7040 Virginia Manor Road
Beltsville, MD 20705, USA
Web: www.abpbio.com
E-Mail: info@abpbio.com

Safety Report

Product and Company Identification

Product Name	**GreenView DNA Gel Stain**
Product Number	**N100**
Unit Size	**500 µL**
Manufacturer/Supplier	**ABP Biosciences** **7040 Virginia Manor Road** **Beltsville, MD 20705, USA** **Web: www.abpbio.com**

Introduction

Ethidium bromide (EB) is the most commonly used nucleic acid stain in molecular biology laboratories for decades. The dye is inexpensive, sensitive and very stable. However, it has been proved to be strong carcinogen and therefore considered hazardous for laboratory personnel and environment.

To overcome the toxicity problem of EB, ABP Biosciences has developed a GreenView DNA Gel Stain as a safer alternative to the traditional EB for detecting nucleic acid in agarose gel. It is as sensitive as EB and can be used exactly the same way in agarose gel electrophoresis with significantly improved safety profiles over EB.

The safety of GreenView DNA Gel Stain has been performed with the following four tests:

1. Ames test;
2. Cell membrane permeability and cytotoxicity test;
3. Glove penetration test;
4. Acute oral toxicity test.

7040 Virginia Manor Road
Beltsville, MD 20705, USA
Web: www.abpbio.com
E-Mail: info@abpbio.com

AMES TEST

Purpose

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

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.

Test Article

GreenView DNA Stain was dissolved in the sterile distilled water, and the concentrations were 0 (control), 5, 10, 25, 50, 100, 250 and 500 μg/mL, respectively. The test volume was 0.1 mL per plate.

The control groups included blank control plates, 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 NaN_3, 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.

Test Procedure

The test substance or control chemical (0.1 mL) and 0.1 mL bacterial suspension with 0.5 mL S9 mixture (+S9) or phosphate buffered saline (-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.

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).

7040 Virginia Manor Road
Beltsville, MD 20705, USA
Web: www.abpbio.com
E-Mail: info@abpbio.com

Table 1. Results of GreenView DNA Stain Ames test

Dosage	TA_{97}		TA_{98}		TA_{100}		TA_{102}	
μg/plate	without S_9	with S_9	without S_9	with S_9	without S_9	with S_9	without S_9	with S_9
0	123	124	35	35	121	124	268	265
0.5	123	124	35	35	120	125	266	265
1	123	125	36	35	121	122	270	263
2.5	125	124	36	38	123	121	272	268
5	124	126	38	40	126	121	275	266
10	128	130	36	41	122	124	268	264
25	129	158	37	44	123	125	270	271
50	132	179	38	48	122	124	274	268
Pos. control	1643[1]	1725[2]	1489[1]	1816[2]	1328[3]	1223[2]	1425[4]	1506[5]

Note: 1. 9-Fluorenone, 0.2 μg/plate;
2. 2-AF, 10 μg/plate;
3. NaN_3, 2.5 μg/plate;
4. Mitomycin C, 4 μg/plate;
5. 1,8-Hydroxyanthraquinone, 50 μg/plate.

Conclusion

GreenView DNA Gel Stain is nonmutagenic over the dose range from 0.5 μg/plate (or 225 ng/mL) to 50 μg/plate (or 22.5 μg/mL) to these *Salmonella typhimurium* strains. GreenView working concentration used in gel staining is 1-5 μg/mL, which is well within the safety range.

7040 Virginia Manor Road
Beltsville, MD 20705, USA
Web: www.abpbio.com
E-Mail: info@abpbio.com

CELL MEMBRANE PERMEABILITY AND CYTOTOXICITY TEST

Purpose

The purpose of this test is to see if GreenView DNA Gel Stain can cross cell membranes to stain nuclear DNA, and how it will affect the cytotoxicity.

Method

Cell membrane permeability test

Hela cells were cultured in 24-well tissue culture plate. Each well was incubated with SYBR Safe, GreenView, GreenView Plus, and RedView, respectively. The dye concentrations were all 1X based on the respective dye concentrations used for gel staining for each dye. The SYBR Safe was used as a control as it is known to be able to stain DNA in live cells. After incubation at 37 ^{o}C for 30 min, cell imaging was taken by fluorescence microscopy using optical filter sets appropriate for each dye.

Cytotoxicity test

Hela cells were cultured in 96-well tissue culture plate. Each well was incubated with SYBR Safe, GreenView, GreenView Plus, and RedView, respectively. The dye concentrations were 4, 10, 20, 40 µM for each dye. As negative control the cells were treated only with medium. The positive control group was treated with phenol. After incubation at 37 ^{o}C for 24 hours, MTT was added in all wells and incubated for 2 hours. After incubation, DMSO was added to the wells and read at 570 nm using a spectrophotometer. Mean value of growth inhibition was calculated by the following formula:

Mean value growth inhibition = $100\% \times [A570_{(\text{Negative control})} - A570_{(\text{test compound})}] / A570_{(\text{Negative control})}$

Results

Cell images obtained following 30 minutes of incubation are shown in Figure 1. Cells stained with SYBR Safe show bright nuclear stain, cells stained with GreenView show weak nuclear stain, and cells stained with GreenView Plus or RedView show no nuclear counterstain.

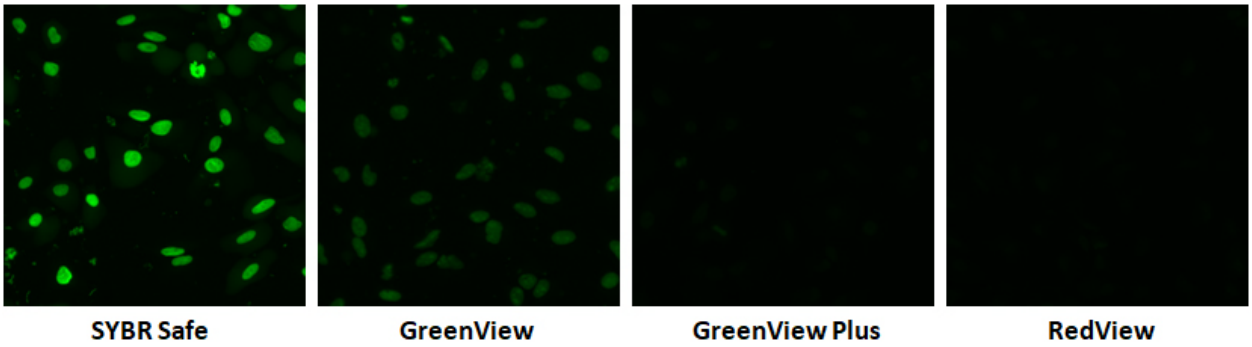

Figure 1. HeLa cells were incubated with 1X of SYBR Safe, GreenView, GreenView Plus, and RedView, respectively. After incubation at 37 ^{o}C for 30 min, cell imaging was taken by fluorescence microscopy, respectively.

Results of cytotoxicity assay are shown in Figure 2. SYBR Safe shows moderate inhibition at low concentration at 4 µM. When the concentration of SYBR Safe is increased to 20 µM, the inhibition becomes significantly. When the concentration of GreenView is below 10 µM, there is no inhibition to cells. But when the concentration of GreenView is increased to 20 µM, GreenView will show moderate inhibition. Instead, even the concentration of GreenView Plus or RedView is over 40 µM, there is no or little inhibition to cells. The dye working concentration used in gel staining is about 2-5 µM.

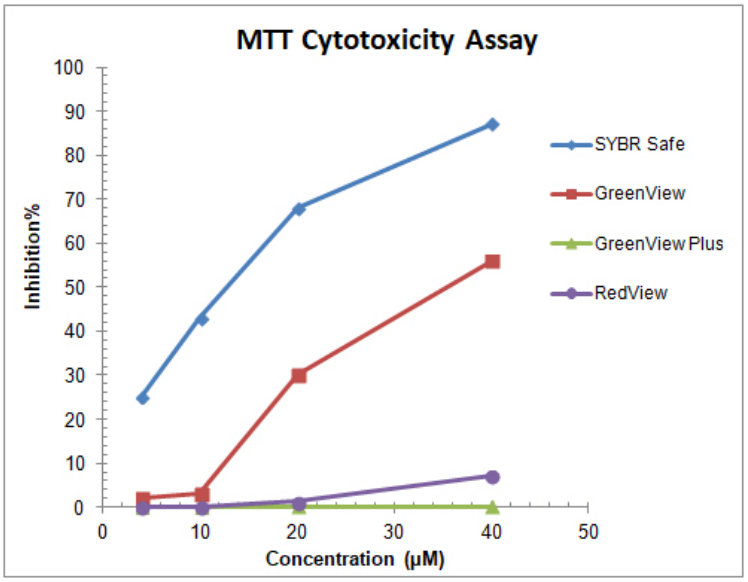

Figure 2. MTT cytotoxicity assay with SYBR Safe, GreenView, GreenView Plus, and RedView.

Conclusion

GreenView DNA Gel Stain is slowly permeable to cell membranes, and has no cytotoxic effects at working concentration.

7040 Virginia Manor Road
Beltsville, MD 20705, USA
Web: www.abpbio.com
E-Mail: info@abpbio.com

GLOVE PENETRATION TEST

Purpose

The purpose of the glove penetration tests is to test the ability of GreenView DNA Gel stain to diffuse through latex gloves.

Method

A finger of a latex glove containing 0.5X TBE buffer was dialyzed against 0.5X TBE buffer containing 5X GreenView DNA Gel stain for 24 hours. The solution in the finger was then analyzed for presence of the dye by fluorescence. As a reference, the fluorescence of the dye at 1X was also measured. To increase the sensitivity of the detection, all fluorescence measurements were made in the presence of 50 µg/mL salmon sperm dsDNA. Maximum excitation and emission wavelengths were chosen for this analysis (490 nm for excitation and 530 nm for emission). The bandwidths of excitation and emission filters are 10 nm.

Results

The result of the test shows GreenView is impenetrable to latex gloves (Figure 3).

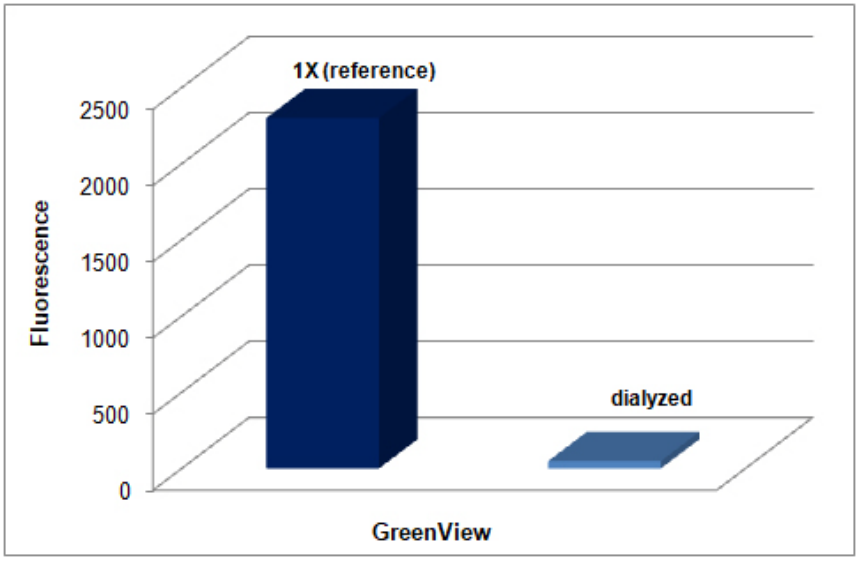

Figure 3. Relative fluorescence of solutions dialyzed in latex glove fingers against 5X GreenView and the relative fluorescence of the corresponding 1X dye solution as a reference.

Conclusion

Handling GreenView with latex gloves is safe.

7040 Virginia Manor Road
Beltsville, MD 20705, USA
Web: www.abpbio.com
E-Mail: info@abpbio.com

ACUTE ORAL TOXICITY TEST

Purpose

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. This procedure is designed to determine the acute oral toxicity of the material under test.

Method

1X GreenView DNA Gel Stain in 0.5X TBE buffer was directly used as test article. A single dosage at 10g/kg was chosen. The animals used in the test were healthy Kuming mice. Before testing, the animals were fasted for 18 hours but water was ad libitum. Food was withheld until 4 hours after dosing in order to facilitate gastrointestinal absorption of the test article. The animals were administrated with 10 g/kg of the test article solution by means of a gavage needle attached to a hypodermic syringe.

After exposure, the animals were observed for mortality, weight change, and toxic signs for a two-week period.

Results

All animals remained healthy with no toxic signs throughout the duration of the study. All animals gained weight during the test period. No abnormalities were observed on all animals at the end of the study.

Conclusion

A single oral administration of GreenView DNA Gel Stain in 0.5X TBE at a limit dose of 10 g/kg to three healthy mice produced no mortalities or toxic signs.