Test Report

Form Code: F03-P32/00
Customer Code: M0314030521004/01

Identification

Company/Applicant	Rasa Salamat Arvand		
Address	National Institute of Genetic Engineering and Biotechnology, Pajoohesh Blvd.		
Date of Receipt	1403.05.21	**Test Duration**	3 Days
Date of Test	1403.05.25	**Number of Received item(s)**	1
Date of Report	1403.05.27	**Test Specific Conditions**	-

Environmental conditions

Temperature: 22 ± 3 °C **Humidity:** NMT 50 RH% **Preparation Conditions:** 37℃ for 24 h

Product picture and Test Result

Product Name:	Revaheal sept
Batch/Lot Number:	042

Received product picture

Test	Result			Acceptable limit	Compliance		Reference	Attachment
	Test Result*	Unit	Uncertainty		Pass	Fail		
Cytotoxicity by MTT (Viability)-Polar	97.14	%	±2.99	> 70%	non-toxic	-	ISO 10993-5, 2009,	Attachment I
Cytotoxicity by MTT (Morphological grade*)	0	-	-	< 2	non-toxic	-		

*** The results are calculated based on the average of Five repetitions.**
*** Morphological Evaluation:** *No observable cytotoxic morphological changes (e.g., rounding, lysis, or detachment) were observed under the microscope in either extract-exposed group compared to the negative control.*

EXAMINER: *S.Ghadami* Sign:
Lab. Manager: *M.Borjian* Sign:
CEO: *M.Borjian* Sign:
Nikopharmed laboratory Sing:

- *Test results are only related to the tested products.*
- *Reproduction of test results without the permission of the laboratory is prohibited.*
- *Sampling has been done by the customer.*
- *This report is not valid without the seal and signature of the CEO.*
- *If the tests were performed by the contractor, the name of the contractor is given in the description section.*
- *Any objection to the issued results can be processed within 7 days after the date of issuance of the result.*
- *If the sample is stable, after the test, the sample will be stored in the laboratory for one month.*
- *Expanded uncertainty (CI: 95% , K=2) is calculated for quantitative tests and included upon customer request.*
- *Nikopharmed Arya Company, National Institute of Genetic Engineering and Biotechnology, Pajuhesh Boulevard, 17km Tehran-Karaj Highway, Tehran, Iran*

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Test Objective

To evaluate the potential cytotoxic effects (Based on the ISO 10993-5) of leachable substances extracted from the test item by determining the viability of **L929 mouse fibroblast cells** *(Source: Pasteur Institute Cell Bank, Iran)* exposed to the polar and non-polar extracts of the sample using the MTT assay.

Description of product

Material composition: This Wound antiseptic solution provides effective disifection while promoting tissue regeneration and accelerating the healing process. It contains silver, Known for its strong antibacterial properties, and chitosan, which enhances wound healing and tissue repair.

Test procedure

Sample Preparation Process and Cell Cytotoxicity Test was done based on the ISO 10993-12 and 10993-5

1 ·Sample Extraction (ISO 10993-12):

Sample Preparation for Cytotoxicity Testing According to ISO 10993-12 (Hemodialyzer PS 160 HF)

Extraction vehicles:

Polar: DMEM (without FBS)

Extraction: The extraction procedure was performed at extraction ratios of 0.2 g/ml.

For the extraction of the internal fiber of the product, and taking into account the absorbent nature of the test material, the water absorption capacity of the sample was first determined. Based on the calculated absorption, the extraction medium was applied at a ratio of 0.2 g/ml.

Extraction temperature and time: 37 ± 1 °C for 24 hours

Extraction method: Dynamic incubation in an incubator

Extraction container: Chemically inert, sterile, and tightly sealed

Post-extraction handling: Extracts were collected aseptically and used directly for cytotoxicity testing according to ISO 10993-5

Post-extraction storage: 2–8 °C, protected from light, used within 24 hours

2. The test was carried out according to ISO 10993-5 standard method.

- Test procedure was done based on following table

Row	Procedure	Incubation time
1	Seed 96-well plates: $1*10^4$ cells/100µl MEM culture medium/well	Incubate 37°C / 5% CO_2 For 24 ± 2 h
2	Remove culture medium	-
3	Treat with ≥4 concentrations of test sample extract in treatment medium (100µl) (Untreated blank=treatment medium)	Incubate 37°C / 5% CO_2 For 24 h
4	Microscopic evaluation of morphological alterations Remove culture medium Add 50µl MTT solution	Incubate 37°C / 5% CO_2 For 2 h
5	Remove MTT solution Add 100 ml isopropanol to each well Sway plate	-
6	Detect absorption at 570 nm (reference 650 nm)	-

Negative control: *Untreated culture medium (DMEM + 10% FBS)*

Positive control: *0.1% Triton X-100 in culture medium*

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Conclusion and Interpretation

The Hemodialyzer PS 160 HF did not exhibit cytotoxic effects under the test conditions. Cell viability remained above the 70% threshold in both polar and non-polar extracts. According to ISO 10993-5, the test item is considered non-cytotoxic.

The results indicate that the sample meets the biocompatibility requirements for in vitro cytotoxicity. No further cytotoxic testing is necessary unless required by other parts of ISO 10993.

Diagram

Polar Solvent Non-Polar Solvent

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