MIDORI^{Green} Advance DNA Stain

Safety Report

IDENTIFICATION OF THE PRODUCT AND OF THE COMPANY

Product name	MIDORI^{Green} Advance DNA Stain
Catalog numbers	MG03 MG04
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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.

Midori^{Green} Advance DNA Stain 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 Midori^{Green} Advance DNA Stain has been controlled with five tests:

I. Ames Test
II. Cytotoxicity Test
III. The Mouse Bone Marrow Micronucleus Test
IV. Chromosome Aberration Test
V. Latex And Nitrile Gloves Penetration Test

I. AMES TEST

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.

Midori^{Green} Advance DNA Stain was dissolved in the sterile distilled water, and the concentrations were 0.5, 1.0, 2.5 and 5 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 mixture, the positive control reference for strains TA97 and TA98 was 9-fluorenone, for TA100 was NaN3, and for TA102 was Mitomycin C. In the presence of the S9 mixture, the positive control reference substance for strains TA97, TA98 and TA100 was 2-AF (Aminofluorene), and 1,8-hydroxyanthraquinone for TA102.

2. Test Procedure

The test substance (0.1 mL) and 0.1 mL bacterial suspension with 0.5 mL S9 mixture (+S9) or without S9 mixture (-S9) were mixed uniformly in test tubes with 1.5 mL overlay agar (liquid, 45°C). 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..

3. Test Result

According to the results of the Ames test (Table 1), in the presence and absence of metabolic activator S9 the increase in the numbers of revertant colonies of strains TA97, TA98, TA100 and TA102 compared to spontaneous revertant colonies was less than 2 times, and there was no dose-response relationship. Appropriate reference mutagens were used as positive controls and they showed a distinct increase of induced revertant colonies (Table 1).

Table 1. Results of Midori^{Green} Advance DNA Stain Ames test (± SD)

Dose level (mg/plate)	TA97		TA98		TA100		TA102	
	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
Neg. control	135 ± 12	156 ± 5	30 ± 1	32 ± 1	136 ± 21	155 ± 11	247 ± 10	275 ± 14
Water	132 ± 16	155 ± 2	30 ± 2	32 ± 1	146 ± 10	158 ± 19	248 ± 7	275 ± 15
0.5	142 ± 9	141 ± 14	31 ± 1	32 ± 2	137 ± 17	154 ± 13	261 ± 9	266 ± 10
1	130 ± 5	151 ± 13	30 ± 1	31 ± 2	140 ± 14	168 ± 10	251 ± 8	258 ± 6
2.5	138 ± 13	151 ± 7	30 ± 1	31 ± 1	142 ± 12	167 ± 7	259 ± 13	254 ± 5
5	136 ± 10	159 ± 2	31 ± 2	32 ± 1	152 ± 10	172 ± 28	254 ± 15	282 ± 6
Pos. control	1475 ± 105[1]	1519 ± 161[2]	2244 ± 36[1]	2306 ± 234[2]	1283 ± 26[3]	1338 ± 166[2]	1464 ± 156[4]	1562 ± 285[5]

[1] 9-Fluorenone, 0.2 µg/plate
[2] 2-AF, 10 µg/plate
[3] NaN3, 2.5 µg/plate
[4] Mitomycin C, 4.0 µg/plate
[5] 1,8-Hydroxyanthraquinone, 50 µg/plate

4. Conclusion

According to the guidelines, negative result was obtained and a significant mutagenic effect of Midori^{Green} Advance DNA Stain could not be detected.

II. THE ACUTE ORAL TOXICIRY TEST

1. Test System

The acute toxicity testing provides information on the biologic activity of the test item that can be used in hazard identification and risk management in the context of production, handling, and use of chemicals. The LD50 value, defined as the statistically derived dose that, when administered in an acute toxicity test, is expected to cause death in 50% of the treated animals in a given period, is the basis for toxicologic classification of chemicals.

Sample: Original liquid of Midori^{Green} Advance DNA Stain.

Animals: The animals used in the test were healthy Kunming mice, body weight were from 18 g to 22 g. Before testing, all animals were fasting but water was ad libitum. All animals were housed in clean animal room (CL). The room temperate was from 18 °C to 22 °C and the relative atmospheric humidity was from 45 to 65%.

2. Test Procedure

Dose Design: According to Horn's Method, the dosages for the test groups were designed to be 1000, 2150, 4640 and 10000 mg/kg. The mice were divided into 4 groups (5 animals/sex/group) at random. Animals were administrated with test item by oral gavage, calculate the amount of exposure according to 0.2 ml/10 g.

Outcome Measures: After exposure, poisoning symptoms and death situations were observed, the observation period was two weeks. In case no deaths are observed within two weeks, the conclusion can be drawn that the LD50 is above 10000 mg/kg.

3. Test Result

Table 2. Results of Acute Oral Toxicity Test with Midori^{Green} Advance DNA Stain.

Sex	Dose (mg/kg)	No. of Animals	No. of Dead Animals	Death Rate (%)
Female	1000	5	0	0
	2150	5	0	0
	4640	5	0	0
	10000	5	0	0
Male	1000	5	0	0
	2150	5	0	0
	4640	5	0	0
	10000	5	0	0

4. Conclusion

After exposure, poisoning symptoms or deaths were not detected. That means LD50 is above 10000 mg/kg and the test item Midori^{Green} Advance DNA Stain was nontoxic.

III. THE MOUSE BONE MARROW MICRONUCLEUS TEST

1. Test System

This test was performed to assess the mutagenic properties of Midori^{Green} Advance DNA Stain 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.

Sample: Original liquid of Midori^{Green} Advance DNA Stain.

Positive Control: Mitomycin C.

Animals: The animals used in the test were healthy Kunming mice (body weight from 25 g to 30 g).

2. Test Procedure

Mice in test groups were administrated orally with the test item twice in 30 hours. The dosages of test groups were 1000, 2000 and 5000 mg/kg, respectively. The negative control was administrated with distilled water and the positive control group was administrated with Mitomycin C (1.5 mg/kg). In each group, five males and five female mice were used. At the interval of 6h after the second administration, the mice were sacrificed. 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 erythrocytes (PCE) were observed for each animal. The number of cells with micronucleus was counted.

3. Test Result

Table 3. Data of the micronucleus test in bone marrow polychromatic erythrocytes of the mouse with Midori^{Green} Advance DNA Stain.

Sex	Group	Dose (mg/kg)	No. of Animals	PCEs	PCEs with micronuclei	The incidences of micronuclei	*P*
Female	Midori^{Green} Advance	1000	5	5000	4	0.80 ± 0.45	>0.05
		2000	5	5000	4	0.80 ± 0.45	>0.05
		5000	5	5000	3	0.60 ± 0.55	>0.05
	Distilled Water	-	5	5000	4	0.80 ± 0.45	-
	Mitomycin C	1.5	5	5000	135	27.0 ± 1.22	<0.01
Male	Midori^{Green} Advance	1000	5	5000	4	0.80 ± 0.45	>0.05
		2000	5	5000	4	0.80 ± 0.45	>0.05
		5000	5	5000	3	0.60 ± 0.55	>0.05
	Distilled Water	-	5	5000	3	0.60 ± 0.55	-
	Mitomycin C	1.5	5	5000	123	24.6 ± 3.05	<0.01

4. Conclusion

The results showed that there was no significant difference (P>0.05) in the incidence of micronuclei between the test groups and negative control while there was significant difference (P<0.01) between Mitomycin C and negative control. Therefore, according to the guidelines the mouse marrow chromophilous erythrocyte micronucleus test with Midori^{Green} Advance DNA Stain was negative.

IV. THE IN VITRO MAMMALIAN CHROMOSOME ABERRATION TEST

1. Test System

The purpose of the in vitro chromosomal aberration test is to identify agents that cause structural chromosome aberrations in cultured mammalian cells. This test was performed to assess the potential of Midori^{Green} Advance DNA Stain to induce chromosomal aberrations in vitro.

Cell strains: Chinese hamster ovary line.
Metabolic activation system: S9 mixture, which is rat liver homogenate induced by both Phenobarbital sodium and Naphthaflavone and some appropriate cofactors.
Test compound: 5.0 mg/ml of MEM stock solution, separately diluted to different concentration by serum-free culture solution (3 hours, presence and absence of S9 mixture) and MEM medium supplemented with 10% fetal bovine serum (24 hours, absence of S9 mixture).

2. Test Procedure

Culture solution: MEM medium supplemented with 10% fetal bovine serum and 100 IU/ml of penicillin and streptomycin.
Maximum final concentration determination: It is shown that the metabolic activation way is +S9 and -S9, and the action time are 3 hours through two-time preliminary experiments. We also confirmed the highest final concentration of the -S9 metabolic activation responding for 24 hours at the two different conditions. Both of the two preliminary experiments contain a test group and a blank control group. Measuring cell activity with resazurin, and determine the final concentration according to cell inhibiting rate.
Chromosome aberration test: Two kinds of test condition, one kind of it is metabolic activation +S9 and –S9, action time is 3 hours, another one is –S9, action time 24 hours.
Cell harvesting: 4 hours before harvesting, 1.0 ug/ml colchicines were added followed by hypotonic treatment, fixation, tabletting and dyeing with Giemsa. 200 normal metaphase cells were selected in test compound and the blank control set, and 100 normal metaphase cells were selected in the positive control set. For the analysis the chromosome aberration type and number were recorded and the chromosome aberration rate was calculated.
Statistical analysis: $\chi 2$ analysis was performed to compare the chromosome aberration rate of each test compound and chromosome aberration rate of negative control set.

3. Test Result

Table 4. The results of in vitro mammalian cell chromosome aberration test (3 hours, -S9).

Group	Final concentration (µg/ml)	Total cell number	Cells with chromosome aberration	Aberration rate (%)
Negative control	-	200	3	1.5
Test compound	1250.0	200	2	1.0
	2500.0	200	4	2.0
	5000.0	200	1	0.5
Mitomycin C	1.0	100	11	11.0*

*P<0.01

Table 5. The results of in vitro mammalian cell chromosome aberration test (3 hours, +S9).

Group	Final concentration (µg/ml)	Total cell number	Cells with chromosome aberration	Aberration rate (%)
Negative control	-	200	1	0.5
Test compound	1250.0	200	0	0
	2500.0	200	0	0
	5000.0	200	0	0
Cyclophosphamide	15.0	100	13	13.0*

*P<0.01

Table 6. The results of in vitro mammalian cell chromosome aberration test (24 hours, -S9).

Group	Final concentration (µg/ml)	Total cell number	Cells with chromosome aberration	Aberration rate (%)
Negative control	-	200	5	2.5
Test compound	31.2	200	3	1.5
	62.5	200	2	1.0
	125.0	200	1	0
Cyclophosphamide	1.6	100	14	14.0*

*P<0.01

4. Conclusion

Under the experimental conditions, in vitro mammalian cell chromosome aberration detection system, compared to negative control group, with or without metabolic activation system, the test compound didn't lead to the higher chromosome aberration rate, so the result of in vitro mammalian cell chromosome aberration test with Midori^{Green} Advance DNA Stain is negative.

IV. LATEX NAD NITRILE GLOVES PENETRATION TEST

1. Test System

The purpose of the glove penetration tests is to test the ability of Midori^{Green} Advance DNA stain to diffuse through latex gloves (e.g. ECO Latex PF250) and nitrile gloves (e.g. Orange Nitril 260) based on Glove Penetration test.

2. Test Procedure

10 µl of 1x Midori^{Green} Advance DNA stain solution were dissolved in 200 µl TAE buffer to make the working solutions of Midori^{Green} Advance. 5 ml of TAE buffer was pipetted into latex and nitrile gloves' fingers to dialyze it against the working solutions. The dialysis was ended 10, 30, 60, 180 and 360 minutes after initiation and every time the solution from two fingers of both glove types were used for analysis. After the dialysis all solutions from fingers were preserved in 15 ml centrifuge tubes. As a reference the pure TAE buffer was also preserved in glove fingers overnight.
The solutions in the glove fingers were analyzed for presence of the dye by fluorescence (Perkin Elmer LS55). As a reference, the fluorescence of the working solutions of the dye was also measured. To increase the sensitivity of the detection, all fluorescence measurements were made in the presence of 100 µg/ml salmon sperm dsDNA.
Maximum excitation and emission wavelengths were chosen for this analysis (502 nm for excitation and 526 nm for emission). The bandwidth of excitation and emission filters were 10 nm.

3. Test Result

For Midori^{Green} Advance DNA stain the fluorescence of dialyzed buffer was less than 1 AU for nitrile glove which is at the fluorescence noise level and equals with the fluorescence of TAE buffer from latex glove. For latex glove the fluorescence intensity of dialyzed buffer was 1.5-3 AU and the fluorescence intensity of undialyzed buffer was 1.3 AU (Figure 2). The fluorescence intensity of Midori^{Green} Advance working solution at the same measuring conditions was 338 AU. So we can say that during the test time this dye does not diffuse through nitrile glove and the diffusion through the latex glove is irrelevant (<1% in 6 hrs).

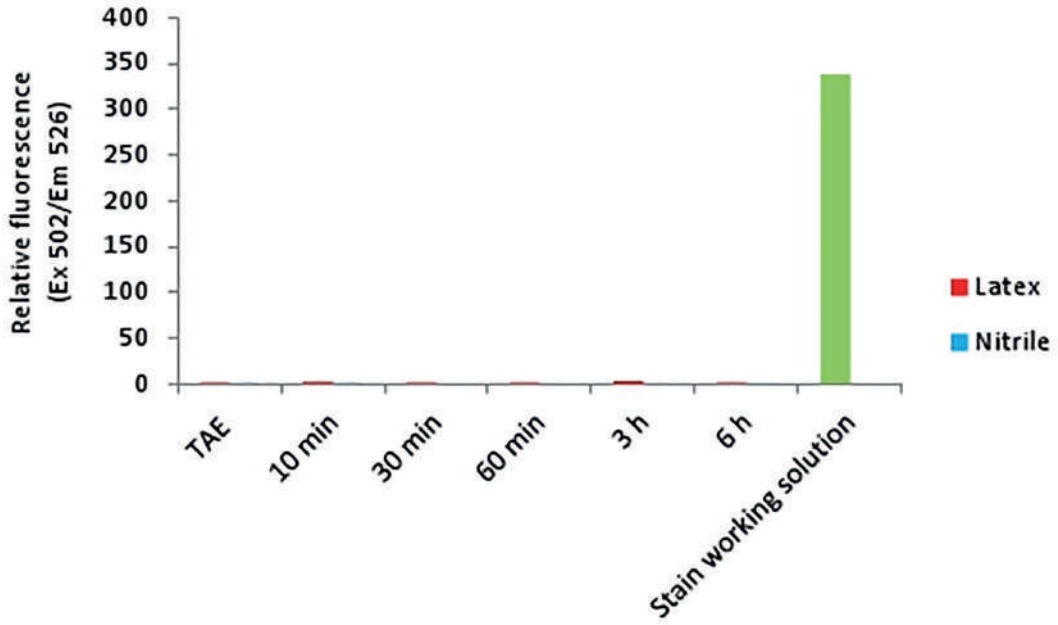

Figure 1. Relative fluorescence of solutions dialyzed in latex (red) and nitrile (blue) glove fingers against Midori^{Green} Advance DNA Stain (working solution) and the relative fluorescence of the corresponding working dye solution as a reference (green).

Other Information

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