

General Manager
Israel Laboratory Accreditation Authority

Date of first recognition:02.05.2002

Date of signature 04/03/2024

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