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 four tests:

a. Ames test
b. Comparison of SERVA DNA Stain G and Ethidium bromide by the Ames test
c. Mouse bone marrow micronucleus test
d. Chromosome Aberration Test

2. Safety Tests

2.1. Ames Test

2.1.1. Test System

The Ames test employed four Salmonella strains, TA97, TA98, TA100 and TA102. 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 concentration was 0, 2.5, 5, 10 and 20 mg/mL, respectively. The test volume was 0.1 mL per plate.

Preliminary assays were performed. 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 control 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 control reference substance for strains TA97, TA98 and TA100 was AF-2 (Aminofluorene), and for TA102 was 2-Hydroxyanthraquinone.

2.1.2. Test Procedure

The test substance, 0.1 mL bacterial suspension and 0.3 mL S9 mixture (for the tests with metabolic activation) were mixed uniformly in the test tubes with 1.5 mL overlay agar (liquid, 45 °C). The mixture was uniformly poured on the surface of a selective agar plate. After solidification, the plates were incubated upside down 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 by an independent licensed testing laboratory at the following concentrations: 0, 250, 500, 1000 and 2000 µg/plate. According to the result of Ames test, 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 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. The numbers of revertant colonies of strains TA98 and TA102 were increased, but did not exceed as twice as those of the spontaneous revertant colonies, and there was no dose-response relationship (Table 1). Appropriate reference mutagens were used as positive controls and they 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
Negative 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

According to the guidelines, negative result was obtained and SERVA DNA Stain G is considered to be non-mutagenic.

2.2. Comparison of SERVA DNA Stain G and Ethidium bromide by the Ames test

2.2.1. Test System

The Ames test was used to compare the mutagenicity of SERVA DNA Stain G and Ethidium bromide. Test was carried out by an independent testing laboratory using one *Salmonella typhimurium* strain TA98 (detects frame-shift mutagens). For the Ames test the OECD guideline (OECD 471, 1997) was followed using the plate incorporation method as described by Maron and Ames (1983)[1]. For both compounds, 9 doses were analysed in presence as well as in absence of exogenous metabolic activation (S9/Cofactor mixture), along with negative control (to detect spontaneous revertants) and positive controls (see below). S9 prepared from the liver of rodents treated with Aroclor 1254 was used (purchased from In vitro Technologies, US).

[1] Maron, D.M. and Ames, B. N. (1983). Revised methods for the Salmonella mutagenicity test. *Mutation Research* 113, 173–215.

2.2.2. Test Procedure

SERVA DNA Stain G and Ethidium bromide were tested under the same conditions. Deionised water (MilliQ, Millipore) was used for dissolving the stock of both dyes (concentration 10 mg/mL) to yield the following concentrations (µg/mL): 0 (negative control), 1, 2.5, 5, 10, 25, 50, 75, 100, 250, and 500. The following was added to each sterile culture tube containing 2.0 mL top agar: 0.1 mL of the overnight cell culture (TA98), 0.1 mL of certain concentration of dye or positive control, and either 0.5 mL of S9/Cofactor mix or 0.5 mL of Phosphate buffered saline. Using the above 10 concentrations for both dyes, the following per plate doses were used: 0, 0.1, 0.25, 0.5, 1, 2.5, 5, 7.5, 10, 25, and 50 µg per plate. Three plates for each concentration of both dyes and positive controls were tested. Positive controls (dissolved in DMSO) were 4-nitro-1,2-phenylene diamine (0.5 µg/plate) used in the absence of S9 and 2-aminoanthracene (3 µg/plate) used in the presence of S9. The tubes were vortexed and poured onto Vogel-Bonner media plates, and evenly distributed. The culture plates were incubated at 37 °C in the dark for 72 hours. Induced and spontaneous revertant colonies per plate were counted.

2.2.3. Test Result

The results of the mutagenicity assay of SERVA DNA Stain G and Ethidium bromide are presented in Table 2 and Fig. 1. Table 2 shows the mean number of revertants per plate and the mutagenicity ratio (MR). MR is the number of revertant colonies growing in the presence of a test sample divided by the number of spontaneous revertant colonies growing in the absence of the test samples (negative control). By the definition of Ames test, a compound is considered mutagenic when MR ≥ 2. Figure 1 illustrates the dose response of strain (TA98) as MR of tested dyes.

Table 2. Number of revertants of *S. typhimurium* TA98 and mutagenicity ratio (MR) of SERVA DNA Stain G and Ethidium bromide (EB).

Dose level (µg/plate)	Number of revertants[a] and mutagenicity ratio (MR)			
	Without metabolic activation (-S9)		With metabolic activation (+S9)	
	SERVA DNA Stain G	EB	SERVA DNA Stain G	EB
Negative control	38 ± 2		45 ± 5	
0.10	34 ± 1 (0.9)	26 ± 1 (0.7)	85 ± 1 (1.9)	149 ± 4 (3.3)
0.25	37 ± 4 (1.0)	26 ± 4 (0.7)	101 ± 10 (2.2)	205 ± 5 (4.5)
0.50	37 ± 3 (1.0)	37 ± 2 (1.0)	134 ± 2 (3.0)	319 ± 12 (7.1)
1.00	41 ± 4 (1.1)	33 ± 4 (0.9)	178 ± 8 (4.0)	441 ± 6 (9.8)
2.50	49 ± 2 (1.3)	33 ± 3 (0.9)	382 ± 23 (8.5)	703 ± 67 (15.6)
5.00	55 ± 9 (1.4)	37 ± 5 (1.0)	456 ± 66 (10.1)	1096 ± 108 (24.4)
7.50	70 ± 6 (1.8)	32 ± 6 (0.8)	472 ± 88 (10.5)	805 ± 74 (17.9)
10.00	67 ± 13 (1.8)	35 ± 8 (0.9)	458 ± 12 10.2)	817 ± 15 (18.1)
25.00	63 ± 10 (1.7)	39 ± 1 (1.0)	241 ± 46 (5.3)	695 ± 80 (15.5)
50.00	60 ± 12 (1.6)	35 ± 4 (0.9)	244 ± 66 (5.4)	504 ± 43 (11.2)
Positive control[b]	134 ± 10		1600 ± 129	

[a] Mean number of revertants per plate ± standard deviation. Each dose was plated in triplicate. In the brackets is the value of mutagenicity ratio (MR).
[b] Positive controls (dissolved in DMSO) were 4-nitro-1,2-phenylene diamine (0.5 µg/plate) used in the absence of S9 and 2-aminoanthracene (3 µg/plate) used in the presence of S9. Quality control criteria were passed in both cases, i.e. the number of induced revertants per plate was in the range indicated by Maron and Ames (1983)[1].

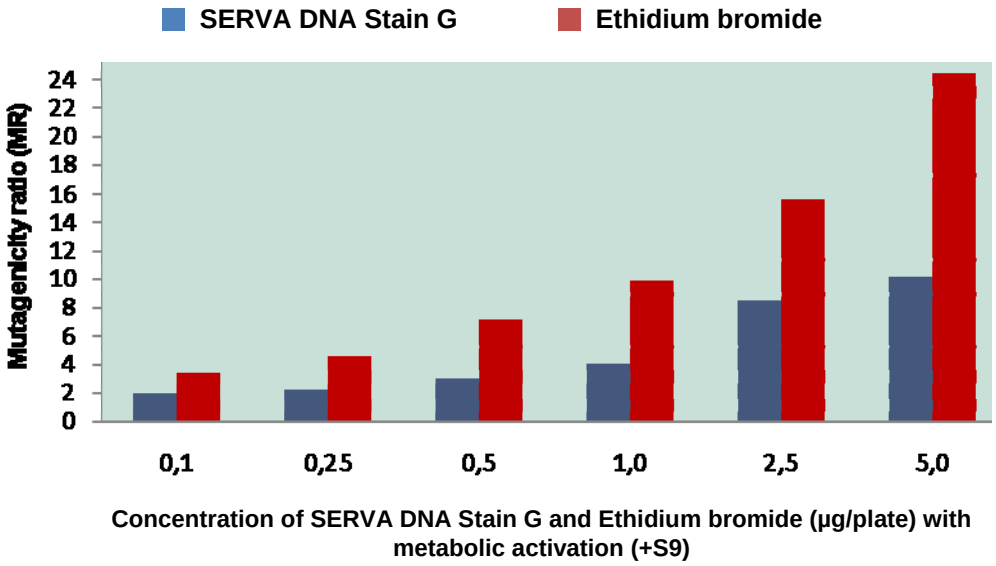

Fig. 1. Mutagenicity ratio of SERVA DNA Stain G and Ethidium bromide with metabolic activation (S9) in TA98 mutagenicity assay. Data are plotted from Table 2.

According to the results of positive and negative controls (Table 2) the assay passed the respective quality control criteria. In absence of metabolic activation system S9 mix, MR<2 was detected at all the tested concentrations of both dyes (Table 2).

In presence of metabolic activation (+S9), a clear dose-dependent increase of the number of revertants was noted (doses ≥7.5 µg per plate were probably toxic to the strain TA98 demonstrated by the decrease in the number of revertants and decreasing mutagenicity ratio). Starting from the concentration 0.25 µg per plate, the mutagenicity ratio increased *more than 2-fold* in case of Ethidium bromide compared to SERVA DNA Stain G (Table 2 and Fig.1).

2.2.4. Conclusion

According to the results of the Ames test, SERVA DNA Stain G is significantly less mutagenic compared to Ethidium bromide.

2.3. Mouse Bone Marrow Micronucleus Test

2.3.1. Test System

This test was performed by an independent licensed testing laboratory to assess 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 concentration was 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, 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, LD_{50} of male and female mice is both above 5000 mg/kg. Thus, the dosages for the test groups were designed to be 1/5, 1/10, and 1/20 of LD_{50}, and there were two control groups including one solvent control group and one positive control group.

2.3.2. Test Procedure

25 healthy Kunming mice of each gender were used, whose initial body weight ranged from 25 to 30g. 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 24h interval. The dosages of 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 6 h after the second administration, the mice were sacrificed by cervical dislocation. The marrow in sternum of the mice was taken, and the marrow suspension was made into microscopic slides. The slides were stained with Giemsa's and examined under the microscope. 1000 polychromatic erythrocyte (PCE) was observed for each animal. The number of cells with micronucleus was counted.

2.3.3. Test Result

1000 polychromatic erythrocyte (PCE) was 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, respectively (Table 3). 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 3. 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

2.3.4. Conclusion

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

2.4. Chromosome Aberration Test

2.4.1. 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 by an independent licensed testing laboratory to assess 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 concentration was 0, 2.5, 5 10 and 20 mg/mL, respectively. The animals used in the test were 30 Kunming male, whose initial body weight ranged from 25 to 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, in which the mice took food and water freely. The room temperature was between 22 – 26 °C and the relative atmospheric humidity was from 40 to 60 %.

2.4.2. 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. Animals were administrated with test item orally daily for a period of 5 days. The dosages of three test groups were 250, 500 and 1000 mg/kg (1/5, 1/10 and 1/20 of LD_{50}), the negative control group was administrated with peanut oil (10 mL/kg) and the positive control group was administrated with Cytophoshamide (40 mg/kg). 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 and 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 minutes 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 glacial acetic acid (2 mL of 60 %). 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 minutes twice. All the supernatant except 1 mL was removed and the left supernatant was mixed with the cell precipitation uniformly. 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 0.05.

2.4.3. Test Result

The chromosome aberration rates of the test groups were 1.8 %, 1.8 % and 2.2 %, respectively (Table 4); that of the solvent control group was 1.8% and the positive control group was 5.6 %. 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 the statistics result indicated that there was no significant difference between the test groups and the solvent control group (P>0.05).

Table 4. 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

2.4.4. Conclusion

According to the guidelines, 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.