TEST REPORT

Report Ref. No.
15-035315-01-1

Name and address of the applicant

NUGA MEDICAL CO., LTD
185 Jiraeul-ro, Jijeong-myeon, Wonju-si, Gangwon-Do, 220-821, Korea
TEL:033-730-0026
FAX:033-748-7447

Standard / Test method

The methods(standards) were requested by the applicant

Test result

Test article(s) complies with the following method(standard)

Tested equipment :

Low frequency electrode

Model/type ref. :

Low frequency electrode

Manufacturer :

NUGA MEDICAL CO., LTD

185 Jiraeul-ro, Jijeong-myeon, Wonju-si, Gangwon-Do, 220-821, Korea

Additional information :

LFE1506001

Issue date : 2015-08-04
Reissue date : 2015-08-05

Tested and reported by

이종찬

Lee Jong-chan, Senior Engineer

Reviewed by

Seok-kyoung, Kong , Technical Manager

KOREA TESTING LABORATORY

※위 마크는 추후 전자확인증 대조 프로그램에서 원본대조시 사용되는 2D코드입니다.

Requirement - Test	Results P F N/A	Remarks	1)T/F
1. Cytotoxicity test (Agar Diffusion Test) Under the conditions of ISO 10993-5:2009, Tests for in vitro cytotoxicity, the test articles should meet the test requirements.	(V) ( ) ( )	See attachment 1	- / -
2. Maximization Sensitization Under the conditions of ISO 10993-10:2010 7.5 Guinea pig maximization test(GPMT), the test articles should meet the test requirements	(V) ( ) ( )	See attachment 2	- / -
3. Intracutaneous Reactivity Under the conditions of ISO 10993-10:2010, 6.4 Animal intracutaneous (intradermal) reactivity test, the test articles should meet the test requirements	(V) ( ) ( )	See attachment 3	- / -

※ Remarks

1. Date of application : 2015.06.17
2. Test location : Korea Testing Laboratory
3. Test period : 2015.06.17. ~ 2015.08.04.
4. Test article preparation : 0.2 g/mL, (70 ± 2) °C, (24 ± 2) h

P: Pass, F: Fail, N/A: Not Applicable, 1)T/F: Technical File

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Attachment 1

STUDY TITLE :

CYTOTOXICITY STUDY
AGAR DIFFUSION METHOD

TEST ARTICLE :

Low frequency electrode

Model/Type Ref. :

Low frequency electrode

IDENTIFICATION NO. :

Lot No. LFE1506001

2015.07.15.

Korea Testing Laboratory

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Study Summary

The Low frequency electrode / Low frequency electrode(Lot No.:LFE1506001) sponsored by NUGA MEDICAL CO., LTD was subjected to a cytotoxicity study for biocompatibility as below.

- ISO 10993-5:2009 Tests for in vitro cytotoxicity
- Ministry of Food and Drug Safety, Notification no. 2014-115

The growth medium in each dish was replaced to equal amounts of double strength minimum essential medium and 2 % agarose. The MEM-agarose mixture(4ml) was then placed in the cell culture plate and allowed to solidify over the cells to form the agarose overlay. The test sample was placed on the solidified agarose surface in separate wells for each sample. Similarly, the negative control, and the positive control were each placed on the solidified agarose surface in separate wells. The cell culture plates were incubated at 37 ℃ in 5 % CO_2 for 24 hours to 48 hours. Following incubation, the cultures were examined macroscopically for cell decolorization around the test article and controls to determine the zone of cell lysis.

Under the conditions of this study, the test article is considered to be suitable for testing standards.

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1. Introduction

■ **Purpose**

The purpose of this study was to determine whether leachable extracted from the test article would cause cytotoxicity.

■ **Dates**

Experiment Start : 2015.07.06.
Experiment End : 2015.07.10.

■ **Testing Guideline**

- ISO 10993-5:2009 Tests for in vitro cytotoxicity
- Ministry of Food and Drug Safety, Notification no. 2014-115

2. Materials

■ **Test Article**

The test article provided by the sponsor was identified and handled as follows:

Test Article	:	Low frequency electrode
Model No.	:	Low frequency electrode
Lot No.	:	LFE1506001
Storage Condition	:	Room temperature
Sponsor	:	NUGA MEDICAL CO., LTD

■ **Controls(supplied by KTL)**

1) Negative Control : HDPE

2) Positive Control : ZDEC

■ **Media(supplied by KTL)**

1) 2X MEM(Minimum Essential Media)
Color : Red, Transparent
Storage condition : Refrigerate
Composition : MEM(with 10 % Fetal bovine Serum, 2 % antibiotics)

■ **Test article Preparation**

The test article was trimmed to approximately 1 cm X 1 cm and it was used in test.

■ **Control Preparation**

1) Negative Control : The negative samples were cut into approximately 10 mm X 10 mm.
2) Positive Control : The positive samples were cut into approximately 10 mm X 10 mm.

■ **Test System**

1) Mouse Fibroblast Cells (ATCC CCL 1, Clone 929, of Strain L, or equivalent source)
2) Test System Management

Cultures of L929 cells were propagated at 37°C in sealed flasks containing MEM supplemented with fetal bovine serum and a 2 % concentration of the antibiotics. For this study, 75-T flasks were seeded, labeled with the passage number and date, and incubated at 37 °C in order to obtain confluent monolayers of cells prior to use.

Justification of Test System:

Mammalian cell cytotoxicity study has been used historically to evaluate cytotoxicity.

3. Methods

■ **Procedure**

The culture plate (6wells) that contained a confluent cell monolayer were selected. The growth medium in each dish was replaced to equal amounts of double strength minimum essential medium and 2 % agarose (final concentration 1 % agarose, 1×MEM). The MEM-agarose mixture was then placed in the cell culture plate and allowed to solidify over the cells to form the agarose overlay.

■ **Observation**

The test sample was placed on the solidified agarose surface in three separate wells for each three samples. Similarly, the negative control, and the positive control were each placed on the solidified agarose. The culture plates were labeled with the corresponding lab number and dosing date, and incubated at 37 °C in 5 % CO_2 for 24 hours to 72 hours(by the request of the sponsor). Following incubation, the cultures were examined macroscopically for cell decolorization around the test article and controls to determine the zone of cell lysis (if any). After macroscopic examination, the cell monolayers were examined microscopically(100×) to verify any decolorized zones and to determine cell morphology in proximity to the article.

Scoring for cytotoxicity was based on the following criteria:

Table 1 Reactivity grade for agar diffusion Study

Grade	Description of reactivity zone
0	No detectable zone around or under specimen
1	Some malformed or degenerated cells under specimen
2	Decoloized zone limited to area under specimen
3	Zone extending specimen size up to 1.0 cm
4	Zone extending farther than 1.0 cm beyond specimen

For the suitability of the system to be confirmed, the negative control must have been a grade of 0 (reactivity none) and the positive controls must have been a grade 3. The test article passed the test if all of the monolayers exposed to the test medium showed no greater than grade 2. The test would have been repeated if the controls did not perform as anticipated and/ or if all test petridishes did met yield the same conclusion.

4. Results

The scores obtained were as follows:

		After 24 h	After 48 h	After 72 h
Test Article	Sample 1	0	0	0
	Sample 2	0	0	0
	Sample 3	0	0	0
Control	Negative Control 1	0	0	0
	Negative Control 2	0	0	0
	Negative Control 3	0	0	0
	Positive Control 1	4	4	4
	Positive Control 2	4	4	4
	Positive Control 3	4	4	4

5. Conclusion

The test article showed no evidence of causing cell lysis or toxicity. The negative controls, and the positive controls performed as anticipated. Under the conditions of this study, the test article was considered to be suitable for testing standards.

6. Records

All raw data pertaining to this study and a copy of the final report are to be retained in designated KTL archive files for a period of 5 years.

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Attachment 2

STUDY TITLE :

GUINEA PIG MAXIMIZATION SENSITIZATION

TEST ARTICLE :

Low frequency electrode

Model/Type Ref. :

Low frequency electrode

IDENTIFICATION NO. :

Lot No. LFE1506001

2015.08.03.

Korea Testing Laboratory

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Study Summary

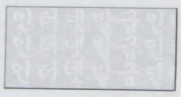
The Low frequency electrode / Low frequency electrode(Lot No.:LFE1506001) sponsored by NUGA MEDICAL CO., LTD was extracted and subjected to a maximization test for biocompatibility as below:

- ISO10993-10:2010, 7.5 Guinea pig maximization test (GPMT)
- Ministry of Food and Drug Safety, Notification no. 2014-115, no.9 6. Skin sensitization tests

The test articles were extracted in a 0.9 % sodium chloride solution and cotton seed oil. Each extract was intradermally injected and occlusively patched to 10 test guinea pigs (per extract) in an attempt to induce sensitization.

The vehicle was similarly injected and occlusively patched to five control guinea pigs (per vehicle). Following a recovery period, the test and control animals received a challenge patch for the appropriate test article extract and the reagent control. All sites were scored at 24 h and 48 h after patch removal.

Under the conditions of this study, the SC test extract and CSO test extract showed no evidence of causing delayed dermal contact sensitization in the guinea pig.



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1. Introduction

■ Purpose

The purpose of this study was to evaluate whether leachable extracted from the test article would cause the potential for delayed dermal contact sensitization in the guinea pig.

■ Dates

Animal Receiving : 2015.07.01.
Experiment Start : 2015.07.06.
Experiment End : 2015.07.31.

■ Testing Guideline

- ISO10993-10:2010, 7.5 Guinea pig maximization test (GPMT)
- Ministry of Food and Drug Safety, Notification no. 2014-115, no.9 6. Skin sensitization tests

2. Materials

■ Test Article

The test article provided by the sponsor was identified and handled as follows:

Test Article	:	Low frequency electrode
Model No.	:	Low frequency electrode
Lot No.	:	LFE1506001
Storage Condition	:	Room temperature
Sponsor	:	NUGA MEDICAL CO., LTD

■ Extraction Vehicles and Control(supplied by KTL)

1) 0.9 % Sodium Chloride Solution, (SC)
Color : Clear
Storage condition : Room temperature
Manufacturer : Daihan Pharm Co. Ltd

2) Cotton Seed Oil, (CSO)
Color : yellow
Storage condition : Room temperature
Manufacturer : SAMCHUN CHEMICAL

3) Positive Control : Dinitrochlororobenzene(DNCB)
Color : yellow
Storage condition : Room temperature
Manufacturer : SIGMA

■ **Additional Materials(supplied by KTL)**

1) Freund's Complete Adjuvant
Color : yellow
Storage condition : Room temperature
Manufacturer : SIGMA

■ **Test Article Preparation**

Test article preparation was conducted according to the requirements of ISO 10993-12:2012.
Extracted the body contact part of Low frequency electrode

Thickness and Form of Test Article	Weight	Volume of extract liquid	Extraction Condition
amorphousness	4 g	20 mL	(70 ± 2) ℃, (24 ± 2) h

=> Test Article was extracted according to the conditions presented by the applicant in consideration of the characteristics of the product.

■ **Control Preparation**

1) Negative Control
The negative control was prepared with the same extraction condition as the test article.

2) Positive Control
The 0.1 % concentration of Dinitrochlororobenzene(DNCB) in 95 % ethanol was prepared same extraction condition as the test article.
(A positive control test was conducted for a period no less than six months, one or more times.)

■ **Test System**

Species	:	Guinea pig
Strain	:	Hartley Hsd:DH
Source	:	ORIENT BIO Inc. / 322,Galmachi-ro, Jungwon-gu, Seongnam-si, Gyeonggi-do, Korea
Sex	:	Female
Number of Animals	:	30
Age	:	No particular age was prescribed for this test
Acclimation Period	:	Minimum 5 days
Identification Method		Picric Acid

Justification of Test System:

The Hartley albino guinea pig has been used historically for sensitization studies (Magnusson and Kligman, 1970). The guinea pig is believed to be the most sensitive animal model for this type of study.

■ **Animal Management:**

Food	:	A commercially available guinea pig feed was provided daily
Source	:	ORIENT BIO Inc. / 322,Galmachi-ro, Jungwon-gu, Seongnam-si, Gyeonggi-do, Korea
Water	:	Reverse osmotic water was provided through an automatic watering system
Housing	:	Animals were housed in groups of five in guinea pig stainless steel cage
Environment	:	The temperature range - (22 ± 3) ℃ The humidity range - (50 ± 15) % R.H. The light cycle - on : 07:00, off : 19:00 The intensity of illumination : (150 ~ 300) Lux

No feed or water analysis was performed, because there were no possible contaminants that could interfere with the study. Animal husbandry and environmental conditions conformed to current Korea Testing Laboratory SOPs.

■ **IACUC**

This study was conducted in compliance with the regulations on management and use of laboratory animals.

- Korea Testing Laboratory, IACUC Guideline (IX-6)
- Korea Food and Drug Agency & Animal, Plant and Fisheries Quarantine and Inspection Agency, IACUC Standard Guideline
- ISO 10993-2:2006

■ **Equipment**

Equipment	No.	Date of Calibration	Note
Electric Balance	0610179	2015.03.26.	
Shaking bath	1110582	2014.11.12.	

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3. Methods

■ **Groups**

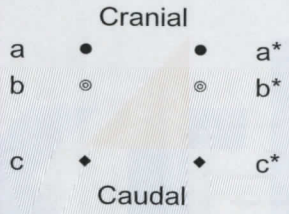
Group	Sex	Number of Animals	No.
SC Test	female	10	01 ~ 10
SC Control	female	5	11 ~ 15
CSO Test	female	10	16 ~ 25
CSO Control	female	5	26 ~ 30

■ **Procedure**

The day prior to treatment, each animal was weighted, identified and the fur over the dorsoscapular region was removed with an electric clipper.

1) Induction I (Intradermal Application) : Day 0

The following day, the test animals were injected with the test article extract and the control animals were injected with the reagent control.



Experimental Group :

a. 0.1 mL FCA 1:1 with the chosen vehicle
b. 0.1 mL Test article extract
c. 0.1 mL Test article extract 1:1 with FCA

Negative Control Group :

a. 0.1 mL FCA 1:1 with the chosen vehicle
b. 0.1 mL Vehicle
c. 0.1 mL Vehicle 1:1 with FCA

Positive Control Group :

a. 0.1 mL FCA 1:1 with Vehicle
b. 0.1 mL 0.1 % DNCB in 95 % Ethanol
c. 0.1 mL 0.1 % DNCB in 95 % Ethanol 1:1 with FCA

2) Introduction II (Topical Application) : Day 6 ± Day 1

On the sixth day (± 1) after the induction I injection, the same area as used during induction I was clipped free of fur and treated with 0.5 to 1 grams of a 10 % sodium lauryl sulfate (SLS) suspension in petrolatum. The suspension was massaged into the skin over the injection site to provoke mild acute inflammation. The area was left uncovered. At 24 hours (±2 hours) after the SLS administration, all remaining SLS residue was gently removed with a gauze pad.

A filter paper, saturated with a freshly prepared test article extract, was then topically applied to the previously injected sites of the test animals. The control animals were similarly patched with the appropriate reagent control. Each patch was secured with a non-reactive tape and the trunk of each animal was wrapped with an elastic bandage. At 48 hours (±2 hours), the binders and patches were removed.

Negative control group animals were exposed to the vehicle without the test article in the same way as the experimental group.

3) Challenge Application : Day 23 ± Day 1

On the day prior to the challenge patch, the fur was removed from the sides and flank. On the 14th day since the removal of the induction patch, the non-woven cotton disk was saturated with the test article extract or reagent control. All patches were topically applied as indicated below:

	CHALLENGE SITE	
TREATMENT GROUP (n)	LEFT FLANK	RIGHT FLANK
Test (10)	Extract Vehicle	Test Extract
Negative Control (5)	Extract Vehicle	Control Extract

Each patch was secured to the skin with a semi-occlusive hypoallergenic adhesive tape. The trunk of each animal was wrapped with an elastic bandage to maintain the well-occluded sites for the (24 ± 2) hours exposure period. The sites were wiped gently with gauze after patch removal.

■ **Observation**

Observations for dermal reactions were conducted at (24 ± 2) h and (48 ± 2) h after the challenge patch was removed. Scores were recorded in accordance with the criteria shown below:

The response, pattern, character, and duration of test animal reactions were compared to reactions in the control conditions. The dermal inflammatory response at the test sites greater than that seen in any control condition was considered evidence of a potential allergic response.

< 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 Swelling	3

4. Results (Table I)

1) Body Weights : All animals gained in body weight.
2) Clinical observation : All animals appeared clinically normal throughout the study.
3) Dermal sensitization : None of the treated or negative control animals exhibited any reaction to the challenge (0 % sensitized).

Results and conclusions apply only to the tested articles. No further evaluation of these results is made by KTL. Any extrapolation of these data to other samples is the responsibility of the sponsor.

5. Conclusion

Under the conditions of this study, the test article extracts showed no evidence of causing delayed dermal contact sensitization in guinea-pig.

6. Records

All raw data pertaining to this study and the copy of the final report are to be retained in designated KTL archive files for a period of 5 years.

Table I. Body Weight & Clinical Observations & Dermal Reaction

Group	An. #	Sex	Wt (g) DAY 0	Wt (g) DAY 25	Signs of Toxicity*	Magnusson and Kligman Scale			
						24 Hour		48 Hour	
						Site A	Site B	Site A	Site B
Test Group (SC Extract)	1	Female	337.9	504.5	None	0	0	0	0
	2	Female	349.2	498.2	None	0	0	0	0
	3	Female	361.5	476.8	None	0	0	0	0
	4	Female	361.8	470.9	None	0	0	0	0
	5	Female	346.0	484.0	None	0	0	0	0
	6	Female	337.0	499.7	None	0	0	0	0
	7	Female	358.1	506.4	None	0	0	0	0
	8	Female	360.4	493.7	None	0	0	0	0
	9	Female	341.5	466.8	None	0	0	0	0
	10	Female	333.0	464.3	None	0	0	0	0
Control Group (SC)	11	Female	361.4	498.2	None	0	0	0	0
	12	Female	342.8	501.4	None	0	0	0	0
	13	Female	347.7	485.5	None	0	0	0	0
	14	Female	357.4	465.2	None	0	0	0	0
	15	Female	352.8	465.7	None	0	0	0	0

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Table I. Body Weight & Clinical Observations & Dermal Reaction

Group	An. #	Sex	Wt (g) DAY 0	Wt (g) DAY 25	Signs of Toxicity*	Magnusson and Kligman Scale			
						24 Hour		48 Hour	
						Site A	Site B	Site A	Site B
Test Group (CSO Extract)	16	Female	335.4	470.9	None	0	0	0	0
	17	Female	351.5	513.9	None	0	0	0	0
	18	Female	359.2	478.1	None	0	0	0	0
	19	Female	346.3	470.2	None	0	0	0	0
	20	Female	360.6	498.9	None	0	0	0	0
	21	Female	344.2	479.8	None	0	0	0	0
	22	Female	350.5	492.3	None	0	0	0	0
	23	Female	358.6	504.5	None	0	0	0	0
	24	Female	360.3	485.5	None	0	0	0	0
	25	Female	349.8	469.4	None	0	0	0	0
Control Group (CSO)	26	Female	350.6	479.3	None	0	0	0	0
	27	Female	361.5	471.7	None	0	0	0	0
	28	Female	332.8	489.6	None	0	0	0	0
	29	Female	359.2	505.7	None	0	0	0	0
	30	Female	355.2	494.5	None	0	0	0	0

Attachment 3

STUDY TITLE :

ISO INTRACUTANEOUS REACTIVITY STUDY IN RABBIT

TEST ARTICLE :

Low frequency electrode

Model/Type Ref. :

Low frequency electrode

IDENTIFICATION NO. :

Lot No. LFE1506001

2015.07.16.

Korea Testing Laboratory

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Study Summary

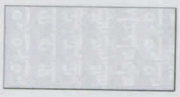
The Low frequency electrode / Low frequency electrode(Lot No.:LFE1506001) sponsored by NUGA MEDICAL CO., LTD was extracted and subjected to a Intracutaneous Reactivity Test for biocompatibility based on below.

- ISO10993-10 : 2010, 6.4 Animal Intracutaneous(Intradermal) Reactivity Test.
- Ministry of Food and Drug Safety, Notification no. 2014-115,no.9 5.4 Animal Intracutaneous(Intradermal) Reactivity Test.

The test articles were extracted in a 0.9 % sodium chloride solution and cotton seed oil. A 0.2 mL dose of the appropriate test article SC extract was injected by intracutaneous route into five separate sites on the left side of the back of each rabbit. Similarly, the corresponding reagent control was injected on the right side of the back of each rabbit. CSO extract was injected to same method on the lower section of the back of each rabbit. Observations for erythema and oedema were conducted at 24 hours, 48 hours, 72 hours after injection.

The difference between test extracts and corresponding control mean scores did not exceed 1.0 at any observation period.

Under the conditions of this study, there was no evidence of significant irritation or toxicity from the test article extracts injected intracutaneously into rabbits.



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1. Introduction

■ Purpose

The purpose of this study was to evaluate whether leachable extracted from the test article would cause local dermal irritant effects following injection into rabbit skin.

■ Dates

Animal Receiving : 2015.07.08.
Technical Initiation : 2015.07.13.
Technical Completion : 2015.07.16.

■ Testing Guideline

- ISO10993-10 : 2010, 6.4 Animal Intracutaneous(Intradermal) Reactivity Test
- Ministry of Food and Drug Safety, Notification no. 2014-115
  5.4 Animal Intracutaneous(Intradermal) Reactivity Test.

2. Materials

■ Test Article

The test article provided by the sponsor was identified and handled as follows:

Test Article	:	Low frequency electrode
Model No.	:	Low frequency electrode
Lot No.	:	LFE1506001
Storage Condition	:	Room temperature
Sponsor	:	NUGA MEDICAL CO., LTD

■ Extraction vehicle (supplied by KTL)

1) 0.9 % Sodium Chloride Solution, (SC)
Color : Clear
Storage condition : Room temperature
Manufacturer : Daihan Pharm Co. Ltd

2) Cotton Seed Oil, (CSO)
Color : yellow
Storage condition : Room temperature
Manufacturer : SAMCHUN CHEMICAL

■ **Test Article Preparation**

Test article preparation was conducted according to the requirements of ISO 10993-12:2012.
Extracted the body contact part of Low frequency electrode

Thickness and Form of Test Article	Weight	Volume of extract liquid	Extraction Condition
amorphousness	4 g	20 mL	(70 ± 2) ℃, (24 ± 2) h

=> Test Article was extracted according to the conditions presented by the applicant in consideration of the characteristics of the product.

■ **Control Preparation**

1) Negative Control

The negative control was prepared with the same extraction condition as the test article.

■ **Test System**

Species	:	Rabbit
Breed	:	New Zealand White
Source	:	ORIENT BIO Inc. / 322,Galmachi-ro, Jungwon-gu, Seongnam-si, Gyeonggi-do, Korea
Sex	:	Male
Number of Animals	:	3
Age	:	No particular age was prescribed for this test
Acclimation Period	:	Minimum 5 days
Body Weight	:	2.20 kg, 2.31 kg, 2.43 kg at injection
Identification Method	:	name card

Justification of Test System:

The intracutaneous injection test in rabbits is specified in the current ISO testing standards and has been used historically to evaluate biomaterial extracts.

■ **Animal Management:**

Food	:	A commercially available rabbit feed was provided daily
Source	:	ORIENT BIO Inc. / 322,Galmachi-ro, Jungwon-gu, Seongnam-si, Gyeonggi-do, Korea
Water	:	Reverse osmotic water was provided through an automatic watering system
Housing	:	Animals were housed in groups of five in guinea pig stainless steel cage
Environment	:	The temperature range - (22 ± 3) ℃ The humidity range - (50 ± 15) % R.H. The light cycle - on : 07:00, off : 19:00 The intensity of illumination : (150 ~ 300) Lux

No feed or water analysis was performed, because there were no possible contaminants that could interfere with the study. Animal husbandry and environmental conditions conformed to current Korea Testing Laboratory SOPs.

■ **IACUC**

This study was conducted in compliance with the regulations on management and use of laboratory animals.

- Korea Testing Laboratory, IACUC Guideline (IX-6)
- Korea Food and Drug Agency & Animal, Plant and Fisheries Quarantine and Inspection Agency, IACUC Standard Guideline
- ISO 10993-2:2006

■ **Equipment**

Equipment	No.	Date of Calibration	Note
Electric Balance	0610179	2015.03.26.	
Shaking bath	1110582	2014.11.12.	

3. Methods

■ **Procedure**

Within a 4 h to 18 h period before testing, closely clip the fur on the backs of the animals, allowing a sufficient distance on both sides of the spine for injection of the extracts.

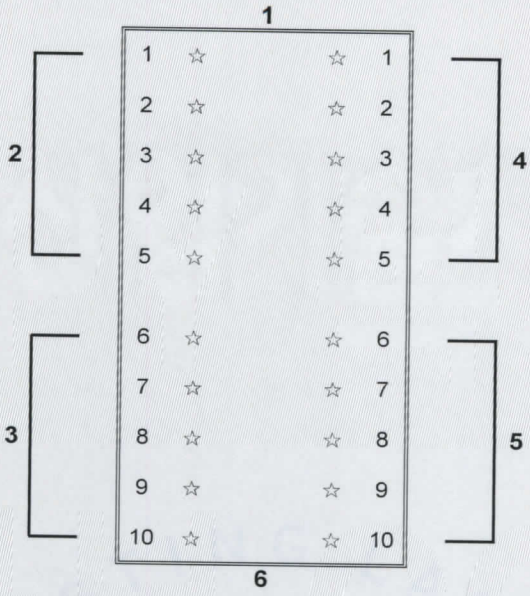
Inject intracutaneously 0.2 mL of the extract obtained with polar or non-polar solvent at five sites on one side of each rabbit. Use the smallest needle appropriate to the viscosity of the test material for the intradermal injections.

An example of the arrangements of the injection sites is presented in Figure 1.

Similarly, inject 0.2 mL of the polar or non-polar solvent control on five sites of the contralateral side of each rabbit (for example, see Figure 1).

If other solvents are used, repeat the above steps for the extract obtained with the other solvents and the solvent controls.

Figure 1. - Arrangement of injection sites



1 Cranial end
2 0.2 mL injections of polar extract
3 0.2 mL injections of non-polar extract
4 0.2 mL injections of polar solvent control
5 0.2 mL injections of non-polar solvent control
6 Caudal end

■ **Observation of animals**

Note the appearance of each injection site immediately after injection and at 24 h, 48 h and 72 h after injection. Grade the tissue reaction for erythema and oedema according to the system given in Table 1. for each injection site and at each time interval observed. and record the results.

Reaction	Numerical Grading
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
Oedema formation	
No Oedema	0
Very Slight Oedema(Barely perceptible)	1
Well-defined Oedema(edges of area well-defined by definite raising)	2
Moderate Oedema(raised approximately 1 mm)	3
Severe Oedema(raised more than 1 mm & extending beyond exposure area)	4
Total possible score for irritation	8
Other adverse changes at the injection sites shall be recorded and reported.	

TABLE 1. Grading system for Intracutaneous(Intradermal) Reactions

■ **Evaluation of Results**

After the (72 ± 2) 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 or blank for each individual animal. To calculate the score of a test sample or blank on each individual animal, divide each of the totals by 15 (3 scoring time points X 5 test or blank sample injection sites). To determine the overall mean score for each test sample and each corresponding blank, add the scores for the three animals and divide by three. The final test sample score can be obtained by subtracting the score of the blank from the test sample score. The requirements of the test are met if the final test sample score is 1.0 or less. If at any observation period the average reaction to the test sample is questionably greater than the average reaction to the blank, repeat the test using three additional rabbits. The requirements of the test are met if the final test sample score is 1.0 or less.

4. Results (Table I)

Results of scores for individual rabbits appear in Table I, Table II. The findings are summarized below :

Extract	Test Site Mean Score	Control Site Mean Score	Difference
SC	0.00	0.00	0.00
CSO	0.20	0.20	0.00

The difference in mean scores was considered acceptable. The difference between test and control site mean scores did not exceed 1.0 at any observation period.

Results and conclusions apply only to the test article tested. No further evaluation of results is made by KTL. Any extrapolation of these data to other samples is the responsibility of the sponsor.

5. Conclusion

Under the conditions of this study, there was no evidence of significant irritation or toxicity from the extracts injected intracutaneously into rabbits. Each test article extract met the ISO requirements.

6. Records

All raw data pertaining to this study and the copy of the final report are to be retained in designated KTL archive files for a period of 5 years.

Table I. Intracutaneous Observations - SC

An. #	Sex	Observation Scores											
		24 Hour				48 Hour				72 Hour			
		Test		Control		Test		Control		Test		Control	
		ER	ED	ER	ED	ER	ED	ER	ED	ER	ED	ER	ED
1	♂	0	0	0	0	0	0	0	0	0	0	0	0
		0	0	0	0	0	0	0	0	0	0	0	0
		0	0	0	0	0	0	0	0	0	0	0	0
		0	0	0	0	0	0	0	0	0	0	0	0
		0	0	0	0	0	0	0	0	0	0	0	0
2	♂	0	0	0	0	0	0	0	0	0	0	0	0
		0	0	0	0	0	0	0	0	0	0	0	0
		0	0	0	0	0	0	0	0	0	0	0	0
		0	0	0	0	0	0	0	0	0	0	0	0
		0	0	0	0	0	0	0	0	0	0	0	0
3	♂	0	0	0	0	0	0	0	0	0	0	0	0
		0	0	0	0	0	0	0	0	0	0	0	0
		0	0	0	0	0	0	0	0	0	0	0	0
		0	0	0	0	0	0	0	0	0	0	0	0
		0	0	0	0	0	0	0	0	0	0	0	0

ER : ERYTHEMA / ED : EDEMA / SC : 0.9% sodium chloride solution

Table II. Intracutaneous Observations - CSO

An. #	Sex	Observation Scores											
		24 Hour				48 Hour				72 Hour			
		Test		Control		Test		Control		Test		Control	
		ER	ED	ER	ED	ER	ED	ER	ED	ER	ED	ER	ED
1	♂	0	0	0	0	0	0	0	0	0	0	0	0
		0	0	0	0	0	0	0	0	0	0	0	0
		0	0	0	0	0	0	0	0	0	0	0	0
		0	0	0	0	0	0	0	0	0	0	0	0
		0	0	0	0	0	0	0	0	0	0	0	0
2	♂	1	0	1	0	0	0	1	0	0	0	0	0
		1	0	1	0	1	0	1	0	1	0	1	0
		1	0	1	0	1	0	1	0	0	0	1	0
		1	0	1	0	1	0	0	0	1	0	0	0
		0	0	0	0	0	0	0	0	0	0	0	0
3	♂	0	0	0	0	0	0	0	0	0	0	0	0
		0	0	0	0	0	0	0	0	0	0	0	0
		0	0	0	0	0	0	0	0	0	0	0	0
		0	0	0	0	0	0	0	0	0	0	0	0
		0	0	0	0	0	0	0	0	0	0	0	0

ER : ERYTHEMA / ED : EDEMA / CSO : Cotton Seed Oil

☐ **Photos of the Article**

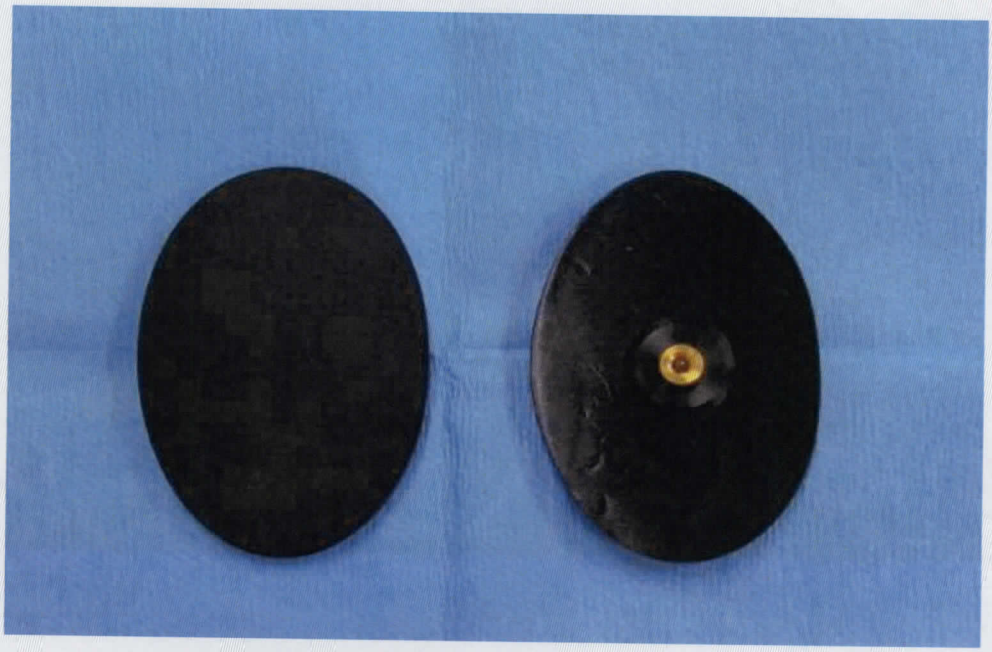


Photo : Low frequency electrode
(REF : Low frequency electrode, LOT No. LFE1506001)

※위 마크는 추후 전자확인증 대조 프로그램에서 원본대조시 사용되는 2D코드입니다.