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**	1400/03/12
Date of Completion	1400/04/22	**Number of tested products**	10

Sample preparation

Sample type	Solid	**Extraction ratio**	6 cm^2/ml ± 10%
Extraction conditions	37±1 °C for 72±2 h in dynamic condition		
Test method	GPMT	**Environmental conditions**	20±2°C
Test materials	**Physiological saline 0.9%** NS002101	**SDS** SD002101	**FCA** FC002101

Animal management

Species	Guinea pigs	**Body weight range**	300 g ~ 500 g
Strain/Type	Albino	**Number of treated animals**	10
Sex	Male	**Number of control animals**	5

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 sensitization test was performed based on the ISO 10993-10 standard method.

- **Sequence of key steps of the process for the sensitization 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.

***Address**: National Institute of Genetic Engineering and Biotechnology, Pajoohesh Blvd*
***Web site**: http:/www.nikopharmed.com*
***Tell**: 02144184570, 09918126355*

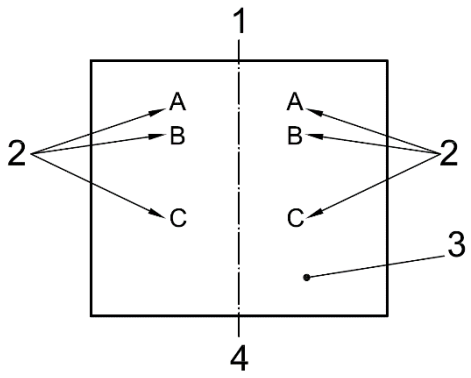
2. Choice of test methods

- There are currently three animal assays available for the determination of the skin sensitizing potential of chemicals. These include two guinea pig assays and one murine assay. So far, the two most commonly used methods for testing for skin sensitization are the Guinea Pig Maximization Test (GPMT) and the closed-patch test (Buehler test). Of these the maximization test is the most sensitive method. The closed-patch test is suitable for topical products.
- The murine Local Lymph Node Assay (LLNA) was internationally accepted for testing single chemicals as a stand-alone alternative to the guinea pig assays, and is now the preferred assay for chemicals.

3. 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 (as specified in Annex A) and ISO 10993-12 standard methods.

Figure 1. Location of skin application sites



1. Cranial end

2. Test sites (0,1 ml intradermal injections)

- *(left side **A1**, **B1**, **C1**; right side **A2**, **B2**, **C2**)*

3. clipped intrascapular region

4. Caudal ends

4. Main test

- Intradermal induction phase
  - Make a pair of 0,1 ml intradermal injections of each of the following, into each animal, at the injection sites (A, B and C), as shown in Figure 1, in the clipped intrascapular region.
  - ***Site A:*** A 50:50 volume ratio stable emulsion of Freund's complete adjuvant mixed with the chosen solvent. Use physiological saline (BP, USP or equivalent) for water-soluble materials.
  - ***Site B:*** The test sample (undiluted extract); inject the control animals with the solvent alone.
  - ***Site C:*** The test sample at the concentration used at site B, emulsified in a 50:50 volume ratio stable emulsion of Freund's complete adjuvant and the solvent (50%); inject the control animals with an emulsion of the blank liquid with adjuvant.

Address: *National Institute of Genetic Engineering and Biotechnology, Pajoohesh Blvd*
Web site: *http:/www.nikopharmed.com*
Tell: *02144184570, 09918126355*

- Topical induction phase
  - At (7±1) d after completion of the intradermal induction phase, administer the test sample by topical application to the intrascapular region of each animal, using a patch of area approximately 8cm² (filter paper or absorbent gauze), so as to cover the intradermal injection sites (Figure 1). If the maximum concentration that can be achieved in "intradermal induction phase" does not produce irritation, pretreat the area with 10 % sodium dodecyl sulfate massaged into the skin (24±2) h before the patch is applied. Remove the dressings and patches after (48±2) h.
  - Freshly prepared extracts are preferred. If an extract is stored longer than (24±2) h, then the stability of the extract under the conditions of storage should be verified.
  - Treat the control animals similarly, using the blank liquid alone.

- Challenge phase
  - At (14±1) d after completion of the topical induction phase, challenge all test and control animals with the test sample. Administer the test sample and a blank by topical application to sites that were not treated during the induction stage, such as the upper flank of each animal, using appropriate patches or chambers soaked in the test sample at the concentration selected in "intradermal induction phase" for site C. Dilutions of this concentration might also be applied to other untreated sites in a similar manner. Secure with an occlusive dressing. Remove the dressings and patches after (24±2) h.

5. Observation of animals

- Observe the appearance of the challenge skin sites of the test and control animals (24±2) h and (48±2) h after removal of the dressings. Use of natural or full-spectrum lighting is highly recommended in order to visualize the skin reactions. Describe and grade the skin reactions for erythema and oedema according to the Magnusson and Kligman grading given in **Table 1** for each challenge site and at each time interval. It is highly recommended that reading be done without knowledge of the treatment, in order to minimize bias in the evaluation of the results.

Table 1. Magnusson and Kligman scale

Patch test reaction	Grading scale
No visible change	0
Discrete or patchy erythema	1
Moderate and confluent erythema	2
Intense erythema and/or swelling	3

6. Evaluation of results

Magnusson and Kligman grades of 1 or greater in the test group generally indicate sensitization, provided grades of less than 1 are seen in control animals. If grades of 1 or greater are noted in control animals, then the reactions of test animals which exceed the most severe reaction in control animals are presumed to be due to sensitization.

Address: *National Institute of Genetic Engineering and Biotechnology, Pajoohesh Blvd*
Web site: *http:/www.nikopharmed.com*
Tell: *02144184570, 09918126355*

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 was 0.**

The Sensitization score of the test article was **all 0.0** as shown at table 2.

Table 2. Skin reaction

Group	Animal No.	Site	Acceptable limit	Sensitization score		Results		
				24±2	*48±2*	*Worst case*	*Response Date*	*PASS / FAIL*
Test Sample	*1*	*A1*	*0*	*0*	*0*	*0*	1400/04/22	*PASS*
		B1		*0*	*0*			
		C1		*0*	*0*			
		A2		*0*	*0*			
		B2		*0*	*0*			
		C2		*0*	*0*			
	2	*A1*	*0*	*0*	*0*	*0*	1400/04/22	*PASS*
		B1		*0*	*0*			
		C1		*0*	*0*			
		A2		*0*	*0*			
		B2		*0*	*0*			
		C2		*0*	*0*			
	3	*A1*	*0*	*0*	*0*	*0*	1400/04/22	*PASS*
		B1		*0*	*0*			
		C1		*0*	*0*			
		A2		*0*	*0*			
		B2		*0*	*0*			
		C2		*0*	*0*			
	4	*A1*	*0*	*0*	*0*	*0*	1400/04/22	*PASS*
		B1		*0*	*0*			
		C1		*0*	*0*			
		A2		*0*	*0*			
		B2		*0*	*0*			
		C2		*0*	*0*			
	5	*A1*	*0*	*0*	*0*	*0*	1400/04/22	*PASS*
		B1		*0*	*0*			
		C1		*0*	*0*			
		A2		*0*	*0*			
		B2		*0*	*0*			
		C2		*0*	*0*			
	Worst case					***0***	***Final Result***	*PASS*

Address: *National Institute of Genetic Engineering and Biotechnology, Pajoohesh Blvd*
Web site: *http:/www.nikopharmed.com*
Tell: *02144184570, 09918126355*

Test Results

Table 2. Skin reaction - continue

Group	Animal No.	Site	Acceptable limit	Sensitization score		Results		
				24±2	*48±2*	*Worst case*	*Response Date*	*PASS / FAIL*
Test Sample	*6*	*A1*	*0*	*0*	*0*	*0*	1400/04/22	*PASS*
		B1		*0*	*0*			
		C1		*0*	*0*			
		A2		*0*	*0*			
		B2		*0*	*0*			
		C2		*0*	*0*			
	7	*A1*	*0*	*0*	*0*	*0*	1400/04/22	*PASS*
		B1		*0*	*0*			
		C1		*0*	*0*			
		A2		*0*	*0*			
		B2		*0*	*0*			
		C2		*0*	*0*			
	8	*A1*	*0*	*0*	*0*	*0*	1400/04/22	*PASS*
		B1		*0*	*0*			
		C1		*0*	*0*			
		A2		*0*	*0*			
		B2		*0*	*0*			
		C2		*0*	*0*			
	9	*A1*	*0*	*0*	*0*	*0*	1400/04/22	*PASS*
		B1		*0*	*0*			
		C1		*0*	*0*			
		A2		*0*	*0*			
		B2		*0*	*0*			
		C2		*0*	*0*			
	10	*A1*	*0*	*0*	*0*	*0*	1400/04/22	*PASS*
		B1		*0*	*0*			
		C1		*0*	*0*			
		A2		*0*	*0*			
		B2		*0*	*0*			
		C2		*0*	*0*			
	Worst case					*0*	*Final Result*	*PASS*

Address: *National Institute of Genetic Engineering and Biotechnology, Pajoohesh Blvd*
Web site: *http:/www.nikopharmed.com*
Tell: *02144184570, 09918126355*

Test Results

Table 2. Skin reaction – continue

Group	Animal No.	Site	Acceptable limit	Sensitization score		Results		
				24±2	*48±2*	*Worst case*	*Response Date*	*PASS / FAIL*
Control	*1*	*B1*	*0*	*0*	*0*	*0*	1400/04/22	*PASS*
		C1		*0*	*0*			
		B2		*0*	*0*			
		C2		*0*	*0*			
	2	*B1*	*0*	*0*	*0*	*0*	1400/04/22	*PASS*
		C1		*0*	*0*			
		B2		*0*	*0*			
		C2		*0*	*0*			
	3	*B1*	*0*	*0*	*0*	*0*	1400/04/22	*PASS*
		C1		*0*	*0*			
		B2		*0*	*0*			
		C2		*0*	*0*			
	4	*B1*	*0*	*0*	*0*	*0*	1400/04/22	*PASS*
		C1		*0*	*0*			
		B2		*0*	*0*			
		C2		*0*	*0*			
	5	*B1*	*0*	*0*	*0*	*0*	1400/04/22	*PASS*
		C1		*0*	*0*			
		B2		*0*	*0*			
		C2		*0*	*0*			
	Worst case					***0***	***Final Result***	*PASS*

Address: *National Institute of Genetic Engineering and Biotechnology, Pajoohesh Blvd*
Web site: *http:/www.nikopharmed.com*
Tell: *02144184570, 09918126355*

Conclusion

The sensitization response category in a Guinea pigs of **Collagen, Type I Rozhan vista mehr** for 48±2 hours **was 0,** So the mean score is **No visible change**.

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

Interperation of results

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

Negative control

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

References

1. ISO 10993-10: 2010, Biological evaluation of medical devices, Part 10: Tests for sensitization 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.*

***Address**: National Institute of Genetic Engineering and Biotechnology, Pajoohesh Blvd*
***Web site**: http:/www.nikopharmed.com*
***Tell**: 02144184570, 09918126355*