Eleclean Disinfectant(Reactive Oxygen Species Solution) *In Vitro* Skin Irritation- Reconstructed Human Epidermis Test

FINAL REPORT

Sponsor: **ELECLEAN Co., Ltd.**
Testing Institution: **SGS Taiwan Ltd.**
Ultra Trace &Industrial Safety Hygiene
Report No.: **UG/2020/51834A-01**

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4. The content of this report is invalid if it is not presented as the entire report.
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6.The report has the Chinese version of translations UG/2020/51834. The Chinese version of this report shall prevail in case of discrepancy between the Chinese version and the English version.

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STUDY SCHEDULE

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Report No.:	UG/2020/51834A-01
Test Article Received Date	2020.05.27
Experimental Starting Date:	2020.06.18
Experimental Completion Date:	2020.07.02
Study Completion Date:	See Study Director's signature date in the report
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SIGNATURE OF PERSONNEL

Eleclean Disinfectant(Reactive Oxygen Species Solution)
In Vitro Skin Irritation-Reconstructed Human Epidermis Test

Approval Signatory:

Benson Liu / SGS Taiwan Ltd. Date 2020.07.24

Laboratory Head:

Shin Jyh Chen / SGS Taiwan Ltd. Date 2020.07.27

＊Approval signatory of this study is the study director.

ABSTRACT

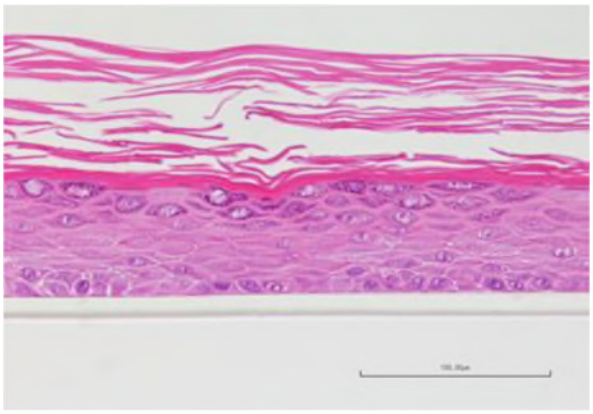
In vitro Skin Irritation-Reconstructed Human Epidermis Test was performed in this study to evaluate the skin irritation potency of "Eleclean Disinfectant(Reactive Oxygen Species Solution)". Treatment of reconstructed human epidermis (RhE) tissue with test article was performed according to OECD 439 guidance. Tissue viability determined by MTT assay showed that the 100% test article solution had in average 70% tissue viability. The results is considered as non-irritant to skin in accordance with UN GHS No Category and suggested that the test article has an expected *in vivo* dermal irritancy potential in the non-irritating range under this test system.

EXPERIMENTAL DESIGN

1. Test System

A. Reconstructed human epidermis: LabCyte EPI-MODEL24 SIT, Batch No.: LCE24-200615-A, supplied by J-TEC.

B. Histology



2. Reagents

	Reagents	Brand	Cat No.:	Lot No.:
A	10X Phosphate buffer solution (PBS)	BIOMAN SCIENTIFIC CO.,LTD	PBS105000	20051805
B.	Dulbecco's Phosphate Buffered Saline (without MgCl2 and CaCl2)	SIGMA	D5652	SLCB9248
C.	LabCyte EPI-MODEL24 SIT	J-TEC	411124	LCE24-200615-A
	Assay medium		402030	1002239874
D.	Isopropyl Alcohol (Isopropanol)	MARCON	3043-10	225262
E.	3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT)	SIGMA	M5655	MKCG3023
F.	Sodium Dodecyl Sufate (SDS)	J.T.Baker	4095-04	0000228028

3. Equipments

	Equipments	Brand	Model	Equipment No.:
A	Balance	OHAUS	PA214C	BAL-17
B.	Biological safety cabinet	NuAire	NU-543-600	BSC-07
C.	CO_2 Incubator	NuAire	NU-5810	INB-16
D.	Water bath	KANSIN	WB212-B2	WAB-02
E.	Microplate Spectrophotometer	BioTek™	Eon	MPS-02

4. Preparation of Test Article and Controls

A. Test Article

The test article was applied directly onto the reconstructed human epidermis. The pH adjustment; filtration and centrifugation were not conducted.

B. Controls

(1) Positive control: 5% SDS.
(2) Negative control: PBS.

5. *In vitro* Skin Irritation-Reconstructed Human Epidermis Test

(1) Equilibrated the LabCyte EPI-MODEL reconstructed epidermis tissue at 37±1℃, 5±1% CO_2 and ≧ 90% humidity for 24±2 hours.
(2) After the appropriate tissue preparation, 25±2 µL test article and controls were added to each well of LabCyte EPI-MODEL tissue. Treatment on 3 tissue replicates per condition.
(3) After 15 min ± 30 sec exposure at room temperature, each insert was individually removed from its well and rinsed with DPBS to remove any residual material.
(4) Transferred tissues to fresh maintenance medium and incubated at 37±1℃, 5±1% CO_2 and ≧ 90% humidity for 42±1 hours.
(5) Transferred tissues to 0.3 mg/mL MTT solution for 3 h ± 15 min at 37±1℃, 5±1% CO_2 and ≧ 90% humidity.
(6) Made biopsy punch of the tissues and extracted the tissue in 500 µL acidified isopropanol at 4±2 for 72h±2.
(7) 200 uL aliquot of each extract was subjected to a microplate reader equipped with a 570 nm filter for colorimetric measurement.
(8) With the absorbance of the negative control defined as 100%, the percent absorbencies of the other article were determined. The percentages listed below directly correlate with the cell metabolism in the LabCyte EPI-MODEL reconstructed epidermis.
(9) All the experiment procedure was according to SGS SOP: TESP-UB-1034.

6. Quality criteria

(1) Positive and negative controls should be included in every test.
(2) Negative control: the mean OD value of the triplicate tissues is ≧0.7 and the standard deviation (SD) of the % viability is ≦18.
(3) Positive control: the mean % viability of the NC is ≦40% and the SD is ≦18.
(4) Test article: the SD of % viability is≦18.

DATA MANAGEMENT

The OD values was used to calculate the percentage of viability normalised to the negative control, which is set to 100%. The quantitative data was showed as mean and standard deviation (SD). As the prediction model, the achievement of relative tissue viability is more than 50% (>50%) of the negative control is considered as non-irritant to skin in accordance with UN GHS No Category.

Prediction model	
Mean tissue viability is ≦ 50%	Category 2, Irritant (I)
Mean tissue viability is > 50%	No Category, Non Irritant (NI)

RESULTS

OD value

Groups	Exp 1	Exp 2	Exp 3	Mean	SD
Negative control	0.721	0.736	0.776	0.743	0.028
Positive control	0.016	0.014	0.027	0.018	0.007
UG/2020/51834	0.571	0.560	0.434	0.520	0.076

Tissue viability (%), normalised to the negative control

Groups	Exp 1	Exp 2	Exp 3	Mean	SD
Negative control	96.9	98.9	104.3	100.0	3.8
Positive control	2.0	1.7	3.4	2.4	0.9
UG/2020/51834	76.7	75.2	58.2	70.0	10.2

CONCLUSION

Tissue viability determined by MTT assay showed that the 100% “Eleclean Disinfectant(Reactive Oxygen Species Solution)” solution had in average 70% tissue viability. The results is considered as non-irritant to skin in accordance with UN GHS No Category and suggested that the test article has an expected *in vivo* dermal irritancy potential in the non-irritating range under this test system.

REFERENCES

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4. SGS SOP: EOMP-USL-0027 Operating procedures of the biosafety cabinet and laminar flow, UV-lamp verification and aerobic plate counts. Version 2.5
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8. SGS SOP: EOMP-USL-0037 Manual of freezer-refrigerators. Version 1.0
9. SGS SOP: EOMP-USL-0038 Microorganism incubator operating Procedures Version 1.0
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TEST ARTICLE PHOTO

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