**Pusat Penyelidikan Teknologi Alam Sekitar**
***Environmental Technology Research Centre***
Building 15, SIRIM Berhad, Shah Alam, Selangor Darul Ehsan
*Tel: 60-3-5544 6550 / 6598 Fax: 60-3-5544 6590 / 6579*

## RESULTS SUMMARY

| | | |
|---|---|---|
| Company Name | : | Trumer Medicare Sdn Bhd (Attn.: Mr. K.C. Yap) |
| Address | : | No 16-2 Jalan SS19/1G, 47500 Subang Jaya, Selangor |
| Request | : | Mutagenicity Study |

| | |
|---|---|
| Your Ref No. | |
| SIRIM Ref. No. | ETRC 257/16/1127 |
| Job No. | J076/2013 |
| Report No. | R 113/2013 |
| Date of Issue | 30 July 2013 |
| No. of pages | 1 |

**SAMPLE DESCRIPTION:**
One (1) liquid samples were received on 18 June 2013 and coded as Nano Colloidal Argentum:

**TEST METHOD:**
OECD Guidelines for Testing of Chemicals Method 471(Adopted 21st July 1997), Bacterial Reverse Mutation Test.

**RESULT:**
Nano Colloidal Argentum was judged to have no reverse mutagenic potential with and without metabolic activation system under the present test conditions.
*Refer to ETRC257/16/1127(R113/2013) for details.*

**INFERENCE:**
The Nano Colloidal Argentum was determined as non-mutagenic and classified as "Not classified" according to the Glabally Harmonized System of Classification and Labelling of Chemicals (GHS), Fourth Revised Edition as shown below:

| Ingredient Classification | Cut-off/concentration limit | | |
|---|---|---|---|
| | Category 1 mutagen | | Category 2 mutagen |
| | Category 1A | Category 1B | |
| Category 1A mutagen | ≥0.1% | - | - |
| Category 1B mutagen | - | ≥0.1% | - |
| Category 2 mutagen | - | - | ≥0.1% |



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*Study Code: J076/2013/48*
*Reference No: ETRC257/16/1127 (R113/2013)*

SIRIM

# TEST REPORT

# BACTERIAL REVERSE MUTATION TEST OF NANO COLLOIDAL ARGENTUM

***Prepared for:***

Trumer Medicare Sdn. Bhd.
No. 16-2, Jalan SS19/1G,
47500 Subang Jaya
Selangor

***Prepared by:***

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**Jul 30, 2013**

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**Statement of ISO/IEC 17025 STR 1.2 Compliance**

I, the undersigned, hereby declare that this report constitutes a true and complete representation of the procedures followed and of the result obtained in this study by ETRC, SIRIM Berhad, and that the study was carried out under my supervision.

The study was conducted according to the ISO/IEC 17025 STR 1.2 requirements.

Hasnah Mohd Zin
Study Director
Environmental Technology Research Centre
SIRIM Berhad

30/7/2013
Signature and Date

## Study Information

**Study Title**
Bacterial Reverse Mutation Test of Nano Colloidal Argentum

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**Study personnel**
i. Wan Yusmin Wan Yusuf
ii. Ahmad Tarmizi Abdullah Zawawi
iii. Ruslan Ibrahim

**Study period**
i. Experimental start date: 10 July 2013
ii. Experimental completion date: 22 July 2013
iii. Study completion date: 30 July 2013

**Sponsor**
Trumer Medicare Sdn. Bhd.
No. 16-2, Jalan SS19/1G
47500 Subang Jaya
Selangor

**Testing Facility**
Mutagenicity Laboratory
Environmental Technology Research Centre
Building 15, SIRIM Berhad
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## Report Information

**Study Title**
Bacterial Reverse Mutation Test of Nano Colloidal Argentum

**This report was prepared by**
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30/7/2013
Signature and Date

**Summary**

The bacteria reverse mutation test of Nano Colloidal Argentum was performed on *Salmonella typhimurium* strains TA100, TA1535, TA98, TA1537 and *Escherichia coli* strain WP2*uvrA* using the pre-incubation method in the presence and absence of an exogenous metabolic activation system.

The results showed the number of revertant colonies for all strains which were treated with the test item were less than twice that of the negative controls with and without S9 Mix.

The number of revertant colonies in the negative control and positive controls were within the background data of ETRC laboratory.

Based on the above results, Nano Colloidal Argentum was judged to have no reverse mutagenic potential with and without metabolic activation system under the present test conditions.

## 1 Introduction

The sample is coded as Nano Colloidal Argentum, a product produced by Trumer Medicare Sdn. Bhd.

The purpose of this study was to determine the mutagenic potential of Nano Colloidal Argentum using *Salmonella typhimurium* and *Escherichia coli* strains. This study was conducted in accordance with Method 471, Bacterial Reverse Mutation Test (Adopted 21st July 1997), OECD GUIDELINE for Testing of Chemicals.

## 2 Materials and Methods

### 2.1 Test Item

(Information as provided by the sponsor)

| | |
|---|---|
| Name: | Nano Colloidal Argentum |
| Other name: | Not applicable |
| CAS No.: | Not applicable |
| Sponsor: | Trumer Medicare Sdn. Bhd. |
| Lot/Batch No.: | Not available |
| Manufactured date: | Not available |
| Date of receipt: | 18th June 2013 |
| Expiry date: | Not available |
| Structural formula or rational formula: | Not available |
| Purity: | Not available |
| Contents of Impurities: | Not available |
| Physicochemical properties as follows: | |
| Appearance at ordinary temperature: | Clear liquid |
| Molecular weight: | Not available |
| Stability: | *No denaturation observed |
| Melting Point: | Not available |
| Boiling Point: | Not available |
| Vapor Pressure: | Not available |
| Partition Coefficient: | Not available |
| Solubility: | No information |
| Degree of Solubility: | |
| Water: | * Soluble at 50 mg/ml |
| DMSO: | * Soluble at 50 mg/ml |
| Acetone: | * Soluble at 100 mg/ml |
| Storage condition: | Store in Cold Room 4°C |
| Stability of test concentrations: | *No denaturation observed |
| Handling care: | Wear mask, glove and lab coat |

Note *: test was conducted as in-house method.

## 2.2 Reference Items

(Information as provided by the chemical suppliers)

2.2.1 Dimethyl sulfoxide (DMSO)

| | |
|---|---|
| Manufacturer: | R&M Chemicals |
| Lot No.: | POHE130612 |
| Properties: | Colourless liquid |
| Purity: | >99.9% |
| Grade: | Analytical grade |

2.2.2 Furylfuramide (AF-2)

| | |
|---|---|
| Manufacturer: | Wako Pure Chemical Industries, Ltd. |
| Lot No.: | STQ3987 |
| Properties: | Green crystalline powder |
| Purity: | 99.7% |
| Grade: | Analytical grade |

2.2.3 Sodium azide ($NaN_3$)

| | |
|---|---|
| Manufacturer: | Sigma-Aldrich |
| Lot No.: | 109K0088 |
| Properties: | White crystalline powder |
| Purity: | 99.7% |
| Grade: | Analytical grade |

2.2.4 Acridine Mutagen (ICR-191)

| | |
|---|---|
| Manufacturer: | Sigma |
| Lot No.: | 031M1947V |
| Properties: | Yellow powder with orange cast |
| Purity: | Not available |
| Grade: | Analytical grade |

2.2.5 2-Nitrofluorene (2-NF)

| | |
|---|---|
| Manufacturer: | Aldrich |
| Lot No.: | S43858 |
| Properties: | Yellow-tan powder |
| Purity: | 97.9% |
| Grade: | Analytical grade |

2.2.6 2-Aminoanthracene (2AA)

| | |
|---|---|
| Manufacturer: | Aldrich |
| Lot No.: | STBB1901 |
| Properties: | Olive green powder |
| Purity: | 97.5% |
| Grade: | Analytical grade |

2.2.7 Storage Conditions

2-NF, AF-2, $NaN_3$ and 2AA were stored at room temperature and ICR-191 was stored in a refrigerator at 4°C.

2.2.8 Care on Handling

Gloves and lab coat were worn when handling the positive controls.

## 2.3 Test System

2.3.1 Source

The *Salmonella typhimurium* strains TA100, TA98, TA1535 and TA1537 and *E. coli* strain WP2*uvrA* were obtained from National Collection of Type Cultures (NCTC)

United Kingdom and National Collection of Industrial, Food and Marine Bacteria (NCIMB) United Kingdom, respectively, on 19th August 2010.

2.3.2 Characterization of the test organism

All *Salmonella* strains were histidine, and the *E. coli* strain was tryptophan dependent. The revertants were identified as colonies that grow in low levels of histidine or tryptophan. Additional genetic markers serve to make the strains more sensitive to certain types of mutagens. A list of these additional genetic markers and strain characteristics were shown in Table 1.

**Table 1: Characteristics of *Salmonella* and *E. coli* Strains Used for the Ames Test**

| Bacterial Strain | Gene Affected | DNA-repaired | LPS | Biotin Requirement | Plasmids | Mutational Event |
|---|---|---|---|---|---|---|
| *S. typh.* TA98 | hisD | uvrB | rfa | Bio (-ve) | PKM101 | Frameshift |
| *S. typh.* TA100 | hisG | uvrB | rfa | Bio (-ve) | PKM101 | Base-pair substitution |
| *S. typh.* TA1535 | hisG | uvrB | rfa | Bio (-ve) | - | Base-pair substitution |
| *S. typh.* TA1537 | hisC | uvrB | rfa | Bio (-ve) | - | Frameshift |
| *E. coli* WP2*uvrA* | trp | uvrA | - | - | - | Base-pair substitution |

The DNA repair mutation (*uvrA/B)* eliminates excision repair, a repair pathway for DNA damage from UV light and certain mutagens. The presence of the *uvrA/B* mutation makes the strains more sensitive to the test articles that induce damage in this manner. The *uvrA/B* mutation is part of a deletion mutation extending into a gene for biotin synthesis; therefore, the biotin requirement is a result of the deletion of this region. The *uvrA/B* mutation is indicated by sensitivity to UV light.

The *rfa* mutation changes the properties of the bacterial cell wall and results in the partial loss of the lipopolysaccharide (LPS) barrier increasing permeability of cells to certain types of chemicals. The *rfa* mutation is indicated by sensitivity to crystal violet. The R factor plasmid (pKM101) makes the strains more responsive to a variety of mutagens. The plasmid carries an ampicillin resistance gene; therefore ampicillin resistance indicates that the strains retain the plasmid.

The characterization of *Salmonella typhimurium* strains TA98, TA100, TA1535 and TA1537 and *E. coli* strain WP2*uvrA* were conducted on 24th July, 2012.

2.3.3 Storage

The tester strains were stored as frozen working stock cultures (0.0875 ml of Dimethyl sulfoxide (DMSO)/ml of broth culture) at -80°C in Ultra-deep Freezer.

## 2.4 Test Condition

2.4.1 Minimal agar plate

Minimal agar plates (Batch No. D13MINIMAL002) were purchased from ISOLAB Sdn. Bhd. with the expiry date on 15th October 2013. The plates were stored at 2°C to 8°C until use.

2.4.2 Overlay agar

The overlay agar was composed of 0.6 % (w/v) agar (Bacto-Agar, Lot No. 348882) and 0.5% (w/v) NaCl. One bottle of 100 ml overlay agar was added with 10 ml a mixture of 0.5 mM histidine and 0.5 mM biotin for *S. typhimurium* strains. Another bottle of 100 ml overlay agar was added with 10 ml 0.5 mM tryptophan for *E. coli* strain. Both have a ratio of 1:10 of amino acid to agar.

2.4.3 Metabolic Activation System (S9 Mix)

A volume of 6 ml rat liver S9 with cofactor and buffer was added into tube contained of 1.2 ml salt solution (1.65M KCl + 0.4M $MgCl_2$), 0.3 ml 1M glucose-6-phosphate, 2.4 ml 0.1M nicotine adenine dinucleotide phosphate (NADP), 30 ml 0.2M phosphate buffer at pH7.4 and 20.1 ml sterile pure water.

The final concentration of S9 mix was 10% and it was prepared freshly before used. The S9 (Lot No. 2837) was purchased from Molecular Toxicology Inc. (Moltox) with the expiry date on 30th September 2013 and was stored at -80°C Ultra-deep Freezer.

2.4.4 Test Item

Prior to the sample preparation the test item was tested for solubility on water, DMSO and acetone. The results show the water is the appropriate as the test item was much homogenized compared to the rest.

The test item was weighed (0.4g) and suspended in water (8ml) with the final concentration 50 mg/ml. In the test, the highest concentration prepared was 5000 µg/plate and continue with 4 other concentrations in geometric series of a factor 2. The test item was in the form of solution and the test item solution was mixed with vortex during transferring into test tube.

The stability of test item solution for denaturation activity with respect to the changes of colour and/or exothermic reaction was observed throughout the experimental time. The test item solution was kept at room temperature within the experimental time.

2.4.5 Reference Items

Sodium azide ($NaN_3$) was dissolved in pure water. Furylfuramide (AF-2), Acridine mutagen (ICR-191), 2-Nitrofluorene (2-NF) and 2-Aminoanthracene (2AA) were dissolved in DMSO. The concentration of positive controls (PC) used as reference items for each of bacterial tester strain were shown in Table 2.

**Table 2: Positive controls used for each of bacterial strain**

| Bacterial Tester Strain | | TA100 | TA1535 | WP2*uvrA* | TA98 | TA1537 |
|---|---|---|---|---|---|---|
| Without S9 Mix | PC | $NaN_3$ | $NaN_3$ | AF-2 | 2-NF | ICR-191 |
| | Conc. (µg/plate) | 0.5 | 0.5 | 0.05 | 1 | 1 |
| With S9 Mix | PC | 2AA | 2AA | 2AA | 2AA | 2AA |
| | Conc. (µg/plate) | 1 | 2 | 10 | 0.5 | 2 |

The working stocks of positive control were stored at -80°C Ultra-deep Freezer.

2.4.6 Bacteria culture

Each of tested bacteria strain culture was prepared by inoculation of 36 µl of bacterial suspension from working stock into a L-tube containing 18 ml nutrient broth No. 2 for *S. typhimurium* (TA1535 and TA1537) and *E. coli* (WP2 *uvrA*) and Nutrient broth No. 2 with Ampicillin for *S. typhimurium* (TA98 and TA100). These cultures were incubated at 37 ± 0.5°C for 6 - 9 h with shaking at 57 times/min in a shaker water-bath.

The optical density reading was determined at 660 nm by spectrophotometer until it reaches the target value at the end of incubation. The optical density values were used to calculate the viable cell against the set value equivalent to 1 x $10^9$. The set values were obtained from growth profile study prepared for each new lot of bacterial strains. The viable cell counts for each of bacterial strain were shown in Table 3.

**Table 3: Viable cell counts of each strain**

| Bacterial Tester Strain | | TA100 | TA1535 | WP2 *uvrA* | TA98 | TA1537 |
|---|---|---|---|---|---|---|
| No. of viable cells (x $10^9$/ml) | Dose finding test | 1.02 | 1.00 | 1.07 | 1.00 | 1.00 |
| | Main test | 1.01 | 1,01 | 1.03 | 1.02 | 1.02 |

2.4.7 Treatment procedures of pre-incubation method

Test item

One set of 15 test tubes containing of test item (0.1 ml) was added with 0.5 ml of S9 Mix. Another set of test tubes containing test item (0.1 ml) was added with 0.5 ml of 0.1 M sodium phosphate buffer (pH 7.4). Lastly, both sets were added with 0.1 ml fresh bacteria culture.

Negative control

One set of test tubes containing of negative control (0.1 ml) was added with 0.5 ml of S9 Mix. Another set was added with 0.5 ml of 0.1 M sodium phosphate buffer (pH 7.4). Lastly, both sets were added with 0.1 ml fresh bacteria culture.

Positive control

One set of test tubes containing of positive control (0.1 ml) was added with 0.5 ml of S9 Mix. Another set was added with 0.5 ml of 0.1 M sodium phosphate buffer (pH 7.4). Lastly, both sets were added with 0.1 ml fresh bacteria culture.

The mixtures were incubated for 20 minutes at 35 ± 2°C and aerated during incubation using shaker. Two ml of soft agar was added into each tube followed by vortexed and poured onto the surface of a minimal agar plates, swirled to form an even layer and allowed to solidify.

The plates were incubated for 48 hours at 37 ± 2°C. The number of revertant colonies were observed and counted.

Triplicate plates were used for the negative control, positive control and test item at each concentration in the presence and absence of metabolic activation system for an adequate estimated of variation.

The contaminant checks were conducted for test item solution, S9 Mix and 0.1 M sodium phosphate buffer. The volume of 0.1 ml of each were poured onto a minimal agar plate and incubated at 37 ± 2°C for 48 hours. The bacterial contamination was observed by the growth of bacterial colony.

2.4.8 Concentration selection

The concentration was selected based on the highest dose at 5000 µg/plate and followed with 2500, 1250, 625 and 313 µg/plate.

2.4.9 Observation and measurement

The form of the revertant colonies, test item precipitation and growth inhibition was examined under a stereo microscope, growth inhibition was classified based on shape of bacterial lawn and described as follows: T1 for slightly rough or changed, T2 for rough, T3 for very rough, and T4 for no revertant and no bacterial lawn observed.

The number of revertant colonies was counted manually. The average number of colonies for each test item concentration, negative control and positive control were calculated as the average plate counts for a set of triplicates. Decimals of the average figures were rounded off.

## 2.5 Statistical Method

Experimental raw data was statistically treated for average and standard deviation using Excel spreadsheet.

## 2.6 Unexpected Situations and Deviations

There was no unexpected situation which might have affected the test results and deviation from study plan.

# 3 Result and Discussion

There was no sign of toxicity effect at the concentration of 5000 µg/plate. Test item concentration from 313 to 5000 µg/plate was shown no precipitation of test item on the surface of agar.

The results for dose finding test without and with S9 mix were shown in Table A1.1 and A1.2 (Appendix A1). Whereas the results for main test without and with S9 mix were shown in Table A2.1 and A2.2 (Appendix A2).

The numbers of revertant colonies for all tested strains TA98, TA100, TA1535, TA1537 and WP2*uvrA*, treated with and without S9 Mix at all concentrations were less than 2-fold of negative control.

The results show no evidence of increasing in the revertant colonies from the 313 and 5000 µg/plate concentration for all bacterial strains treated with and without S9 Mix in both tests (DFT and MT).

The positive controls showed the number of revertant colonies more than 2-fold of negative control. The positive controls with and without metabolic activation system (S9 mix) for TA98 TA100, TA1535, TA1537 and WP2*uvrA* were within the background data in the range of ± 2 SD.

The absence of any microorganisms in the contaminant check shows the test study was free from any contamination.

## 4 Conclusion

Nano Colloidal Argentum was judged to have no reverse mutagenic potential with and without metabolic activation system under the present test conditions.

## 5 Storage and Retention of Records/Materials

All study-specific records are kept in ETRC (address as indicated on the front page of this report) for a period of 6 years from the date of study completion.

All records can be claimed by the sponsor with official notification before the expiry date of the storage and retention time, or upon termination of test facility's storage and retention provision.

## 6 References

i Method 471 Bacteria Reverse Mutation Test (Adopted 21st July 1997), OECD Guideline for Testing of Chemicals.

ii Green M.H.L. and W.J. Muriel (1976) Mutagen testing using Trp + reversion in *Escherichia coli,* Mutation Res., 38: 3-31.

iii Maron, D.M. and B.N. Ames (1983) Revised methods for the Salmonella Mutagenicity test, Mutation Res., 113: 173-215.

Appendix A1

Table A1.1: Result of Dose Finding Test Without S9 Mix for Nano Colloidal Argentum

| Test item concentration (µg/plate) | Number of revertants [(number of colonies/plate) ± SD] | | | | |
|---|---|---|---|---|---|
| | Base-pair substitution type | | | Frameshift type | |
| | TA100 | TA1535 | WP2 *uvrA* | TA98 | TA1537 |
| Control (Water) Concurrent | 214 ± 10 | 16 ± 2 | 30 ± 5 | 42 ± 5 | 9 ± 2 |
| Control (Water) (*[5]Historical) | 162 ± 40 | 19 ± 10 | 25 ± 7 | 33 ± 10 | 12 ± 5 |
| 313 | 195 ± 19 | 17 ± 2 | 34 ± 10 | 43 ± 5 | 13 ± 3 |
| 625 | 187 ± 2 | 18 ± 2 | 41 ± 4 | 45 ± 4 | 10 ± 2 |
| 1250 | 183 ± 13 | 17 ± 1 | 37 ± 2 | 41 ± 3 | 10 ± 3 |
| 2500 | 192 ± 6 | 13 ± 2 | 34 ± 3 | 42 ± 7 | 13 ± 4 |
| 5000 | 209 ± 19 | 14 ± 2 | 36 ± 5 | 39 ± 3 | 12 ± 1 |
| Positive control | *[1]$NaN_3$ | *[1]$NaN_3$ | *[2]AF-2 | *[3]2-NF | *[4]ICR-191 |
| Concentration (µg/plate) | 0.5 | 0.5 | 0.05 | 1 | 1 |
| Number of revertant (Concurrent) | 856 ± 44 | 657 ± 27 | 1850 ± 118 | 877 ± 70 | 340 ± 37 |
| Number of revertant (*[5]Historical) | 1137 ± 330 | 550 ± 183 | 1182 ± 465 | 518 ± 192 | 634 ± 220 |

Note: *[1]$NaN_3$: Sodium azide
*[2]AF-2: 2-Furylfuramide
*[3]2-NF: 2-Nitrofluorene
*[4]ICR-191: Acridine mutagen
*[5]Historical: Accumulative historical data from Year 2000 to this current study

Table A1.2: Result of Dose Finding Test With S9 Mix for Nano Colloidal Argentum

| Test item concentration (µg/plate) | Number of revertants [(number of colonies/plate) ± SD] | | | | |
|---|---|---|---|---|---|
| | Base-pair substitution type | | | Frameshift type | |
| | TA100 | TA1535 | WP2 *uvrA* | TA98 | TA1537 |
| Control (Water) Concurrent | 208 ± 4 | 21 ± 5 | 25 ± 1 | 38 ± 3 | 10 ± 3 |
| Control (Water) (*[2]Historical) | 160 ± 45 | 19 ± 8 | 26 ± 7 | 40 ± 12 | 14 ± 6 |
| 313 | 213 ± 4 | 20 ± 4 | 32 ± 8 | 37 ± 1 | 13 ± 3 |
| 625 | 207 ± 9 | 21 ± 3 | 39 ± 2 | 40 ± 8 | 17 ± 2 |
| 1250 | 208 ± 13 | 23 ± 7 | 39 ± 5 | 29 ± 3 | 14 ± 3 |
| 2500 | 187 ± 6 | 20 ± 6 | 35 ± 8 | 43 ± 8 | 8 ± 1 |
| 5000 | 205 ± 7 | 17 ± 4 | 28 ± 5 | 37 ± 3 | 13 ± 2 |
| Positive control | *[1]2AA | *[1]2AA | *[1]2AA | *[1]2AA | *[1]2AA |
| Concentration (µg/plate) | 1 | 2 | 10 | 0.5 | 2 |
| Number of revertant (Concurrent) | 469 ± 12 | 228 ± 7 | 233 ± 12 | 332 ± 10 | 165 ± 17 |
| Number of revertant (*[2]Historical) | 522 ± 159 | 159 ± 82 | 128 ± 38 | 193 ± 67 | 137 ± 60 |

Note: *[1]2AA: 2-Aminoantharacene
*[2]Historical: Accumulative historical data from Year 2000 to this current study

Appendix A2

Table A2.1: Result of Main Test Without S9 Mix for Nano Colloidal Argentum

| Test item concentration (µg/plate) | Number of revertants [(number of colonies/plate) ± SD] | | | | |
|---|---|---|---|---|---|
| | Base-pair substitution type | | | Frameshift type | |
| | TA100 | TA1535 | WP2 *uvrA* | TA98 | TA1537 |
| Control (Water) Concurrent | 142 ± 17 | 15 ± 5 | 38 ± 2 | 41 ± 6 | 10 ± 3 |
| Control (Water) (*[5]Historical) | 162 ± 40 | 19 ± 10 | 25 ± 7 | 33 ± 10 | 12 ± 5 |
| 313 | 151 ± 14 | 18 ± 1 | 34 ± 8 | 32 ± 1 | 13 ± 3 |
| 625 | 154 ± 14 | 15 ± 7 | 35 ± 4 | 37 ± 6 | 18 ± 3 |
| 1250 | 157 ± 17 | 15 ± 2 | 35 ± 5 | 36 ± 2 | 11 ± 2 |
| 2500 | 166 ± 10 | 14 ± 7 | 33 ± 1 | 31 ± 5 | 14 ± 5 |
| 5000 | 150 ± 10 | 20 ± 9 | 36 ± 3 | 31 ± 1 | 13 ± 4 |
| Positive control | *[1]$NaN_3$ | *[1]$NaN_3$ | *[2]AF-2 | *[3]2-NF | *[4]ICR-191 |
| Concentration (µg/plate) | 0.5 | 0.5 | 0.05 | 1 | 1 |
| Number of revertant (Concurrent) | 623 ± 24 | 636 ± 51 | 1135 ± 37 | 865 ± 252 | 396 ± 79 |
| Number of revertant (*[5]Historical) | 1137 ± 330 | 550 ± 183 | 1182 ± 465 | 518 ± 192 | 634 ± 220 |

Note: *[1]$NaN_3$: Sodium azide
*[2]AF-2: 2-Furylfuramide
*[3]2-NF: 2-Nitrofluorene
*[4]ICR-191: Acridine mutagen
*[5]Historical: Accumulative historical data from Year 2000 to this current study

Table A2.2: Result of Main Test With S9 Mix for Nano Colloidal Argentum

| Test item concentration (µg/plate) | Number of revertants [(number of colonies/plate) ± SD] | | | | |
|---|---|---|---|---|---|
| | Base-pair substitution type | | | Frameshift type | |
| | TA100 | TA1535 | WP2 *uvrA* | TA98 | TA1537 |
| Control (Water) Concurrent | 152 ± 17 | 19 ± 0 | 33 ± 3 | 49 ± 3 | 14 ± 3 |
| Control (Water) (*[2]Historical) | 160 ± 45 | 19 ± 8 | 26 ± 7 | 40 ± 12 | 14 ± 6 |
| 313 | 171 ± 21 | 19 ± 7 | 39 ± 12 | 45 ± 6 | 14 ± 2 |
| 625 | 166 ± 17 | 22 ± 4 | 37 ± 10 | 53 ± 4 | 15 ± 2 |
| 1250 | 164 ± 7 | 20 ± 3 | 39 ± 5 | 39 ± 6 | 19 ± 3 |
| 2500 | 150 ± 5 | 15 ± 0 | 32 ± 2 | 41 ± 3 | 15 ± 4 |
| 5000 | 149 ± 10 | 22 ± 2 | 31 ± 4 | 42 ± 4 | 20 ± 3 |
| Positive control | *[1]2AA | *[1]2AA | *[1]2AA | *[1]2AA | *[1]2AA |
| Concentration (µg/plate) | 1 | 2 | 10 | 0.5 | 2 |
| Number of revertant (Concurrent) | 447 ± 14 | 350 ± 40 | 243 ± 13 | 394 ± 1 | 107 ± 9 |
| Number of revertant (*[2]Historical) | 522 ± 159 | 159 ± 82 | 128 ± 38 | 193 ± 67 | 137 ± 60 |

Note: *[1]2AA: 2-Aminoantharacene
*[2]Historical: Accumulative historical data from Year 2000 to this current study