

SERVA DNA Stain G

Cat. No. 39803

1. INTRODUCTION

Ethidium bromide (EtBr) is most commonly used nucleic acid stain in molecular biology laboratories. It has been proved to be strong carcinogen and therefore considered hazardous for laboratory personnel and environment.

SERVA DNA Stain G is a nucleic acid stain which can be used as a safer alternative to the traditional Ethidium bromide stain for detecting nucleic acid in agarose gels. It is as sensitive as Ethidium bromide and can be used exactly the same way in agarose gel electrophoresis with some extra possibilities.

The safety of SERVA DNA Stain G has been controlled with three tests:

- a. Ames test
- b. Mouse bone marrow micronucleus test
- c. Chromosome Aberration Test

2. Safety Tests

2.1. Ames Test

2.1.1. Test System

The test employed four Salmonella strains, TA97, TA98, TA100 and TA102, all carrying mutation(s) in the operon encoding for histidine biosynthesis. When these bacteria are exposed to mutagenic agents, under certain conditions reverse mutation from amino acid (histidine) auxotrophy to prototrophy occurs, giving colonies of revertants. In order to test the mutagenic toxicity of metabolised products, S9 fraction, a rat liver extract, was used in the assays. The S9 fraction contains a mixture of several enzymes and is known to be able to convert some chemicals into mutagens. SERVA DNA Stain G was dissolved in the sterile distilled water, and the dosages were 0, 2.5, 5, 10 and 20 mg/ml, respectively. Preliminary assays were performed. Five concentrations were tested for cytotoxicity and mutation induction with three plates each, the dosages were 0, 250, 500, 1000 and 2000 µg/plate, respectively. The control groups included blank control plates, solvent control plates (sterile distilled water) and positive control plates. In the absence of S9 mix, the positive reference for strains TA97 and TA98 was 2,4,7-Trinitro-9-fluorenone, and for TA100 and TA102 was MMS (Methyl methanesulfonate).

In the presence of S9 mix, the positive reference for strains TA97, TA98 and TA100 was AF-2 (Aminofluorene), and for TA102 was 2-Hydroxyanthraquinone.

2.1.2. Test Procedure

The test substance and 0.1 ml nutritive broth (promote the growth of bacteria) were orderly added into the tubes containing 2 ml agar in the top layer, melting and incubating in 42 – 45 °C. For the tests with metabolic activation, 0.3 ml S9 mixture was added to the overlay agar after the addition of the bacteria and the test substance. The contents of each tube were mixed and poured over the surface of a selective agar plate. Overlay agar was allowed to solidify. All plates were incubated at 37 °C for 48 h. At the end of the incubation, revertant colonies were counted. All plating was done in triplicate. The number of revertant colonies of the test sample twice or more higher than spontaneous revertant colony plate counts indicates a positive result for mutagenicity.

2.1.3. Test Result

SERVA DNA Stain G was tested at the following concentrations: 0, 250, 500, 1000 and 2000 µg/plate. According to the result, in absence of metabolic activation system S9 mix, the numbers of revertant colonies of strains TA97, TA98, TA100 and TA102 were close to the spontaneous revertant colony plate counts. In presence of metabolic activation S9 mix, the numbers of revertant colonies of strains TA97 and TA100 were close to the spontaneous revertant colony plate counts. And those of the strain TA98 and TA102 were increased, but the increase in the number of revertants were not higher twice the spontaneous revertant colony plate counts, and there was no dose-response relationship (Table 1). Appropriate reference mutagens were used as positive controls and showed a distinct increase of induced revertant colonies (Table 1). In conclusion, it can be stated that the test item SERVA DNA Stain G is considered to be non-mutagenic.

Table 1. Results of SERVA DNA Stain G Ames test (x ± SD)

Dose Level [µg/plate]	TA97		TA98		TA100		TA102	
	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
Blank control	136 ± 10	118 ± 9	33 ± 3	33 ± 4	141 ± 25	153 ± 16	212 ± 34	313 ± 33
Water	132 ± 9	121 ± 13	33 ± 3	34 ± 3	145 ± 13	143 ± 7	218 ± 14	313 ± 21
250	121 ± 14	160 ± 19	34 ± 2	59 ± 7	178 ± 8	178 ± 7	268 ± 60	369 ± 28
500	114 ± 9	170 ± 12	34 ± 3	50 ± 8	166 ± 14	167 ± 16	312 ± 10	553 ± 87
1000	123 ± 11	180 ± 23	32 ± 3	46 ± 6	124 ± 17	180 ± 8	296 ± 36	525 ± 86
2000	98 ± 8	174 ± 10	30 ± 2	49 ± 9	128 ± 38	173 ± 28	303 ± 25	524 ± 35
Positive control	3014 ¹	2754 ²	2358 ¹	5176 ²	1737 ³	2876 ²	2175 ³	932 ⁴

¹: 2,4,7-Trinitro-9-fluorenone, 0.2 µg/plate

²: AF-2, 20 µg/plate

³: MMS, 3 µl raw liquid

⁴: 2-Hydroxyanthraquinone, 50 µg/plate

x is the mean of revertant colony counts of the 6 plates

2.1.4. Conclusion

SERVA DNA Stain G, was tested for retro-mutation of Salmonella typhimurium (Ames test). Negative result is obtained and SERVA DNA Stain G is considered to be non-mutagenic.

2.2. Mouse Bone Marrow Micronucleus Test

2.2.1. Test System

This test was performed to access the mutagenic properties of SERVA DNA Stain G by means of the micronucleus test in bone marrow cells of the mouse on the incidence of micronuclei of bone marrow polychromatic erythrocytes of the mouse. The micronucleus test is one of the most widely applied short term test used in genetic toxicology and has become one of the most important tests to evaluate the genotoxic potential of new chemical compounds. The test detects agent-induced chromosomal damage or damage of the mitotic spindle apparatus.

The test item SERVA DNA Stain G was dissolved in distilled water, and the dosages were 12.5, 25 and 20 mg/ml, respectively. The animals used in the test were healthy Kunming mice. Quality of the mice was observed for 4 days and the healthy animals were selected for the test. All animals were housed in clean animal room and the cage is of stainless steel, in which the mice took food and water freely. The room temperature was between 22 – 26 °C and the relative atmospheric humidity was between 40 – 60 %.

According to the information from the acute oral toxicity study of the test item, LD50 of male and female mice is both more than 5000 mg/kg. Thus, the dosages for the test groups were designed to be 1/5, 1/10, and 1/20 of LD50, and there were two control groups including one solvent control group and one positive control group.

2.2.2. Test Procedure

25 healthy Kunming mice of each gender were used, whose initial body weight ranged from 25 – 30 g. Mice of each gender were divided randomly into five groups, 5 animals for each group. During the test period, mice in test groups were administrated orally with the test item twice with 24 h interval. The dosage for test groups were 250, 500 and 1000 mg/kg, respectively, the positive control group was administrated orally with Cyclophosphamide (40 mg/kg), and the negative control group was administrated orally with salad oil (10 ml/kg). At the interval of 6h after the second administration, the mice were sacrificed by cervical dislocation. The marrow in sternum of the mice was taken after their death, and the marrow suspension was placed on microscopic slides. The slides were stained with Giemsa's and examined under the microscope. 1000 polychromatic erythrocyte (PCE) were observed for each animal. The number of cells with micronucleus was counted.

2.2.3. Test Result

1000 polychromatic erythrocyte (PCE) were observed for each animal, the incidences of micronuclei of the test groups were 2.0 ‰, 2.2 ‰ and 2.4 ‰ for female, and 2.4 ‰, 2.6 ‰ and 2.6 ‰ for male mice, respectively (Table 2). The incidence of micronuclei of the solvent control was 1.6‰ for male mice and 1.4 ‰ for female, and that of the positive control was 40.60 ‰ for male and 35.60 ‰ for female, which were standards-compliant. Compared with the solvent control group and analyzed statistically, there was no significant difference in the incidence of micronuclei between the test groups of SERVA DNA Stain G and solvent control.

Table 2. Data of micronucleus test in bone marrow polychromatic erythrocytes of the mouse with SERVA DNA Stain G.

Sex	Group	Dose (mg/kg)	PCEs	PCEs with micronuclei	The incidences of micronuclei	P
Male	SERVA DNA Stain G	250	5000	12	2.4 ± 0.55	> 0.05
		500	5000	13	2.6 ± 0.55	> 0.05
		1000	5000	13	2.6 ± 0.55	> 0.05
	Distilled water	10 ml	5000	8	1.6 ± 0.89	-
	Cyclophosmamide	40	5000	203	40.6 ± 4.16	< 0.01
Female	SERVA DNA Stain G	250	5000	10	2.0 ± 1.00	> 0.05
		500	5000	11	2.2 ± 0.45	> 0.05
		1000	5000	12	2.4 ± 0.89	> 0.05
	Distilled water	10 ml	5000	7	1.4 ± 0.55	-
	Cyclophosmamide	40	5000	178	35.6 ± 2.30	< 0.01

2.2.4. Conclusion

The result of micronucleus test in bone marrow polychromatic erythrocytes from the mouse with SERVA DNA Stain G is negative.

2.3. Chromosome Aberration Test

2.3.1. Test System

The mammalian in vivo chromosome aberration test is used for the detection of structural chromosome aberrations induced by the test substance to the bone marrow cells of animals. This in vivo assay was performed to access the potential of SERVA DNA Stain G to induce chromosomal aberrations in mice testicular spermatocytes.

SERVA DNA Stain G was dissolved in distilled water, and the dosages were 12.5, 25 and 50 mg/ml, respectively. The animals used in the test were 30 Kunming male, whose initial body weight ranged from 25 - 30 g. Quality of the mice was observed for 4 days before the experiment and the healthy animals were used in the experiment. All animals were housed in clean animal room and the cage is of stainless steel, in which the mice tool food and water freely. The room temperature was between 22 – 26 °C and the relative atmospheric humidity was between 40 to 60 %.

2.3.2. Test Procedure

The mice were divided into five groups (5 mice per group) at random, including three test groups, one positive control group and one negative control group. The route of administration was intragastric administration. The dosages of three test groups were 250, 500 and 1000 mg/kg (1/5, 1/10 and 1/20 of LD₅₀), the negative control group was administrated with peanut oil (10 ml/kg) and the positive control group was administrated with Cytophoshamide (40 mg/kg). The frequency of administration is once a day for 5 days consecutively. At the interval of 14 days after the last treatment, the mice were sacrificed by cervical dislocation, and injected with Colchicine (8 mg/kg) intraperitoneally at 6 h before sacrificed. The bilateral testes of the mice were taken after their death. After removal of bloodiness, the testes were put into 1 % Trisodium salt of citrated acid solution. Testes were decapsulated by ophthalmic tweezers and the spermiducts were separated. And the separated spermiducts were treated in low osmotic solution for 30 min at room temperature. Then the low osmotic solution was removed, and the spermiducts were fixed in a solution conforming methanol: glacial acetic acid (3:1) for 20 min. After that, the fixing solution was removed and the spermiducts were softened in the 2 ml of 60 % glacial acetic acid. After softened, the doubled volume of fixing solution was added in the glacial acetic acid. Then the spermiducts were beaten up uniformly and moved into the centrifuge tubes to be centrifuged at the rotation speed 1000 r/min for 10 min. The centrifuge was repeated twice. After centrifuge, all the supernatant except 1 ml was removed and the left supernatant was mixed with the cell precipitation uniformly, so that the cell suspension was prepared for the next step. The cell suspension was dropped on the cold slides uniformly and 4 slides were prepared for each mouse. The slides were dried in the air and then stained by Giemsa. For each mouse, 100 spermatocytes in the metaphase of mitosis of each mouse were observed under microscope.

Numbers and types of sex chromosomes and euchromosome aberration were observed and counted. Then the rates of aberration were calculated. The results were analyzed statistically with X^2 test and the significance level was $P < 0.05$.

2.3.3. Test Result

The chromosome aberration rates of the test groups were 1.8 %, 1.8 % and 2.2 %, respectively (Table 3); that of the solvent control group was 1.8 % and the positive control group was 5.6 %, which were standards-compliant. The chromosome aberration rates of the test groups with the test item SERVA DNA Stain G were compared with those of the solvent control group and analyzed statistically with X^2 test and the statistics result indicated that there was no significant difference between the test groups and the solvent control group ($P > 0.05$).

Table 3. Results of chromosome aberration test in mice testicular spermatocytes with SERVA DNA Stain G.

Group	Number of animals	Cells observed	Cells with chromosome aberration	Chromosome aberration rate (%)	P
Negative control	5	500	9	1.8	
250 mg/kg	5	500	9	1.8	> 0.05
500 mg/kg	5	500	9	1.8	> 0.05
1000 mg/kg	5	500	11	2.2	> 0.05
Positive control	5	500	28	5.6	< 0.01

2.3.4. Conclusion

The result of chromosome aberration test in mice testicular spermatocytes with SERVA DNA Stain G is negative.

3. OTHER INFORMATION

This information is believed to be accurate and represents the best information currently available to us. However, this shall not constitute a guarantee for any specific product features and shall not establish a legally valid contractual relationship. SERVA Electrophoresis GmbH is not held liable for any damage resulting from handling or from contact with the above mentioned products.