MIDORI^{Green} Xtra DNA Stain

Safety Report

Identification of the Product and of the Company

Product name	MIDORI^{Green} Xtra DNA Stain
Catalog numbers	MG09 MG10
Supplier	NIPPON Genetics EUROPE GmbH Mariaweilerstraße 28-30 52349 Düren, Germany Phone: +49 2421 554960 E-mail: info@nippongenetics.eu

Introduction

Ethidium bromide (EB) 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} Xtra DNA Stain is a nucleic acid stain which can be used as a safe alternative to the traditional Ethidium bromide (EB) stain for detecting nucleic acid in agarose gels. It is flexible in use for both in-gel and post staining. Midori^{Green} Xtra is as sensitive as EB and can be used in the exact same way for agarose gel electrophoresis as EB. With this report, we will show that Midori^{Green} Xtra is safe for the user and the DNA dye is also not dangerous for the environment. Since this dye can be used with Blue/Green LED light or blue light instead of UV light for DNA visualization, it is also harmless to your experiments. Moreover, it does not interfere with downstream applications.

Table 1. Summary of Midori^{Green} Xtra DNA Stain safety test results.

Test	Results
I) Hazardous waste screening (aquatic toxicity test)	Non-toxic to aquatic life at working concentration
II) Cloning efficiency test	Possibility to use blue light instead of UV light
III) Mammalian cell viability/ cytotoxicity test	Non-cytotoxic and non-cytostatic at working concentration
IV) AMES test	No mutagenic effect

I. Hazardous waste screening (aquatic toxicity test)

Daphnia magna acute immobilisation test

Purpose:

This test assesses the acute toxicity of Midori^{Green} Xtra DNA Stain to aquatic life.
The results of the test are used to determine if the dye can be directly released into the environment for disposal.

Method:

OECD Guideline for Testing of Chemicals. *Daphnia sp.*, Acute Immobilisation Test 202. Adopted 13 April, 2004.
The OECD 202 test is one of 3 the most relevant and internationally agreed ecotoxicity testing method used to identify and characterise potential hazards of chemicals to the environment.

System:

Test organism: *Daphnia magna*
Test duration: 48 hours
Compound test concentration:
0.0003%-0.01%

Results:

Results are summarized in Table 2 and Figure 1 below. At working concentration (0.00016%-0.00032%) Midori^{Green} Xtra DNA Stain does not pose a risk to aquatic organisms since significant mortality of *Daphnia magna* was not observed during 48 hours.

Conclusion:

At working concentration (0.00016%-0.00032%) Midori^{Green} Xtra DNA Stain does not pose a risk to environment.
All working solutions can be disposed in regular laboratory waste, according to local regulation.

Table 2. *Daphnia magna* immobilisation (an average value ± standard deviation (n=2)) at 2 timepoints at different Midori^{Green} Xtra DNA Stain concentrations.

Midori^{Green} Xtra concentration [%]	*Daphnia magna* immobilisation [%]	
	24 h	48 h
0.0003	0 ± 0	0 ± 0
0.0006	5.0 ± 0	13.0 ± 4.2
0.00125	15.0 ± 21.8	51.7 ± 37.5
0.0025	45.0 ± 31.2	70.0 ± 30.0
0.005	65.3 ± 32.9	95.0 ± 5.0
0.01	100 ± 0	100 ± 0

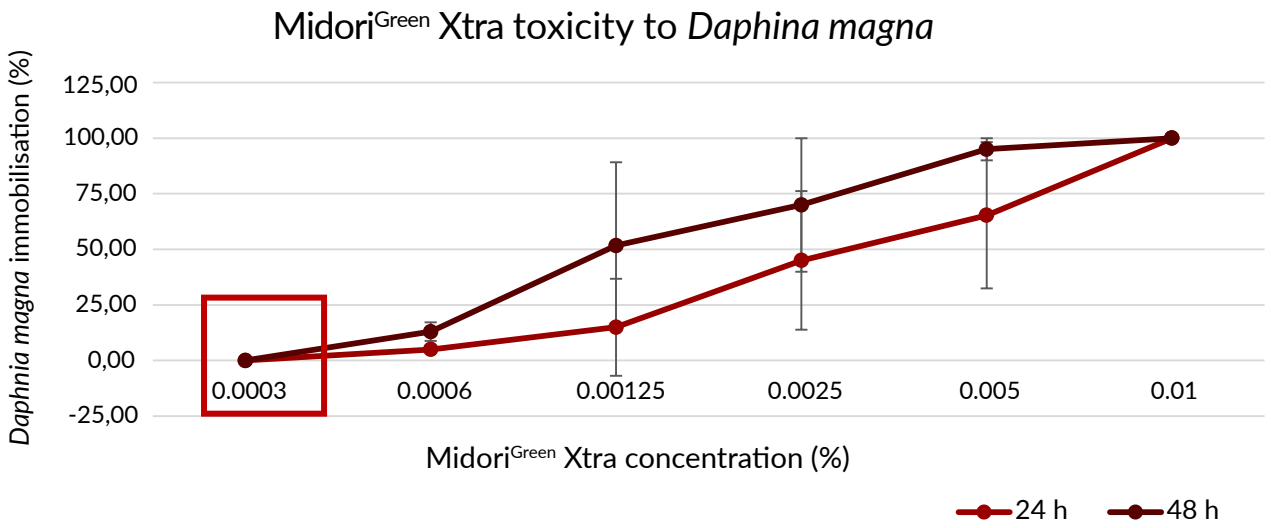

Figure 1. *Daphnia magna* 24-and 48-hours incubation dose-dependent graph (average value ± standard deviation (n=2)).

II. Cloning efficiency

Purpose:
To find out how much the cloning efficiency increases if the blue light transilluminator is being used (instead of the UV transilluminator) for visualization of DNA fragments when cutting those fragments out from the gel during the cloning process.
To find out if Midori^{Green} Xtra DNA stain inhibits the growth of bacteria during cloning process.

System:
The used basic vector was BAEU6 (3200 bp). The used wavelength for UV was 315 nm and for blue light 470 nm. The vectors were exposed to the light for 15, 30, 45 and 120 seconds in both treatments.

Test procedure:
The vector was restricted and separated in agarose gel electrophoresis. After being exposed to UV or blue light the DNA bands (both insert and vector part) were purified from the gel. Ligated vectors were transformed in to *E. coli* strain by using "heat shock" method and plated in to the ampicillin containing LB plates. After 16 hours at 37 °C the viable (Ampicillin resistance) bacterial colonies were counted.

Results:
The number of colonies (y-axis) after UV and blue light treatment are shown in figure 2. Exposing vector DNA to UV light for 30 sec decreased the number of colonies 1.5 times, 45 sec treatment decreased number of viable colonies 5 times. After 120 sec of treatment the number of viable colonies was less than 5% of the number of colonies treated 15 sec with UV. Exposing vector DNA to blue light for even 120 sec did not affect the number of initial colonies.

Conclusion:
The exposure to UV light declines the number of colonies drastically. In those cases, using the blue light transilluminator may increase the cloning efficiency more than 20 times.

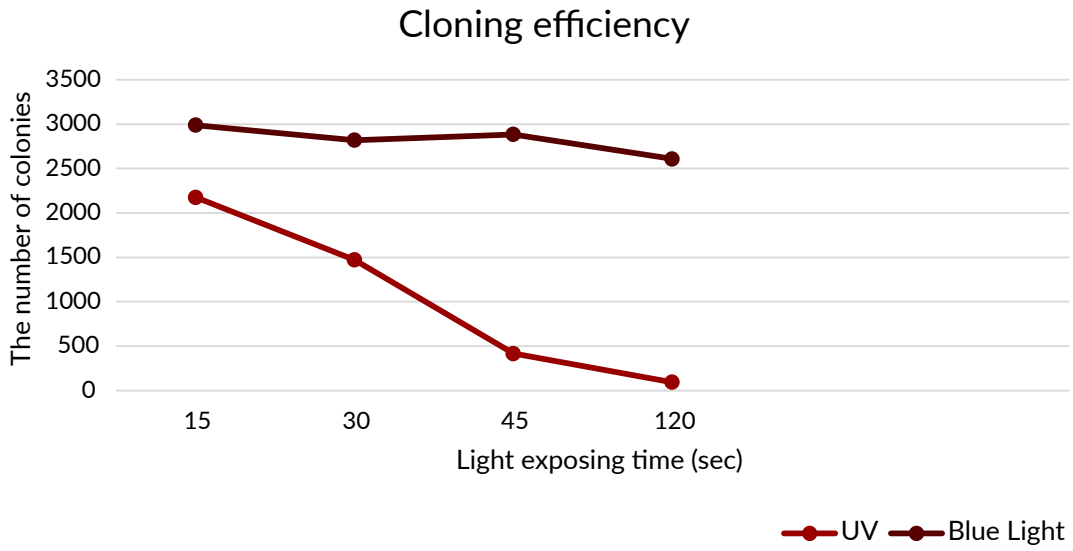

Figure 2. Amount of *E. coli* colonies after exposing transfected DNA to UV or blue light in different time points.

III. Mammalian cell viability/cytotoxicity test

Purpose:
To find out how much Midori^{Green} Xtra DNA Stain affects the viability of cells if it is not directly introduced into the cells.

Method:
Different amount of Midori^{Green} Xtra DNA Stain and EB were added to the media. HeLa cells were used for measuring mammalian cell viability. Cell viability was measured at different time points with XCelligence Roche machine.

System:
Every measured sample consisted a different amount of Midori^{Green} Xtra DNA Stain or EB: 0 µl (as a control), 0.5 µl, 5 µl, 7.5 µl per 10 ml of media. The total amount of media was 250 µl in every assay plate. HeLa cells viability at different time points was measured with XCelligence Roche machine.

Results:
Midori^{Green} Xtra DNA Stain in concentration 0.5 µl per 10 ml is not cytotoxic to HeLa cells in any timepoint. Concentrations up to 5 µl per 10 ml are rather cytostatic but not cytotoxic (Figure 3). On the other hand, all EB concentration used in this experiment are toxic to HeLa cells (Figure 4).
In the light of this experiment it can be argued that Midori^{Green} Xtra DNA Stain is not affecting further experiments in cell culture and it is also safe to the environment.

Conclusion:
At the average working concentration (0.5 µl per 10 ml) Midori^{Green} Xtra DNA Stain is not cytotoxic or cytostatic to mammalian cells.

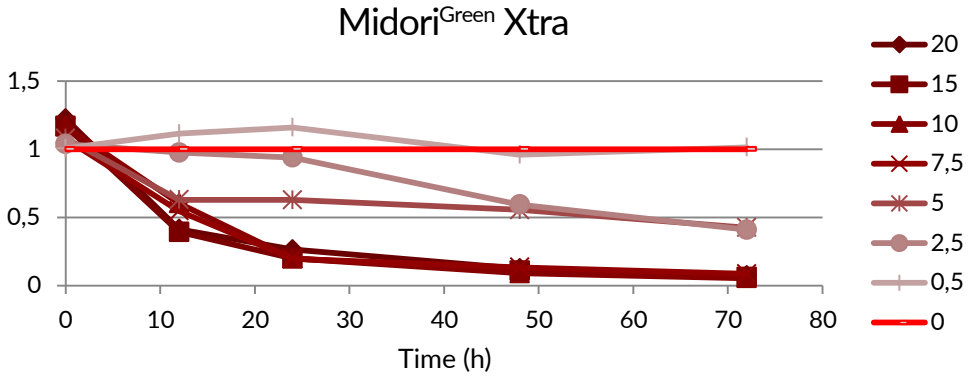

Figure 3. HeLa cells viability measured at different time points (with Midori^{Green} Xtra DNA Stain in the cells growth media).

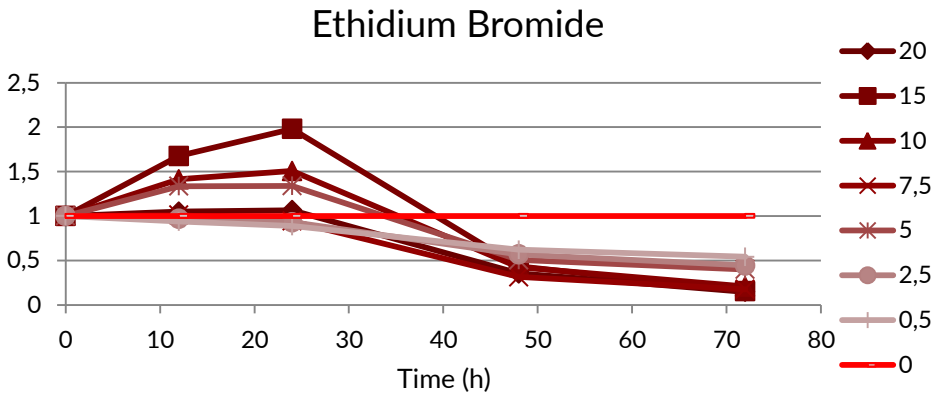

Figure 4. HeLa cells viability at different time points (with Ethidium Bromide in the cells growth media).

IV. AMES test

Purpose:
To find out how much Midori^{Green} Xtra DNA Stain has mutagenic effect to the test strain and therefore may act as a carcinogen.

Method:
The Ames test employed four Salmonella strains, TA97a, TA98, TA100, TA102 and TA1535. 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.

System:
Midori^{Green} Xtra DNA Stain was dissolved in DMSO, and the concentrations were 0.313; 0.625, 1.25, 2.5 and 5 µL/plate, respectively. The control groups included solvent control plates (DMSO) and positive control plates. 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

+ with S9
- without S9

Spot test:
The test was performed by adding test article solution on sterile paper discs, which were placed on S. typhimurium plates. Plates were incubated at 37±2°C for 24 to 48 hours and the bacteriostasis ring (cell toxicity) or circularity formed by large number of colonies (mutation) was checked.

Plate incorporation test:
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 2 mL overlay agar (liquid, 45°C, containing 0.5 mM histidine/biotin). 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. 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 higher than the average of the negative control group.

Results:

In the spot test there were no obvious bacteriostasis ring (cell toxicity) or circularity formed by large numbers of colonies (mutation) around the disc in each test group, so the 5µL/plate, 2.5µL/plate, 1.25 µL/plate, 0.625 µL/plate and 0.313 µL/plate dosage were used in plate incorporation test. This test showed 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.

Conclusion:

The results showed that the revertant numbers of the tested reagent Midori^{Green} Xtra DNA Stain appeared to be two times less than that of the negative control group in Salmonella typhimurium TA97a, TA100 and TA1535. Midori^{Green} Xtra DNA Stain causes no mutagenic effects to these Salmonella typhimurium strains. The result of the Ames test does not show a mutagenic effect of Midori^{Green} Xtra DNA Stain.

Test Strain			TA97a		TA98		TA100		TA102		TA1535	
S9			+	-	+	-	+	-	+	-	+	-
Colony Number (CFU/plate)	Negative Control (NC)	plate 1	172	84	50	57	104	135	504	492	10	11
		plate 2	187	85	55	43	114	110	528	412	11	12
		plate 3	205	93	53	49	120	91	736	388	12	16
		Avg	188	87,3	52,7	49,7	112	112	589,3	430,7	11	13
		SD	16,5	4,9	2,5	7	22,1	22,1	127,6	54,5	1	2,6
	2X Avg. Of NC		376	174,7	105,3	99,3	224	224	1178,7	861,3	22	26
	Positive Control	plate 1	412	576	168	640	300	300	1352	1648	171	246
		plate 2	418	624	161	512	272	272	1304	1480	150	262
		plate 3	426	822	152	496	264	264	1256	1464	137	269
		Avg	418,7	666,7	160,3	549,3	278,7	278,7	1304	1530,7	152,7	259
		SD	7	117,9	8	78,9	18,9	18,9	48	101,9	17,2	11,8
	Test Article Solution (µL/plate) 5	plate 1	187	107	54	51	92	92	608	488	16	10
		plate 2	208	101	46	38	93	93	664	428	12	10
		plate 3	209	91	49	35	94	94	800	380	11	10
		Avg	201,3	99,7	49,7	41,3	93	93	690,7	432	13	10
		SD	12,4	8,1	4	8,5	1	1	98,7	54,1	2,6	0
	2,5	plate 1	207	87	46	36	100	53	720	488	13	18
		plate 2	201	85	52	45	106	65	732	416	13	16
		plate 3	195	84	56	47	119	65	752	404	16	16
		Avg	201	85,3	51,3	42,7	108,3	61	734,7	436	14	16,7
		SD	6	1,5	5	5,9	9,7	6,9	16,2	45,4	1,7	1,2
	1,25	plate 1	189	52	26	30	96	65	584	484	22	10
		plate 2	148	54	30	30	96	53	608	480	16	10
		plate 3	131	70	31	34	78	50	680	464	15	11
		Avg	165	58,7	29	31,3	90	56	624	476	17,7	10,3
		SD	29,8	9,9	2,6	2,3	10,4	7,9	50	10,6	3,8	0,6
	0.625	plate 1	210	66	26	30	106	55	592	516	8	10
		plate 2	226	64	26	23	97	57	776	464	9	12
		plate 3	223	56	24	22	89	65	816	460	11	13
		Avg	219,7	62	25,3	25	97,3	59	728	480	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	plate 1	181	68	24	26	75	59	704	382	14	14
		plate 2	203	64	24	23	90	57	656	384	13	14
		plate 3	235	62	26	20	103	52	624	408	13	14
		Avg	206,3	64,7	24,7	23	89,3	56	661,3	388	13,3	14
		SD	27,2	3,1	1,2	3	14	3,6	40,3	18,3	0,6	0

Colony Number (CFU/plate)