Safety Report

FluoroVue™ Nucleic Acid Gel Stain (NS1000)

Test System

The Ames Test study is conducted by SGS Laboratory Co., Ltd., New Taipei City, Taiwan according to the guidance of OECD guideline for the testing of chemicals # 471：Bacterial reverse mutation test (1997). Five bacteria strains (*Salmonella typhimurium* TA97a, TA98, TA100, TA102 and TA1535) are used to evaluate the mutagenicity of test article. The *Salmonella* reverse mutation test uses histidine requiring strains of *Salmonella typhimuriu*m 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 screen for genotoxic activity and, in particular, for point mutation-inducing activity.

Purpose

The Ames test is a standard assay to assess the mutagenic potential of chemicals. As cancers are often associated with DNA damage, this test can be used to estimate the carcinogenic potential of a chemical compound.

Materials and Methods

- **Test Substance:**
  - Chemical name: FluoroVue™ Nucleic Acid Gel Stain (NS1000)
  - Negative control: DMSO
  - Positive control: Different positive control articles are used based on different bacteria strains. The description of bacteria strains and individual concentration of mutagens are shown below:

Mutagen	S9	Concentration (μg/plate)	Strain
9-aminoacridine	-	50	TA97a
2-nitrofluorence	-	5	TA98
sodium azide	-	0.4	TA100, TA1535
mitomycin C	-	0.5	TA102
2-aminoanthracene	+	4.0	TA1535, TA102
benzo [a] pyrene	+	4.0	TA98
2-aminofluorene	+	4.0	TA97a, TA100

- **Bacterial Strains:**
  - *Salmonella Typhimurium,* TA97a, TA98, TA100, TA102 and TA1535

- **Methods:**
  - **Spot test:**
    The test was performed by adding test article solution on sterile paper disc, which placed on *S. typhimurium* plates. Incubate at 37±2℃ for 24 to 48 hrs and check the bacteriostasis ring (cell toxicity) or circularity formed by large number of colonies (mutation).
  - **Plate incorporation test:**
    The following was added to each sterile culture tube containing 2.0 mL top agar: The 0.1 mL of test substance, 0.1 mL of bacterial suspension, and 0.5 mL of S9 mixture (+S9) or 0.5 mL of without S9 mixture (-S9). The mixture was uniformly poured on the prepared underlay agar plates. After solidification, the plates were incubated at 37°C for 48 h. At the end of the incubation, revertant colonies per plate were counted. All plating was done in triplicate. If the number was more than twice the spontaneous revertant colonies counts and showed a dose-response relationship, positive result for mutagenicity could be concluded.
  - **Quality criteria**
    A. All of the test procedures are operated in the biological safety cabinet (BSC).

B. Strain identification test, spot test and plate incorporation test were conduct in the same time. If the strain identification failed or the dosage used in plate incorporation test cause cell toxicity, all the three tests must be reconducted.
C. The spontaneous mutation colonies of negative control group must be in the reasonable range and the reverse mutation colonies of each positive control must be two times more than the average of the negative control group, otherwise the test is failed and must redo.
D. At the end of the testing, all the test material, raw data, results, and protocols were properly maintained under the guidance of Good Laboratory Practice (GLP). All the experiment procedure was referring to SGS SOP: TESP-UB-1010.

– **Data management**

1. For each dosage of test article, negative control group and positive control group, the reverse mutation colonies were counted and calculated. The spontaneous mutation colonies of negative control group must in the reasonable range.
2. Criteria of evaluation for positive reaction: Whether the S9 added or not, at least one strain has more than one dosage group with two times increase in the number of reverse mutation, consider as positive reaction.
3. Criteria of evaluation for negative reaction: The reverse mutation number of all strains in all dosage groups does not attain the criteria of positive reaction were considered as negative reaction.

Results

– **Spot test:**

There is no obvious bacteriostasis ring (cell toxicity) or circularity formed by large number of colonies (mutation) around the disc in each test group, so the 5 µL/plate, 2.5 µL/plate, 1.25 µL/plate, 0.625 µL/plate, and 0.313 µL/plate dosages were used in plate incorporation test.

– **Plate incorporation test:**

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

Colony Number (CFU/plate)	Test Strain		TA97a		TA98		TA100		TA102		TA1535	
	S9		+	-	+	-	+	-	+	-	+	-
	Negative control (NC)	Avg	188.0	87.3	52.7	49.7	112.7	112.0	589.3	430.7	11.0	13.0
		SD	16.5	4.9	2.5	7.0	8.1	22.1	127.6	54.5	1.0	2.6
	2X Avg. of NC		376.0	174.7	105.3	99.3	225.3	224.0	1178.7	861.3	22.0	26.0
	Positive control	Avg	418.7	666.7	160.3	549.3	270.7	278.7	1304.0	1530.7	152.7	259.0
		SD	7.0	117.9	8.0	78.9	13.3	18.9	48.0	101.9	17.2	11.8
Test Article Solution (µL/ plate)	5 µL /plate	Avg	201.3	99.7	49.7	41.3	117.3	93.0	690.7	432.0	13.0	10.0
		SD	12.4	8.1	4.0	8.5	13.3	1.0	98.7	54.1	2.6	0.0
	2.5 µL /plate	Avg	201.0	85.3	51.3	42.7	108.3	61.0	734.7	436.0	14.0	16.7
		SD	6.0	1.5	5.0	5.9	9.7	6.9	16.2	45.4	1.7	1.2
	1.25 µL /plate	Avg	156.0	58.7	29.0	31.3	90.0	56.0	624.0	476.0	17.7	10.3
		SD	29.8	9.9	2.6	2.3	10.4	7.9	50.0	10.6	3.8	0.6
	0.625 µL /plate	Avg	219.7	62.0	25.3	25.0	97.3	59.0	728.0	480.0	9.3	11.7
		SD	8.5	5.3	1.2	4.4	8.5	5.3	119.5	31.2	1.5	1.5
	0.313 µL /plate	Avg	206.3	64.7	24.7	23.0	89.3	56.0	661.3	388.0	13.3	14.0
		SD	27.2	3.1	1.2	3.0	14.0	3.6	40.3	18.3	0.6	0.0

Conclusion

The results showed that the revertant numbers of the test article "FluoroVue™ Nucleic Acid Gel Stain" appeared to be two times less than that of the negative control group in *Salmonella typhimurium* TA97a, TA100 and TA1535. "FluoroVue™ Nucleic Acid Gel Stain" caused no mutagenic effects to these *Salmonella typhimurium* strains.

References

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