Summary of Mutagenic Toxicity Test Results for GelRed™ and GelGreen™

Compiled by Biotium, Inc. from the results of an independent testing service:

Litron Laboratories, Inc., Rochester, NY

Biotium

Overview

When our scientists designed GelRed™ and GelGreen™, sensitivity was not their only objective—safety of the dyes was also their top priority.

Ethidium bromide (EB) is still a commonly used nucleic acid gel stain in most of the bioscience laboratories because of its low cost and reasonable sensitivity for most of the experimental needs. However, EB is a known powerful mutagen and potential carcinogen that can pose a major safety problem to researchers and an environmental hazard during disposal. Although there have been other commercial gel stains that are marketed as safer alternatives to EB, these alternative gel stains often have significantly compromised sensitivity.

So, how did we make GelRed™ and GelGreen™ safer without sacrificing their sensitivity? Before we began the project, we reasoned that, for a DNA dye to be mutagenic, it must be able to cross cell membrane. Since cell membrane-permeability is not required for a gel stain, a membrane-impermeable gel stain should have improved safety. Thus, as a first line of defense, we built an innovative structure feature into the new gel stain molecules so that the dyes not only have improved sensitivity for gel staining but also become extremely difficult to cross cell membranes. Additionally, we also recognized that once a DNA dye enters a cell it may be subject to metabolism, which may convert the dye into a chemical that could be either more mutagenic (as in the case of EB) or less mutagenic than the unmetabolized dye. Thus, as a second line of defense, our chemists incorporated into the structures of GelRed™ and GelGreen™ chemical bonds at strategic positions so that on enzymatic cleavage the dyes will become very weak DNA-binding molecules. We believe that these unique structure features of GelRed™ and GelGreen™ are at least partially responsible for the observed low mutagenicity and low cytotoxicity of the dyes.

Using standard Ames test, as measured in two bacterial strains, both GelRed™ and GelGreen™ have been confirmed to be substantially safer than EB. GelGreen™ was not mutagenic at all 10 different doses ranging from 0.1 μg/plate (or 0.037 μg/mL) to 50 μg/plate (or 18.5 μg/mL) in the presence or absence of a mammalian S9 fraction. GelRed™ also was not mutagenic at all dosages in the absence of the S9 fraction. With S9 metabolic activation, GelRed™ showed weak mutagenicity only at the highest dosage (50 μg/plate or 18.5 μg/mL), well above the normal concentration used for gel staining. Under the same condition, EB was found to be highly mutagenic in the presence of the S9 fraction, consistent with the known toxicity of the dye.

This document is intended to provide a brief summary of the safety data on GelRed™ and GelGreen™ obtained by Litron Laboratories, an independent toxicity test service in Rochester, New York. For more detailed information, please download the original test reports at our website: www.biotium.com.

Test System Description

The test employed two Salmonella strains, TA98 and TA1537, both of which carry 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. Both strains of bacteria used in the assays are among those recommended by OECD 471 for use in the Ames test. These two strains of S. typhimurium have been shown to be reliably and reproducibly responsive between laboratories.

In order to test the mutagenic toxicity of metabolized 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 Articles and Vehicle Description

GelRed™, GelGreen™ and SYBR® Green I along with ethidium bromide (EB) as a reference were tested under the same conditions. DMSO was used for dissolving each dye to give the following stock concentrations: 0 (control), 1, 2.5, 5, 10, 25, 50, 75, 100, 250 and 500 µg/mL.

Test Procedure

The following was added to each sterile culture tube containing 2.0 mL top agar: 0.1 mL of overnight cell culture (TA98 or TA1537), 0.1 mL of each dye concentration for each dye or control chemical, and either 0.5 mL of S9/Cofactor mix or 0.5 mL of phosphate buffered saline. By using the above 10 stock solutions for each dye plus the control, the following per plate doses for each dye were used: 0, 0.1, 0.25, 0.5, 1, 2.5, 5, 7.5, 10, 25, and 50 µg/plate. These doses corresponded to a final dye concentration of: 0, 0.04, 0.09, 0.19, 0.37, 0.93, 1.85, 2.78, 3.7, 9.3, and 18.5 µg/mL, respectively.

The contents of each tube were vortexed, poured onto Vogel-Bonner media plates, and evenly distributed. The agar on the test plates was allowed to harden. The plates were inverted and incubated at 37 °C for 2 days.

Revertant colonies were counted using a New Brunswick Biotran III automatic colony counter.

Mutagenicity Tests in Salmonella Strain TA98 without S9 Metabolic Activation

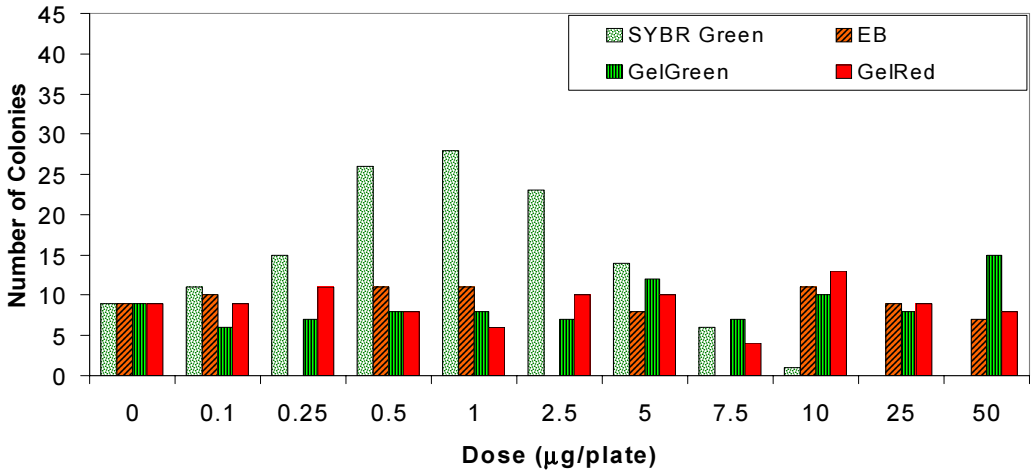

Figure 1. Comparison of mutagenicity of GelGreen™, GelRed™, SYBR® Green I and EB in +1 frameshift indicator strain TA98 without the presence of S9 fraction. Tests were performed by Litron Laboratories Inc., Rochester, NY.

Conclusion

- GelGreen™ and GelRed™ are not mutagenic over the dose range in +1 frameshift indicator strain TA98 without S9 metabolic activation.
- EB is not mutagenic without S9 metabolic activation, consistent with earlier reports (McCann, et al. *Proc. Natl. Acad. Sci. USA* **72**, 5135)1975)).
- SYBR® Green I shows weak dose-dependent mutagenic response at up to 1 µg/plate (or 0.37 µg/mL) and becomes cytotoxic thereafter, consistent with earlier reports (Singer, et al. *Mutat. Res*. **439**, 37(1999)).

Mutagenicity Tests in Salmonella Strain TA98 with S9 Metabolic Activation

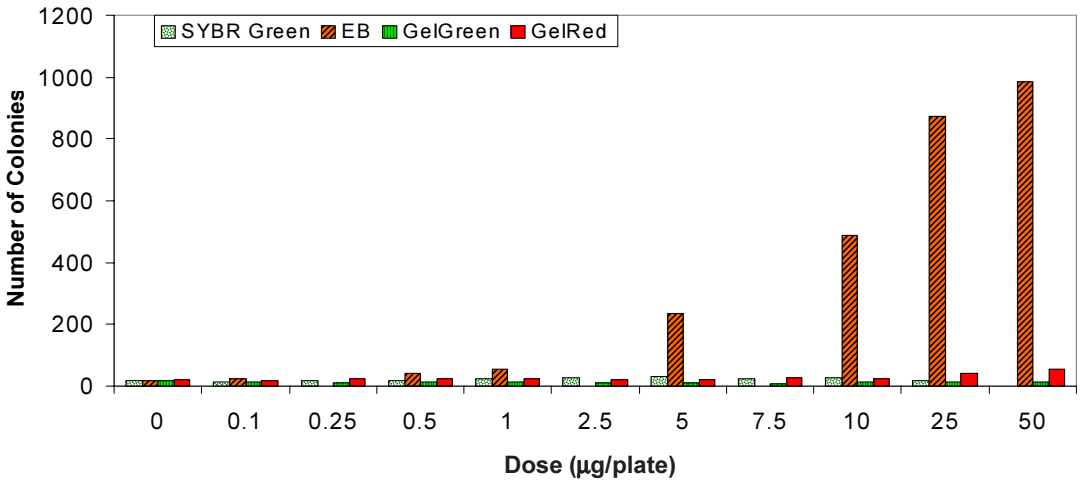

Figure 2. Comparison of mutagenicity of GelGreen™, GelRed™, SYBR® Green I and EB in +1 frameshift indicator strain TA98 with the presence of S9 fraction. Tests were performed by Litron Laboratories Inc., Rochester, NY.

Conclusion

- GelGreen™ is not mutagenic over the dose range in +1 frameshift indicator stain TA98 with S9 metabolic activation.
- GelRed® is only weakly mutagenic with S9 metabolic activation at very high dose, 50 µg/plate or 18.5 µg/mL, which is well above the 3X (<5 µg/mL) dye concentration recommended for post gel staining.
- SYBR® Green I is not mutagenic, but becomes cytotoxic at higher doses (≥ 25 µg/plate or 9.3 µg/mL) when S9 fraction was present.
- EB is highly mutagenic with S9 metabolic activation, consistent with the known toxicity of the dye.

Mutagenicity Tests in Salmonella Strain TA1537 without S9 Metabolic Activation

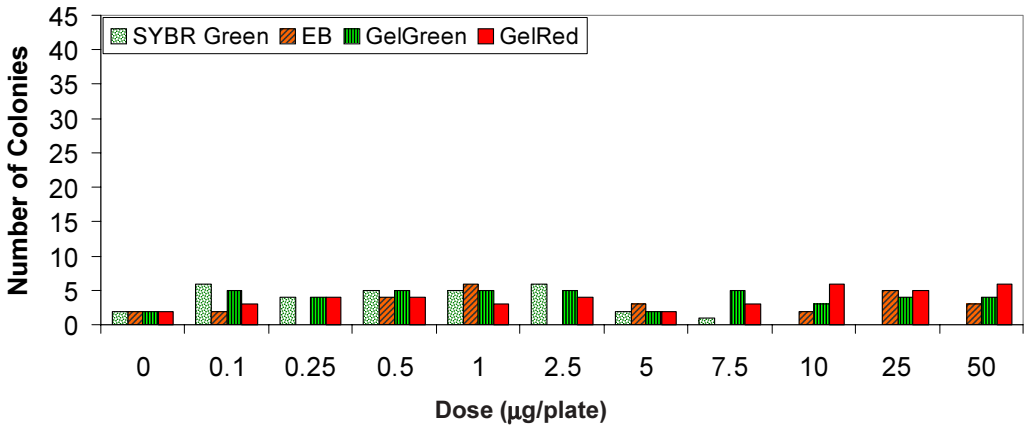

Figure 3. Comparison of mutagenicity of GelGreen™, GelRed™, SYBR® Green I and EB in -1 frameshift indicator strain TA1537 without the presence of S9 fraction. Tests were performed by Litron Laboratories Inc., Rochester, NY.

Conclusion

- GelGreen™ and GelRed™ are not mutagenic over the dose range in -1 frameshift indicator strain TA1537 without S9 metabolic activation.
- SYBR® Green I is not mutagenic, but becomes cytotoxic at higher doses (≥ 2.5 µg/plate or 0.93 µg/mL) without S9 metabolic activation.
- EB is not mutagenic over the dose range in -1 frameshift indicator strain TA1537 without S9 metabolic activation.

Mutagenicity Tests in Salmonella Strain TA1537 with S9 Metabolic Activation

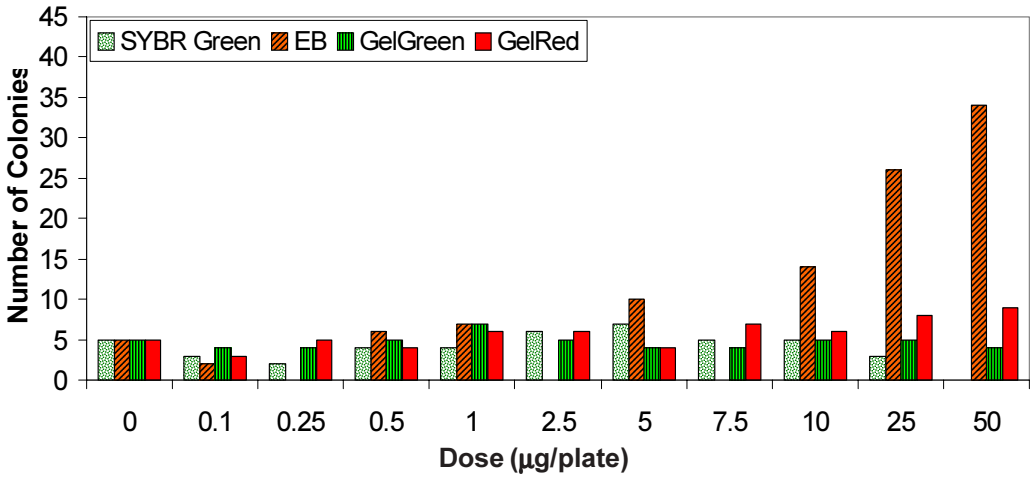

Figure 4. Comparison of mutagenicity of GelGreen™, GelRed™, SYBR® Green I and EB in -1 frame-shift indicator strain TA1537 with the presence of S9 fraction. Tests were performed by Litron Laboratories Inc., Rochester, NY.

Conclusion

- GelGreen™ and GelRed™ are not mutagenic over the dose range in -1 frameshift indicator strain TA1537 with S9 metabolic activation.
- SYBR® Green I is not mutagenic, but becomes cytotoxic at higher doses (≥ 25 µg/plate or 9.3 µg/mL) when S9 fraction was present.
- EB is mutagenic with S9 metabolic activation, consistent with the known toxicity of the dye.