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	**Model**	1090
Date of receipt	1400/03/11	**Date of Test**	1400/03/12
Date of Completion	1400/03/19	**Number of tested products**	3

Sample preparation and Environmental conditions

Sample type	Solid	**Extraction ratio**	6 cm^2/ml ± 10%
Extraction conditions	37±1 °C for 72±2 h in dynamic condition	**Environmental conditions**	20±2°C

Special irritation tests Not-specified ■ Implant ☐ Ocular ☐ Oral ☐ Penile ☐ Vaginal ☐ Rectal ☐

Test materials **Physiological saline 0.9%** NS002101 **SDS** - **FCA** -

Animal management

Species	Rabbit with a healthy intact skin	**Body weight range**	2.3 kg ~ 2.6 kg
Strain/Type	Albino	**Number of treated animals**	3
Sex	Male	**Number of control animals**	-

Test System

Food	Standard pellet provided from the authorized supplier
Housing	Healthy animals were acclimatized to the laboratory conditions before the treatment, and then they were Individually housed in stainless steel suspended cages identified by a card indicating the Identification number of the test article and the first treatment date.
Personnel	Associates involved were appropriately qualified and trained.
Selection	Only healthy, previously unused animals were selected.
Environmente	The room temperature and humidity was monitored daily.

Procedure

The invivo irritation test was performed based on the ISO 10993-10 standard method.

- **Sequence of key steps of the process for the irritation test**

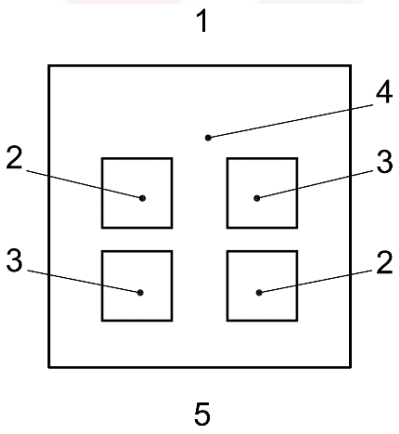
1. Preparation of animals

➢ The condition of the skin is a critical factor. Use only animals with healthy intact skin. Fur is generally clipped within 24 h to 4 h of testing on the backs of the animals, a sufficient distance on both sides of the spine for application and observation of all test sites (approximately 10cm×15cm). Fur may be re-clipped to facilitate observation and/or to accommodate repeated exposures. Depilatories may be used by trained technicians, if the process has been validated at the testing facility.

2. Sample preparation and application of the test sample

- Test sample and negative control was applied according to the experimental design as shown in the figure1.
- Sample preparation was done based on the sample type as mentioned in "Sample preparation" part at page 1 according to the ISO 10993-10 and ISO 10993-12 standard methods.

Figure 1. Location of skin application sites



1. Cranial end
2. Test sites
- *(left side is **A**; right side is **B**)*

3. Control sites
- *(left side is **A**; right side is **B**)*

4. Clipped dorsal regions
5. Caudal ends

3. Observation of animals

- Use of natural or full-spectrum lighting is highly recommended to visualize the skin reactions. Describe and score the skin reactions for erythema and oedema according to the scoring system given in Table 1, for each application site at each time interval, and record the results for the test report.

Table 1. Scoring system for skin reaction

Reaction	Irritation score
Erythema and eschar formation	
No erythema	**0**
Very slight erythema (barely perceptible)	**1**
Well-defined erythema	**2**
Moderate erythema	**3**
Severe erythema (beet-redness) to eschar formation preventing grading of erythema	**4**
Edema formation	
No edema	**0**
Very slight edema (barely perceptible)	**1**
Well-defined edema (edges of area well-defined by definite raising)	**2**
Moderate edema (raised approximately 1 mm)	**3**
Severe edema (raised more than 1 mm and extending beyond exposure area)	**4**
Maximal possible score for irritation	**8**

4. Evaluation of results

- For single exposure tests, determine the primary irritation index (PII) as follows. Use only (24±2) h, (48±2) h and (72±2) h observations for calculations. Observations made prior to dosing or after 72 h to monitor recovery are not used in the determination. After the 72 h grading, all erythema grades plus oedema grades (24±2) h, (48±2) h and (72±2) h are totalled separately for each test sample and blank for each animal. The primary irritation score for an animal is calculated by dividing the sum of all the scores by 6 (two test/observation sites, three time points).
- The primary or cumulative irritation index is characterized by number (score) and description (response category) given in Table 2. In case different extracts have been tested, the one giving the highest PII determines the response category.

Table 2. Irritation response categories in a rabbit

Mean score	Response category
Negligible	0 to 0,4
Slight	0,5 to 1,9
Moderate	2 to 4,9
Severe	5 to 8

Test Results

All animals appeared clinically normal throughout the study. All sites of the test extract and the reagent control appeared normal; the score of the test extract and the reagent control **all were 0**.

The Primary Irritation Index (PII) of the test article was **all 0.0** as shown at table 3.

Table 3. Skin reaction

Animal No.	Group	Site	Acceptable limit	Irritation score						Results		
				Erythema			Edema			Average score	Response Date	PASS / FAIL
				24±2	48±2	72±2	24±2	48±2	72±2			
1	Test sample	A	0 - 0.4	0/0	0/0	0/0	0/0	0/0	0/0	0/0	1400/03/19	PASS
		B		0/0	0/0	0/0	0/0	0/0	0/0			
	Control	A		0/0	0/0	0/0	0/0	0/0	0/0	0/0		PASS
		B		0/0	0/0	0/0	0/0	0/0	0/0			
2	Test sample	A	0 - 0.4	0/0	0/0	0/0	0/0	0/0	0/0	0/0	1400/03/19	PASS
		B		0/0	0/0	0/0	0/0	0/0	0/0			
	Control	A		0/0	0/0	0/0	0/0	0/0	0/0	0/0		PASS
		B		0/0	0/0	0/0	0/0	0/0	0/0			
3	Test sample	A	0 - 0.4	0/0	0/0	0/0	0/0	0/0	0/0	0/0	1400/03/19	PASS
		B		0/0	0/0	0/0	0/0	0/0	0/0			
	Control	A		0/0	0/0	0/0	0/0	0/0	0/0	0/0		PASS
		B		0/0	0/0	0/0	0/0	0/0	0/0			
Total Average of test samples				**0/0**	**0/0**	**0/0**	**0/0**	**0/0**	**0/0**	**0/0**	1400/03/19	PASS

Conclusion

The irritation response category in a rabbit of **Collagen, Type I Rozhan vista mehr** for 72±2 hours **were 0**, So the mean score is **Negligible**.

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

Interperation of results

The product to be examined complies with the test for irritation test YES ■ NO ☐

Negative control

The result of the examined negative control for irritation is Negligible ■ Slight to Severe ☐

References

1. ISO 10993-10: 2010, Biological evaluation of medical devices, Part 10: Tests for irritation and skin sensitization.
2. ISO 10993-12: 2012, Biological evaluation of medical devices, Part 12: Sample preparation and reference materials.
3. ISO 10993-2: 2006, Biological evaluation of medical devices, Part 2: Animal welfare requirements.

PHOTOGRAPH OF THE TEST ARTICLE

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.*