Identification

Applicant	Rozhan vista mehr		
Address	Technopark, rasht, gilan-Iran		
Phone Number	+98 13 33473142	**Customer name**	Ms. Bahare padekan
Product Name	Collagen, Type I	**Batch Number**	1090
Date of receipt	1400/03/11	**Date of Test**	1400/03/21
Date of Completion	1400/03/24	**Number of tested products**	3

Cell line

L929 mouse fibroblast NCTC clone 929 strain L **Environmental conditions** 23±3℃

Cytotoxicity test of the examined product

Technique applied for cytotoxicity test	MTT ■ XTT ☐ CFU ☐ NRU ☐
Culture medium	Minimum essential medium (MEM)
Included anti-microbial agent	YES ☐ NO ■ **Nutrilizing agent/method** -
Sterilization test method	Gamma ☐ Ethylene oxide ☐ Autoclave ☐ **Other** -
Preparation of sample extract	Samples are extracted in accordance with ISO 10993-12

Procedure

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*10⁴ 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)	-

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Test Results

Table 1. Qualitative morphological grading and quantitative measurements of cytotoxic effects by MTT assay after **24 hours**

Repeat / Sample	Optical density (570nm)	Acceptable limit		Results			Director	Response Date
		Viability %	*Morphological grade*	*Morphological grade*	*Viability %*	*Average Viability %*		
Sample *One*	*1.085*	*>70%*	*< 2*	*0*	*96.88%*	*95.51%*	*S. Ansary*	*1400/03/24*
Sample *Two*	*1.058*			*0*	*94.63%*		*S. Ansary*	*1400/03/24*
Sample *Three*	*1.050*			*0*	*95.02%*		*S. Ansary*	*1400/03/24*
Negative control	*1.120*	*100%*	*0*	*0*	*100%*	*100%*	*S. Ansary*	*1400/03/24*
Negative control	*1.118*			*0*	*100%*		*S. Ansary*	*1400/03/24*
Negative control	*1.105*			*0*	*100%*		*S. Ansary*	*1400/03/24*
Total Average of test sample				***0***	***95.51%***			

Morphological grade

0: No reactivity

- None Discrete intracytoplasmic granules, no cell lysis, no reduction of cell growth

1: Slight reactivity

- Slight Not more than 20% of the cells are round, loosely attached and without intracytoplasmic granules, or show changes in morphology; occasional lysed cells are present; only slight growth inhibition observable.

2: Mild reactivity

- Mild Not more than 50% of the cells are round, devoid of intracytoplasmic granules, no extensive cell lysis; not more than 50% growth inhibition observable.

3: Moderate reactivity

- Moderate Not more than 70% of the cell layers contain rounded cells or are lysed; cell layers not completely destroyed, but more than 50 % growth inhibition observable.

4: Severe reactivity

- Severe Nearly complete or complete destruction of the cell layers

$$\text{Viability \%} = \frac{100 \times OD_{570t}}{OD_{570c}}$$

OD570t: is the mean value of measured optical density of the test material after subtracting blank (medium control)

OD570c: is the mean value of measured optical density of the negative control after subtracting blank (medium control)

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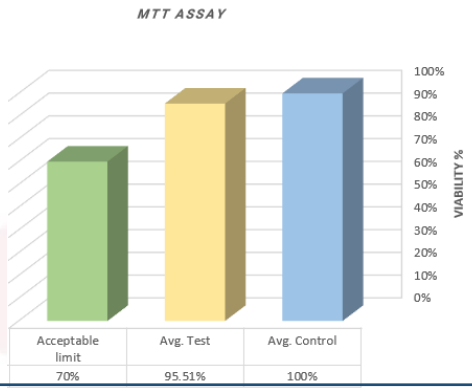

Conclusion

The *in-vitro* cytotoxicity response in L929 mouse fibroblast NCTC for the **Collagen, Type I** product of **Rozhan vista mehr** shown **95.51% viability**, and **No reactivity** in morphological grade for 24 hours.

The results provide evidence to support that the **Collagen, Type I** is **Non-Toxic**.

Interperation of results

The product to be examined complies with the test for *in-vitro* cytotoxicity test YES ■ NO ☐

Negative control

The result of the examined negative control for in-vitro cytotoxicity is Negligible ■ Slight to Severe ☐

References

1. ISO 10993-5, 2009, Biological evaluation of medical devices -Part 5: Tests for in vitro cytotoxicity
2. ISO 10993-1, 2018, Biological evaluation of medical devices -Part 1: Evaluation and testing
3. ISO 10993-12, 2012, Biological evaluation of medical devices -Part 12: Sample preparation and reference materials

Description: *For information*

Technical Manager:
Sign&Date:

Laboratory Manager:
Sign&Date:

Final Result

- *Test results are only related to the tested products.*
- *Sampling has been done by the customer.*
- *Test results should not be replicated without the laboratory permission.*
- *If the tests were performed by the contractor, the name of the contractor is given in the description section.*

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