OnePCR

[Lot No.: MB10370015]

Salmonella Reverse Mutation Test

FINAL REPORT

Client: **TAQKEY Science**
Testing Institution: **SGS Taiwan Ltd.**
Report No. : **UB/2013/80653**
Report Date: **2013.10.24**

Note:
1. The content of this report is invalid if it is not presented as the entire report.
2. Any unauthorized alteration, forgery or falsification of the content or appearance of this report is unlawful and offenders may be prosecuted to the fullest extent of the law.
3. The results shown in this test report refer only to the test article(s) tested.

.

STUDY SCHEDULE
Salmonella Reverse Mutation Test
OnePCR

Report No.:	UB/2013/80653
Study Initiation date:	2013.09.03
Experimental starting date:	2013.09.23
Experimental completion date:	2013.10.24
Study completion date:	See Study Director's signature date in the report
Study Personnel:	CoCo Lin

Testing Institution

Name: SGS TAIWAN LTD.

Address: No. 38, Wu Chyuan 7th Rd., New Taipei Industrial Park, Wu Ku Dist., New Taipei City 24890, Taiwan (R. O. C.)

Client / Sponsor

Name: TAQKEY Science

Address: 1F., No. 60, Jiabei 2nd St., Zhunan Township, Miaoli County 350

INFORMATION FOR TEST ARTICLE

INFORMATION FOR ☒TEST ARTICLE/ ☐CONTROL ARTICLE

Sponsor Company Name	TAQKEY Science
Sponsor Address	1F., No.60, Jiabei 2nd St., Zhunan Township, Miaoli County 350
Contract study item	☒ Base on the contract ☐Others _____
Name of Test article/ Control article	OnePCR
Batch/Lot number	☒Base on the specific number on the package : MB10370015 ☐Base on the date on the package : _____ ☐Base on the arrived date ☐Others : _____
Specification & Amount	1125ul / vial* 6 vials (e.g.10ml / bottle * 6 bottles)
Retention amount (Note 2)	The amount of the same lot is sufficient for☐One test ☒Two test (for retention)
External features	External features: ☒liquid ☐powder ☐tablet ☐capsule ☐Other_____ Color : blue
Major components & Purity	Major components: water Purity: up to 90%
Solvent and solubility	N/A
Storage condition	☐Room temperature☒4℃☐Dry☒Light sensitive☐Others_____
Expiration date(Note 3)	☒Date: 2014 / 03 / 20 (YYYY/MM/DD) or ☐Period :_____year_____month_____day
Attachment(Note 4)	☐ Certificate of Analysis ☐ Material Safety Data Sheet ☐ Stability Test Result ☐ Other : _____ ☒ No attachment (Note4)
Sterilization	Has been sterilized ☐YES ☒NO (If Yes, please select the following item) Methods☐EOsterilization☐Gamma sterilization☐Steam sterilization☐Other_____
Categorization of devices (The column is only for device used)	1.☐Contact with intact skin or mucosa (cumulative contact duration) ☐ Short-term (no greater than 4 hr) ☐ Long-term (exceeding 4 hr) Maximum duration is_____hrs 2.☐ Implanted device
Specific requirement (Note 5)	N/A

Sponsor Signature/ Date : 2013.08.14

Note 1. Above all information is disclosure by the sponsor.
Note 2. If the sponsor doesn't provide the retention of test article/control article, the retention of a reserved test article/control article from each batch of test article /control article is the responsibility of the Sponsor.
Note 3. If the effective period is less than 5 years, the test article/control article will be retained till the expiry date. If the effective period is longer than 5 years, the test article/control article will be retained for 5 years only.
Note 4. Determination and documentation of identity, strength, purity, stability, composition, method of synthesis, fabrication, derivation or other characteristics of the test article/control article are the responsibility of the Sponsor.
Note 5. The test article/control article which has been destroyed or cutting will be discarded after the end of experiment. For retention or return of the kind of test article/control article, please indicate in the "special requirement". The human intake suggests or dose requested by the sponsor also can fill in the "special requirement". Note treatment method after test if the test article need to be retreated
Note 6. The code number of test article is the same as the report number.
Note 7. Note 'N/A' if not applicable. Do not leave blank.

版次：3.1 試驗-對照物質資料表 Information for test article-control article
發行日期：2013.06.14

TABLE OF CONTENTS

SGS

STATEMENT OF GLP COMPLIANCE

All study activities performed by SGS Taiwan Ltd, are carried out in compliance with the GLP (Good Laboratory Practices) for Nonclinical Laboratory Studies (Department of Health, Taiwan, 2006), current OECD Principles of Good Laboratory Practice (Organization for Economic Cooperation and Development, Paris, ENV/MC/CHEM (98) 17) and U.S. Food and Drug Administration Good Laboratory Practice Regulations, 21 CFR Part 58 but expect for the OECD guideline for the testing of chemicals # 471 : Bacterial reverse mutation test. The study is conducted in accordance with the protocol and standard operating procedures and monitored in conformity with the protocol. All laboratory data are accurately recorded and verified. SGS Taiwan makes no GLP compliance claim for characterization and verification of the test article identity and properties; this is the responsibility of the sponsor.

Study Director:

Howard Kao

Howard Kao / SGS Taiwan Ltd.

2013.12.04

Date Completed

Deputy of
Facility Manager:

Amy Liu

Amy Liu / SGS Taiwan Ltd.

2013.12.04

Date Completed

SGS

QUALITY ASSURANCE STATEMENT

UB/2013/80653
OnePCR
Salmonella Reverse Mutation Test

This study is audited by the Quality Assurance personnel of SGS Life Science Service. The QA inspection report includes the protocol review, study-based audit result, and results of the raw data and study report audit. The process of study didn't completely follow OECD guideline for the testing of chemicals # 471 : Bacterial reverse mutation test. The audit report is issued upon the completion of final report of testing.

QA :

Melissa Lin / SGS Taiwan Ltd.

2013.12.04
Date Completed

Inspection Type	Inspection date	Study phase	Date to facility manager and study director
Study base	2013.09.03	Protocol	2013.09.03
Study base	2013.09.24	Strain identification	2013.09.24
Study base	2013.11.19	Raw data & Final report	2013.11.19

ARCHIVING

All the study-related raw data, records, protocol and the final report will be kept in the archives room of SGS (TAIWAN) LTD for 5 years. Furthermore, retention of the test articles will be in the Sample Storage Room for its expiration date or 5 years. All of the records and test articles are handled according to the GLP guideline. Agent authorized by the sponsor can apply for the review according to SGS procedure.

Address:

No. 38, Wu Chyuan 7th Rd., New Taipei Industrial Park, Wu Ku Dist., New Taipei City 24890, Taiwan (R. O. C.)

Archiving List	
Final report	Final Report Copy
Raw Data	*Salmonella* Reverse Mutation Test (Ames test) Data Sheet
Records	Application Form Information for test article-control article GLP Test Article Control From and other supplementary record
Protocol	Protocol

ABSTRACT

The study was to assess the test article “OnePCR” in causing the mutagenicity of microorganism by using the *Salmonella typhimurium* reverse mutation test. The test result could be used for reference in evaluating probabilities of gene mutation caused by the test article extract.

The 1X test article extract of “OnePCR” was used in the plate incorporation test. The strains included *Salmonella typhimurium* TA98, TA100 and TA1535. For each strain, the experiments of positive control group, negative control group, and test group were treated in duplication. The test results demonstrated that whether the rat liver enzyme metabolic system was treated or not, all test data were within the effective range. Furthermore, the revertant numbers of the test group did not appear to be significantly higher than that of the negative control group. The overall results showed that under the test system, the “OnePCR” had low probability in inducing reverse mutation in *Salmonella typhimurium* whether treated with metabolic activation process or not.

PURPOSE

The test system is designed according to the guidance of OECD guideline for the testing of chemicals # 471：Bacterial reverse mutation test (1997). Three bacteria strains (*Salmonella typhimurium* TA98、TA100、TA1535) are used to evaluate the mutagenicity of test article.

The *Salmonella* reverse mutation test uses histidine- requiring strains of *Salmonella typhimurium* to detect point mutations, which involve substitution, addition or deletion of one or a few DNA base pairs. The *Salmonella* reverse mutation test is commonly employed as an initial screening for genotoxic activity and, in particular, for point mutation-inducing activity.

EXPERIMENTAL DESIGN

1. Test system:

A. Test strains:

The strains used in this study are originated from *Salmonella typhimurium*, including TA98, TA100 and TA1535. All strains are purchased from MOLECULAR TOXICOLOGY, INC. The strain numbers are shown below:

a. *Salmonella typhimurium* TA98：#71-098L (Bank No.: 20101210-98-38-5)

b. *Salmonella typhimurium* TA100：#71-100L (Bank No.: 20101210-100-43-5)

c. *Salmonella typhimurium* TA1535：#71-1535L (Bank No.: 20110121-1535-38-5*)*

B. *Strain identificatio*n:

Strain identification is to determine if the genotypes are appropriate to this study. This is conducted by evaluating the requirement of histidine (*his*) and biotin (*ΔuvrB*), and the growth inhibition under ampicillin (R-factor), tetracycline (*pAQ1*) and crystal violet (*rfa*). The details of genotype and strain identification are described in Appendix 1 and Appendix 2.

2. Reagents :

A. 9-aminoacridine (Sigma, Cat. No.A7295) (Lot #:106F06681;Expiry date: 2014.05.04)

B. 2-aminoanthracene (Sigma-ALDRICH, Cat. No. AL-A38800) (Lot #:STBB1901;Expiry date: 2013.10.24)

C. 2-aminofluorene (Sigma, Cat. No.A55500) (Lot #:S90850V;Expiry date: 2014.08.30)

D. Benzo [a] pyrene (Sigma, Cat. No.B1760) (Lot #:090M1400V;Expiry date: 2014.06.10)

E. DMSO (Sigma, Cat. No.3494) (Lot #:SZBB1040V;Expiry date: 2014.09.24)

F. D-Glucose-6-phosphate (Sigma, Cat. No.G7772) (Lot #:080M5161V;Expiry date: 2014.05.20)

G. NB No.2 medium (Oxoid, Cat. No.CM0067) (Lot #:1315350;Expiry date: 2014.07.18)

H. β-Nicotinamide adenine dinucleotide phosphate sodium salt hydrate, NADP (Sigma, Cat. No.N3886) (Lot #:020M7010V;Expiry date: 2018.08.23)

I. 2-nitrofluorene (Sigma-ALDRICH, Cat. No.AL-N16754) (Lot #:S43858;Expiry date: 2013.10.24)

J. Sodium azide (Sigma, Cat. No.13412) (Lot #:83180;Expiry date: 2013.10.24)

K. Biotin (Sigma, Cat. No.B4639) (Lot #:021M11542V;Expiry date: 2015.02.05)

L. Crystal violet (Sigma, Cat. No.32675) (Lot #:BCBG3382V;Expiry date: 2017.12.20)

M. Glucose (J.T. Baker, Cat. No.JT-1916-01) (Lot #:17509;Expiry date: 2017.08.28)

N. Histidine (Sigma, Cat. No.SI-H6034) (Lot #:SLBC5293V;Expiry date: 2016.03.23)

O. Ampicillin (Sigma, Cat. No.SI-A9393) (Lot #:069K0421;Expiry date: 2013.10.11)

P. Magnesium chloride (Merck, Cat. No.8.14733.0100) (Lot #:S5573033221;Expiry date: 2015.09.30)

Q. Potassium chloride (J.T.Baker, Cat. No.4001-01) (Lot #:H35474;Expiry date: 2014.01.05)

R. Sodium dihydrogen phosphate (J.T.Baker, Cat. No.3818-01) (Lot #:G22149;Expiry date: 2016.11.28)

S. Dibasic Sodium Phosphate (J.T.Baker, Cat. No.3818-69) (Lot #: J05145;Expiry date: 2014.08.03)

T. Tetracycline (Sigma, Cat. No. SI-T7660) (Lot #:081M1598V;Expiry date:2015.08.22)

U. Rat liver microsome S-9 fraction (Aroclor 1254-induced, Moltox) (Lot #:3129;Expiry date: 2015.08.04)

3. Equipments:

A. Incubator (PRECISION, CI-60, Equipment No.: INB-6)

B. Biological safety cabinet (Labconco, 3460801, Equipment No.: BSC-5)

C. Autoclave (TOMY, SX-700, Equipment no.AUC-3；ALP, MC-30L, Equipment No.: AUC-2)

D. Analytical balance (Mettler, ML4002, Equipment no.BAL-20；Mettler, ML204, Equipment No.: BAL-25)

4. Preparation of test article and control article:

A. Test article:

a. Solid: According to the OECD #471 guidance, dissolve 0.5 g of test article into 10 mL DMSO, vortex and do the 2- fold serial dilution. The final concentrations of this test are 5 mg/plate, 2.5 mg/plate, 1.25 mg/plate, 0.625 mg/plate, and 0.3125 mg/plate.

b. Liquid: According to the OECD #471 guidance, dissolve 50 µL of test article into 1 mL DMSO, vortex and do the 2- fold serial dilution. The final concentrations of this test are 5 µL/plate, 2.5 µL/plate, 1.25 µL/plate, 0.625 µL/plate, and 0.3125 µL/plate.

B. Control article:

a. Positive control: Different positive control articles are used based on different bacteria strains. The description of bacteria strains and individual concentrations of mutagens are shown below:

Mutagen	S9	Concentration (µg/plate)	Strain
4-nitroquinoline-N-oxide	−	0.5	TA98
sodium azide	−	0.4	TA100, TA1535
2-aminoanthracene	+	4.0	TA1535
benzo [a] pyrene	+	4.0	TA98
2-aminofluorene	+	4.0	TA100

+: Positive

−: Negative

b. Negative control: DMSO.

5. Salmonella reverse mutation test:

A. Spot test:

a. The test is performed by adding the test article extract on the sterile paper disc, which is

placed on the culture medium. Incubate at 37℃ for 24 to 48 hrs and check the bacteriostasis ring (cell toxicity) or circularity formed by large number of colonies (mutation).

B. Plate incorporation test:

Add 0.1 mL of the control article or test article and 0.1 mL of the overnight culture bacteria suspension to 2 mL of the soft agar (containing 0.5 mM histidine/biotin). For those to be treated with S9, 0.5 ml of S9 solution is added. Otherwise, 0.5 ml of 0.2 M sodium phosphate buffer is added. After mixing, the solution is added evenly onto the minimal glucose agar plate. After the soft agar has solidified, the petri-dish is placed in the 37℃ incubator for 48 hrs. All of the tests are treated as replicates.

C. All of the test procedures are operated according to the SGS Taiwan SOP TESP-UB-1010 *Salmonella* Reverse Mutation Test- Ames test.

6. Quality requirement:

A. All of the test procedures are operated in the biological safety cabinet (BSC).

B. The correct genotypes of three test bacteria strains are identified before test.

C. The spontaneous mutation colonies of the negative control group must be in the reasonable range and the reverse mutation colonies of the positive control group must be two times more than the negative control group, otherwise the test is considered failed and must be redone.

DATA MANAGEMENT

A. For each dosage of test the article, negative control group and positive control group, the reverse mutation colonies are counted and calculated. The spontaneous mutation colonies of the negative control group must be in the reasonable range. If the reverse mutation colonies of the test group and positive control group are two times greater than the spontaneous mutation colonies of negative control group, record the data as "> 2X colonies of negative control group".

B. Criteria of evaluation for positive reaction:

Whether the S9 mixture is added or not, at least one strain has more than one dosage group with a two- times increase in the number of reverse mutation, considered as a positive reaction.

C. Criteria of evaluation for negative reaction:

The reverse mutation number of all strains in all dosage groups, which does not attain the criteria of positive reaction, will be considered as negative reaction.

RESULTS

1. Strain identification:

   The genotypes of the 3 bacterial strains used in this study (*Salmonella typhimurium* TA98, TA100 and TA1535) were found to comply with the requirement of Appendix 1 and Appendix 2.

2. Spot test:

   There is no bacteriostasis ring (cell toxicity) or circularity formed by a large number of colonies (mutation) around the disc in each test group.

3. Plate incorporation test:

   The test results demonstrated that whether the rat liver enzyme metabolic system was treated or not, all test data were within the effective range. Furthermore, the revertant numbers of the test group did not appear to be significantly higher than that of the negative control group and did not reach positive reaction criteria. The results are shown below:

Test strain			TA98		TA100		TA1535	
S9			+	—	+	—	+	—
Colony number (CFU/plate)	Negative control		32	43	336	260	16	17
			36	42	349	250	18	15
	2X Average of Negative control		68	85	685	510	34	32
	Positive control		>68	>85	>685	>510	>34	>32
			>68	>85	>685	>510	>34	>32
	Test Article Concentration (mg/plate)	5	52	54	305	276	16	18
			46	40	314	268	12	14
		2.5	57	49	338	257	16	12
			32	50	299	231	12	8
		1.25	35	58	287	246	18	11
			38	57	266	252	18	11
		0.625	55	50	300	293	18	8
			48	46	262	308	15	10
		0.3125	41	42	291	251	12	13
			43	41	292	278	11	8

CONCLUSION

The results showed that the revertant numbers of the test article “OnePCR” did not appear to be significantly higher than that of the negative control group in *Salmonella typhimurium* TA98, TA100 and TA1535. It had the low probability in inducing the reverse mutation in the *Salmonella typhimurium* whether treated with metabolic activation process or not.

DEVIATIONS AND INVESTIGATIONS

There were no deviation and investigation during the test period of this study.

PROTOCOL AMENDMENTS

There was no protocol amendment during the test period of this study.

REFERENCES

1. Assessment of Healthy Food Safety (Department of Health, Taiwan, 1999).

2. Guideline for the Nonclinical pharmacology/toxicology studies for medicinal products applications. Department of Health (2000) the Executive Yuan.

3. Biosafety assessment of health food. Department of Health (2005) the Executive Yuan. (Information was from the web site:www.doh.gov.tw/EN/webpage/index.aspx)

4. Maron DM, Ames BN (1983) Revised methods for the *Salmonella* mutagenicity test. Mutation Res. 113, 173-215.

5. Bacterial reverse mutation test, OECD guideline for the testing of chemicals. # 471 (1997) OECD.

6. Good Laboratory Practice for Nonclinical Laboratory Studies. Title 21 of the U.S. Code of Federal Regulations, Part 58. United States Food and Drug Administration.

7. Current OECD Principles of Good Laboratory Practice (Organization for Economic Cooperation and Development, Paris, ENV/MC/CHEM (98) 17)

8. Biological evaluation of medical devices-Part 3 : Tests for genotoxicity，carcinogenicity and reproductive toxicity ISO 10993 (2003 E) ISO.

9. Biological evaluation of medical devices-Part 12：Sample preparation and reference materials ISO 10993 (2012) ISO.

10. TESP-UB-0224 The management and validation of medium preparation. Version 1.1

11. TESP-UB-0225 Operating Procedures of the strains'activation, verification, preservation and thaw. Version 2.2

12. TESP-UB-1010 *Salmonella* Reverse Mutation Test (Ames test) Version 1.5

13. EOMP-USL-0027 Operating procedures of the biosafety cabinet and laminar flow, UV-lamp verification and aerobic plate counts. Version 2.2

14. EOMP-USL-0025 Operating Procedures and Verification of the autoclave. Version 1.0

15. EOMP-USL-0003 Operating and analyzed Procedures of the Balance Version 1.2

APPENDIXES

Appendix 1. Genotypes of test bacterial strains

Strain	Genotype
Salmonella typhimurium TA98	*hisD3052*, *rfa*,Δ*uvr*B, +R
Salmonella typhimurium TA100	*hisG46*, *rfa*,Δ*uvr*B, +R
Salmonella typhimurium TA1535	*hisG46*, *rfa*,Δ*uvr*B, -R

Appendix 2. Identification of test strains

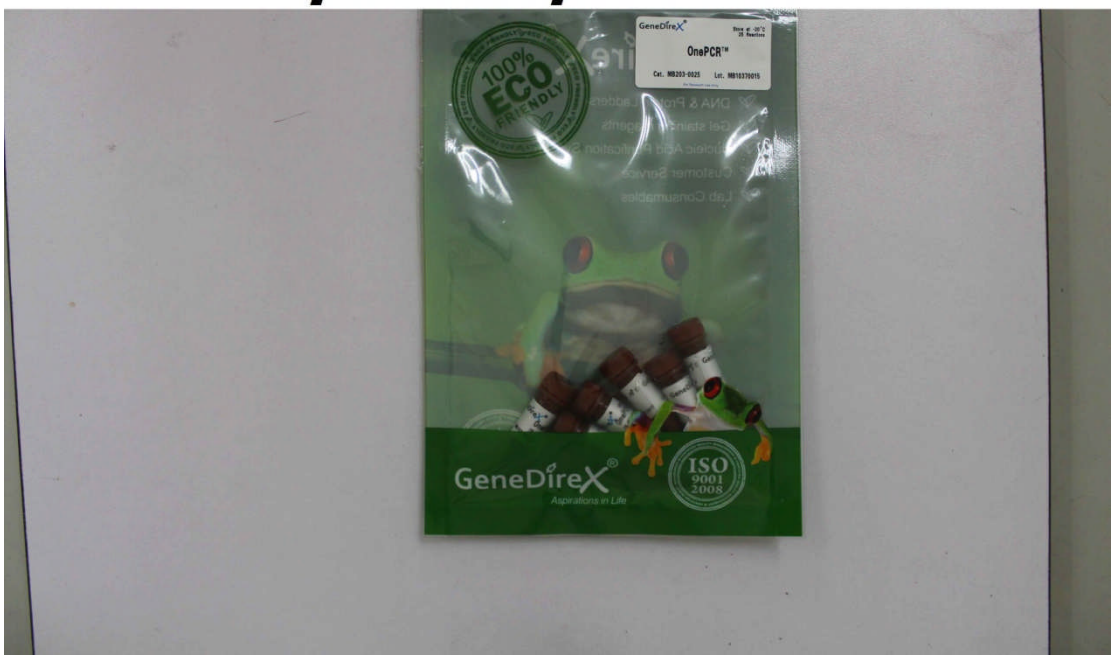
Strain	Histidine requirement	Δ*uvrB* mutant	*rfa* mutant	Ampicillin resistant	Tetracycline resistant
TA98	＋	＋	＋	＋	－
TA100	＋	＋	＋	＋	－
TA1535	＋	＋	＋	－	－

＋: Positive

－: Negative

TEST ARTICLE PHOTO

UB/2013/80653



UB/2013/80653