OnePCR

[Lot No.: MB10370015]

In Vitro Mammalian Chromosomal Aberration Test

FINAL REPORT

Client: **TAQKEY Science**
Testing Institution: **SGS Taiwan Ltd.**
Report No. : **UB/2013/80653A-01**
Report Date: **2013.12.19**

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
In Vitro Mammalian Chromosomal Aberration Test
OnePCR

Report No.:	UB/2013/80653A-01
Study Initiation date:	2013.09.03
Experimental starting date:	2013.09.30
Experimental completion date:	2013.12.18
Study completion date:	See Study Director's signature date in the report
Study Personnel:	Stella Chang

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

SGS

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

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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. 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 / SGS Taiwan Ltd. — Date Completed: 2013.12.27

Facility Manager:

Yuanmin Wen / SGS Taiwan Ltd. — Date Completed: 2013.12.27

SGS

QUALITY ASSURANCE STATEMENT

UB/2013/80653A-01

OnePCR

In Vitro Mammalian Chromosomal Aberration Test

This study was audited by Quality Assurance personnel of SGS Life Science Service. The QA inspection report includes the result of a study-based audit, and results of the raw data and study report audit. The audit report was issued upon the completion of final report of testing.

QA :

Melissa Lin / SGS Taiwan Ltd.

2013. 12. 27

Date Completed

Inspection Type	Inspection date	Study phase	Date to facility manager and study director
Study base	2013.11.20	Preparation of cell slide	2013.11.20
Study base	2013.12.25	Raw data & Final report	2013.12.25

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 procedures.

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	*In Vitro* Mammalian Chromosomal Aberration 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 evaluate the test article “OnePCR” for its genotoxicity using Chinese hamster ovary cells (CHO-K1). The test result could be used for reference in evaluating probabilities of gene mutation caused by the test article extract.

The test article extract was processed with the S9 metabolic activation system for 3 hours and without the S9 metabolic activation system for 3 and 18 hours. Each dosage group was treated in duplicate, and 200 cells at the metaphase were observed with the microscope. The results show that the number of cells with the abnormal chromosome in the positive control group was significantly higher than that of the negative control group ($p<0.05$), and the number of cells with the abnormal chromosome in the negative control group was less than 3%. The results of the test article demonstrated that whether the S9 metabolic activation system was treated or not, the test article was non-genotoxic to the Chinese hamster ovary cells (CHO-K1). The overall results showed that under the test system, the “OnePCR” had no genotoxic effects on and chromosome aberration in the Chinese hamster ovary cells (CHO-K1), whether treated with the S9 metabolic activation system or not.

PURPOSE

The test system was designed according to the guidance of OECD guidelines for the testing of chemicals #473：*In Vitro* Mammalian Chromosome Aberration Test (1997). The Chinese hamster ovary cell (CHO-K1) was used to evaluate the *in vitro* chromosomal aberration of the test article.

EXPERIMENTAL DESIGN

1. Test system:

A. Test cell line: The cell line used in this study was the Chinese hamster ovary cell (CHO-K1) (Strain No.: BCRC 60006, Bank No.:20130326-6-E-3) which was purchased from the Food Industry Research and Development Institute.

B. Morphology: Adherent epithelial cell.

C. Incubation condition: Use the F-12(ham) medium with 10% fetal bovine serum and incubate at 37±1°C in the presence of 5±1% CO_2.

D. Other characteristics: The cell cycle is about 12~14 hrs, and the number of chromosome per cell is 20.

2. Reagents :

A. Acetic acid, glacial (J.T.Baker, Cat. No.JT-9508-03) (Lot #:0000044299; Expiry date: 2018.04.29)

B. Benzo [a] pyrene (Sigma, Cat. No.B1760) (Lot #:SLBC6864V; Expiry date:2014.01.20)

C. Denecolcine (Sigma-ALDRICH, Cat. No.SI-D1925) (Lot #:RNBC8294; Expiry date: 2014.04.08)

D. Dibasic Sodium Phosphate (J.T.Baker, Cat. No.3818-69) (Lot #:K52173; Expiry date: 2013.12.31)

E. DMSO (Sigma, Cat. No.3494) (Lot #: SZBB2720V; Expiry date: 2014.09.13)

F. F-12 Nutrient Mixture (Ham) medium (GIBCO, Cat. No.11765-054) (Lot #:1406872; Expiry date: 2014.07.30)

G. Fetal bovine serum (GIBCO, Cat. No.10437-028) (Lot #:706740; Expiry date: 2014.10.31)

H. Giemsa stain (Sigma-ALDRICH, Cat. No.SI-GS500) (Lot #:SLBH0211V; Expiry date: 2015.06.30)

I. D-Glucose-6-phosphate (Sigma, Cat. No.G7772) (Lot #:SLBF1833V; Expired date: 2018.12.09)

J. Mitomycin C (Sigma, Cat. No.M4287) (Lot #:SLBB7481V; Expiry date: 2016.06.18)

K. MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium, Sigma, Cat. No. M5655) (Lot #:MKBHK674V; Expiry date: 2016.02.03)

L. β-Nicotinamide adenine dinucleotide phosphate sodium salt hydrate, NADP (Sigma, Cat. No.N3886) (Lot #:020M7010V; Expiry date: 2016.09.17)

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

N. Methanol (Merck, Cat. No.1.06007.4000) (Lot #:0000042094; Expiry date: 2018.04.02)

O. Penicillin (Sigma, Cat. No. SI-A9393) (Lot #:1266347; Expiry date: 2013.12.31)

P. Phosphate buffer solution (PBS) (UniRegion Bio-Tech, Cat. No.UR-PBS001) (Lot #:PBS001-5B; Expiry date: 2018.04.14)

Q. Potassium chloride (J.T.Baker, Cat. No.4001-01) (Lot #:G20640; Expiry date: 2013.12.15)

R. Rat liver microsome S-9 fraction (Aroclor 1254-induced, Moltox) (Lot #:3150; Expiry date: 2015.09.28)

S. Sodium dihydrogen phosphate (J.T.Baker, Cat. No.3818-01) (Lot #:K44152; Expiry date: 2017.07.20)

T. Streptomycin (USP, Cat. No.U-62300-3) (Lot #:1266347; Expiry date: 2013.12.31)

3. Equipments:

A. CO_2 Incubator (ASTEC, SCA-165DS, Equipment No.: INB-1)

B. Biological safety cabinet (LABCONCO, 3450801, Equipment No.: BSC-1)

C. Inverted Microscope (OLYMPUS, CKX41SF, Equipment No.: MIS-2)

D. Balance (Denver, TB-214, Equipment No.: BAL-13)

E. Microplate Spectrophotometer (BioTek™, Eon, Equipment No.: MPS-2).

4. Preparation of the cell culture medium:

Add 50 mL fetal bovine serum and 5 mL antibiotic solution (10,000U/mL penicillin and 10 mg/mL streptomycin) to 445 mL F12 (Ham) medium. The cell culture medium was prepared to use or stored at 4°C.

5. Preparation of the test article and control article:

A. Test article:

a. Solid: According to the OECD #473 guidance, dissolve 0.05 g of test article into 10 mL F-12 medium containing 0.25% DMSO and 10% fetal bovine serum, vortex and do 2-fold serial dilution. The final concentrations of this test were 5 mg/mL, 2.5 mg/mL, 1.25 mg/mL, 0.625 mg/mL, and 0.3125 mg/mL.

b. Liquid: According to the OECD #473 guidance, dissolve 50 µL of test article into 10 mL F-12 medium containing 0.25% DMSO and 10% fetal bovine serum, vortex and do 2 -fold serial dilution. The final concentrations of this test were 5 µL/mL, 2.5 µL/mL, 1.25 µL/mL, 0.625 µL/mL, and 0.3125 µL/mL.

B. Control article:

a. Positive control: In the presence of the S-9 metabolic activation system, benzo (a) pyrene was used as the positive control article. In the absence of the S-9 metabolic activation system, Mitomycin C was used as the positive control article.

b. Negative control: F-12 medium.

6. *In Vitro* Mammali*an Chromo*somal Aberration Test:

A. Test groups: Details of test groups are shown below.

Reaction Time	S9-mixture	Groups	Concentration
3 hours	−	Negative control	−
		Positive control Mitomycin C	1 μg /mL
		Test article	5, 2.5, 1.25, 0.625, 0.3125 mg/mL 5, 2.5, 1.25, 0.625, 0.3125 μL/mL
3 hours	+	Negative control	−
		Positive control Benzo(a)pyrene	25 μg /mL
		Test article	5, 2.5, 1.25, 0.625, 0.3125 mg/mL 5, 2.5, 1.25, 0.625, 0.3125 μL/mL
18 hours	−	Negative control	−
		Positive control Mitomycin C	1 μg /mL
		Test article	5, 2.5, 1.25, 0.625, 0.3125 mg/mL 5, 2.5, 1.25, 0.625, 0.3125 μL/mL

−: Without S-9 mixture

+: With S-9 mixture

B. Cytotoxicity Test:

a. Sow 5×10^4 cells/mL per well and incubate overnight, then add the control article and test article to the corresponding experimental groups.

b. According to the corresponding experimental groups, remove the culture medium and wash the cells with PBS at the 3rd or 18th hours. Add new cell culture medium and incubate in the CO2 incubator (37℃, 5% CO2).

c. Examine the cell morphology with the microscope and measure the cell viability by MTT assay at the 24th hour.

C. Chromosome aberration test:

a. Sow 2×10^5 cells/mL per well and incubate overnight, then add the control article and test article to the corresponding experimental groups

(1) 3-Hour Groups:

Remove the culture medium and wash the cells with PBS at the 3rd hour of the reaction period, then add new cell culture medium and incubate in the CO_2 incubator (37 ± 1^oC, $5\pm1\%$ CO_2). At the 18th hour of incubation, add 0.1 μg/mL denecolcine and react for 2 hours, then harvest the cells.

(2)18-Hour Grups:

Remove the culture medium and wash the cells with PBS at the 18th hour of the reaction period, and then add the new cell culture medium and 0.1 μg/mL denecolcine. Then harvest the cells after reacting for 2 hours. .

b. Preparation of the cell slide:

Harvest the cells from each experimental group and centrifuge to discard the supernatant. Then treat the cells with KCl and fix cells with the fixing solution (methanol: glacial acetic acid= 3:1) at 4^oC. Prepare the cell slide and stain with 5% Giemsa solution.

c. Microscopic observation:

All experimental groups were treated in duplicates. 100 cells at the metaphase under different conditions are observed under the 1000X microscope. The chosen cells had chromosome evenly distributed, and the chromosome numbers were 18~22.

d. The observed structure and morphological abnormality of chromosomes included chromosome breakage (csb), chromosome exchange (cse), chromatid breakage (ctb), chromatid exchange (cte), and other abnormalities.

D. All of the test procedures were operated according to the SGS Taiwan SOP TESP-UB-1011 *In Vitro* Mammalian Chromosomal Aberration Test.

7. Quality requirement:

A. Make sure the test cells are mycoplasma-free.

B. The cell viability of the positive control group and test group should be more than 50% of the negative control group, otherwise, the chromosome aberration test must be discontinued because of the cytotoxic effect.

C. After calculating the data with the Chi-square test, the result of the negative control must have significant differences in comparison with the positive control, otherwise, the test should be re-conducted.

DATA MANAGEMENT

A. Assessment of cytotoxicity: If the test article didn't have cytotoxicitic effect, the test article extract would be the test article of the chromosome aberration test. If the test article had cytotoxicitic effect, the test group with a greater than 50% cell viability would be the test article of chromosome aberration test.

B. Chromosome aberration test: Analyze the data with the Chi-square test to evaluate the significant differences between the test groups and negative control groups.

RESULTS

A. Cytotoxicity Test:

The survivability of CHO-K1 cells in each dosage group was compared with the positive control group and negative control group. The cytotoxicity of test group in each dosage was less than 50%, and the cytotoxicity of test article did not increase as the dosage increased. The results are shown below:

Dosage	Reaction Time	S9	Cytotoxicity (%)
5 mg/mL	3 hr	+	13.24
2.5mg/mL			23.77
1.25mg/mL			0.00
0.625mg/mL			1.47
0.3125mg/mL			9.25
Negative control			0.00
Positive control			6.43
5 mg/mL	3hr	−	2.61
2.5mg/mL			0.00
1.25mg/mL			3.08
0.625mg/mL			6.95
0.3125mg/mL			0.00
Negative control			0.00
Positive control			11.77
5 mg/mL	18 hr	−	7.35
2.5mg/mL			0.00
1.25mg/mL			14.04
0.625mg/mL			12.94
0.3125mg/mL			4.49
Negative control			0.00
Positive control			12.56

B. Chromosome aberration test:

a. The number of cells with abnormal chromosomes in the negative control group was less than 3% and the positive control group was significantly higher than that of the negative control group (p<0.05). There was no significant difference in cell numbers between the negative control group and dosage groups (p＞0.05) in terms of cells with abnormal chromosomes. The results of the abnormal chromosomes within 200 cells are shown below:

Dosage	Cell number with abnormal chromosome	
	Treat for 3hr S9 (＋)	*P* value*
5 mg/mL	1；1	1.000
2.5 mg/mL	2；1	0.477
1.25 mg/mL	1；1	1.000
0.625 mg/mL	1；1	1.000
0.3125 mg/mL	1；2	0.477
Negative control	1；1	1.000
Positive control	11；10	0.000

Dosage	Cell number with abnormal chromosome	
	Treat for 3hr S9 (－)	*P* value*
5 mg/mL	2；1	0.477
2.5 mg/mL	1；2	0.477
1.25 mg/mL	1；2	0.477
0.625 mg/mL	1；1	1.000
0.3125 mg/mL	1；1	1.000
Negative control	1；1	1.000
Positive control	8；7	0.000

Dosage	Cell number with abnormal chromosome	
	Treat for 18hr S9 (－)	*P* value*
5 mg/mL	2；1	0.477
2.5 mg/mL	1；1	1.000
1.25 mg/mL	1；1	1.000
0.625 mg/mL	2；1	0.477
0.3125 mg/mL	2；1	0.477
Negative control	1；1	1.000
Positive control	8；6	0.000

* The *p* values are compared with the Negative Control using the Chi-square test.

There are no significant differences with the Negative Control when $p > 0.05$.

b. The results of the chromosome abnormality in the 3-hour treatment with S9 are shown below:

Dosage	Types of abnormal chromosome (3 hr, S9+)					Sum of abnormal numbers
	ctb	cte	csb	cse	others	
5 mg/mL	0	0	1	0	0	1
	0	0	1	0	0	1
2.5 mg/mL	0	0	2	0	0	2
	0	0	0	1	0	1
1.25 mg/mL	0	0	0	1	0	1
	0	0	1	0	0	1
0.625 mg/mL	0	0	1	0	0	1
	0	0	1	0	0	1
0.3125 mg/mL	0	0	1	0	0	1
	0	0	2	0	0	2
Negative control	0	0	1	0	0	1
	0	0	1	0	0	1
Positive control	2	0	9	0	0	11
	3	0	7	0	0	10

ctb: chromatid breakage, cte: chromatid exchange, csb: chromosome breakage, cse: chromosome exchange.

c. The results of the chromosome abnormality in the 3-hour treatment without S9 are shown below:

Dosage	Types of abnormal chromosome (3 hr, S9－)					Sum of abnormal numbers
	ctb	cte	csb	cse	others	
5 mg/mL	0	0	2	0	0	2
	0	0	1	0	0	1
2.5 mg/mL	1	0	0	0	0	1
	0	0	2	0	0	2
1.25 mg/mL	0	0	1	0	0	1
	1	0	1	0	0	2
0.625 mg/mL	0	0	1	0	0	1
	0	0	1	0	0	1
0.3125 mg/mL	0	0	1	0	0	1
	0	0	1	0	0	1
Negative control	0	0	1	0	0	1
	0	0	1	0	0	1
Positive control	1	0	7	0	0	8
	1	0	6	0	0	7

ctb: chromatid breakage, cte: chromatid exchange, csb: chromosome breakage, cse: chromosome exchange.

d. The results of the chromosome abnormality in the 18-hour treatment without S9 are shown below:

Dosage	Types of abnormal chromosome (18 hr, S9－)					Sum of abnormal numbers
	ctb	cte	csb	cse	others	
5 mg/mL	0	0	2	0	0	2
	0	0	1	0	0	1
2.5 mg/mL	0	0	1	0	0	1
	0	0	1	0	0	1
1.25 mg/mL	0	0	1	0	0	1
	0	0	1	0	0	1
0.625 mg/mL	0	0	1	0	1	2
	0	0	1	0	0	1
0.3125 mg/mL	0	0	2	0	0	2
	0	0	1	0	0	1
Negative control	0	0	1	0	0	1
	0	0	1	0	0	1
Positive control	0	0	7	0	1	8
	0	0	6	0	0	6

ctb: chromatid breakage, cte: chromatid exchange, csb: chromosome breakage, cse: chromosome exchange.

CONCLUSION

The overall results show that under the test system, the “OnePCR” has no genotoxic effects on Chinese hamster ovary cells (CHO-K1), whether treated with the S9 metabolic activation system 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

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4. *In Vitro* Mammalian Chromosome Aberration Test. OECD guideline for the testing of chemicals. # 473 (1997).
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7. Current OECD Principles of Good Laboratory Practice (Organization for Economic Cooperation and Development, Paris, ENV/MC/CHEM (98) 17).
8. TESP-UB-217 Operating Procedures of the cells' activation and verification. Version 1.0.
9. TESP-UB-1011 *In Vitro* Mammalian Chromosomal Aberration Test. Version 1.5.
10. EOMP-USL-029 Operating Procedures of Microplate Spectrophotometer-BioTek EON. Version 1.0.
11. EOMP-USL-0003 Operating and analyzed Procedures of the Balance. Version 1.2.
12. EOMP-USL-0030 Maintenance and operating procedures of the microscope. Version 1.1.
13. EOMP-USL-0027 Operating procedures of the biosafety cabinet and laminar flow, UV-lamp verification and aerobic plate counts. Version 2.2.

TEST ARTICLE PHOTO

UB/2013/80653

UB/2013/80653