Title: *Skin Sensitization Test of Finished Products* *Report Form*

Customer Code: **M0983031114005/01**
Form Code: **F03-P36**

Identification

Applicant	Tirooj Kimia Daroo		
Address	Science and Technology Park Of Kurdistan, Zakaryaye Razi Blvd, Ghane Sq, Baharan, Sanandaj, Kurdistan, Iran		
Product Name	Molar-Etch		
Batch Number	ME241214		
Date of Receipt	1403.11.14	**Test Duration**	30-35 Days
Date of Test	1403.11.14	**Number of Received items**	6 Samples
Date of Report	1403.12.11	**Number of Tested animals**	15 Repeats

Animal management

Species	Guinea Pig (Albino)	**Body weight range**	300 - 500 g
Strain/Type	single outbred	**Number of Treated animals**	10
Sex	Male or Female	**Number of Control animals**	5

Sample preparation and Environmental conditions

Extraction conditions	37±1 °C for 72±2 h in dynamic condition
Test method	Guinea pig maximization test (GPMT)
Environmental conditions	20±2°C

Test System

Food	Standard pellet provided by the authorized supplier
Housing	Healthy animals were acclimatized to the laboratory conditions before the treatment. 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	The associates involved were appropriately qualified and trained.
Selection	Only healthy, previously unused animals were selected.
Environment	The room temperature and humidity were monitored daily.

Procedure

The in vivo 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
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.

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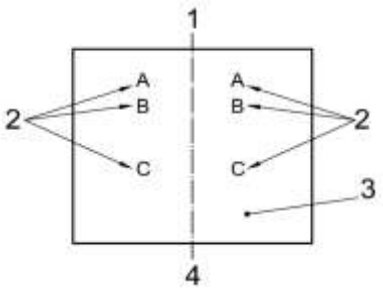
3. Sample preparation and application of the test sample

- Test sample and negative control were applied according to the experimental design as shown in the Figure 1.
- Sample preparation was done based on the sample type as mentioned in the "Sample preparation" part on page 1 according to the ISO 10993-10 (as specified in Annex A) and ISO 10993-12 standard methods.



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

Figure 1. Location of skin application sites

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.
- Topical induction phase
- Challenge phase

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. 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.

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

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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.

Test Results

Table 2. Skin reaction

Animal Group	Acceptable limit	Sensitization score			Results	
		24±2	*48±2*	*72±2*	*Worst case*	*PASS / FAIL*
Control	*0*	*0*	*0*	*0*	*0*	*PASS*
		0	*0*	*0*		
		0	*0*	*0*		
		0	*0*	*0*		
		0	*0*	*0*		
Test Sample	*0*	*0*	*0*	*0*	*0*	*PASS*
		0	*0*	*0*		
		0	*0*	*0*		
		0	*0*	*0*		
		0	*0*	*0*		
		0	*0*	*0*		
		0	*0*	*0*		
Final Result						*PASS*

PHOTOGRAPH OF THE TEST ARTICLE

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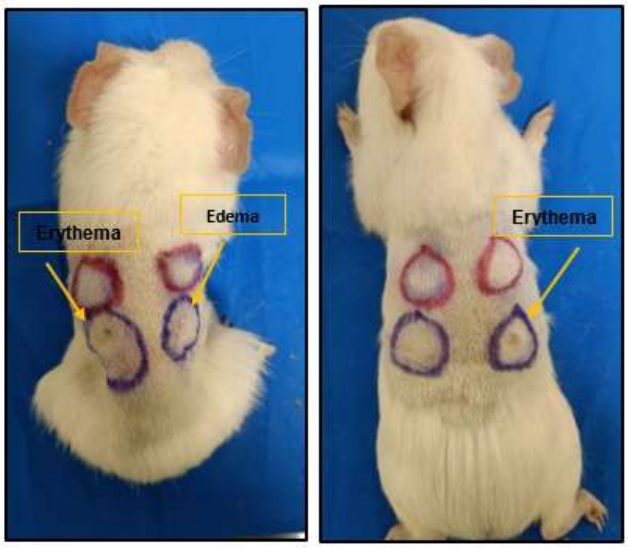
Conclusion

The sensitization response category in a Guinea pig of the examined product for 72±2 hours **was 0,** So the mean score is **No visible change**. The results provide evidence to support that the product is **Non-Sensitizing.**

Also, after intradermal injection and 24±2h before phase II patch application the sample showed a reaction according to the picture below.



References

1. ISO 10993-10: 2010, Biological evaluation of medical devices, Part 10: Tests for irritation and skin sensitization.
2. ISO 10993-12: 2021, Biological evaluation of medical devices, Part 12: Sample preparation and reference materials.
3. ISO 10993-2: 2022, Biological evaluation of medical devices, Part 2: Animal welfare requirements.

Description This result was done based on the customer's request

Test Performer: *Z. Sayyahi*	**Technical Manager:** *M. Daliri*	**CEO:** *M.Borjian*	**Nikopharmed laboratory sign**
Sign:	Sign:	Sign:	

- *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's permission.*
- *If the tests were performed by the contractor, the name of the contractor is given in the description section.*
- *If the sample matrix is stable, the sample will be stored in the laboratory for three months after testing.*
- *Any objection to the issued results can be processed within 7 days after the date of issuance of the result.*

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