SmartGlow™ Stain, Ames Test and Mammalian Genotoxicity Analysis

Introduction

Ethidium Bromide (EB) is a toxic mutagen that is widely used in electrophoretic procedures. In recent years, EB has become both a personal and environmental concern. ***Accuris SmartGlow™ Nucleic Acid Stains,*** developed by Benchmark Scientific, represent alternative DNA/RNA staining dyes that not only address the concerns about EB, but also improve the process of DNA/RNA electrophoresis.

SmartGlow Nucleic Acid Stains are green fluorophores used to tag DNA and RNA in agarose and polyacrylamide gels. They possess excellent sensitivity for their targets and have the advantage of visualization by either UV or blue light. Two formulations are available:

SmartGlow PS Pre Stain is used in the same manner as EB, by adding a small amount to the gel solution prior to casting, and also adding a small amount to the running buffer.

SmartGlow LD Loading Dye is added directly to the samples, eliminating the need to add dyes to both the gel matrix and the running buffer. This reduces fluorescent dye contamination of electrophoresis tanks and glassware.

Below are details of the toxicity results between our ***SmartGlow™*** products vs. EB.

Ames Test System Description

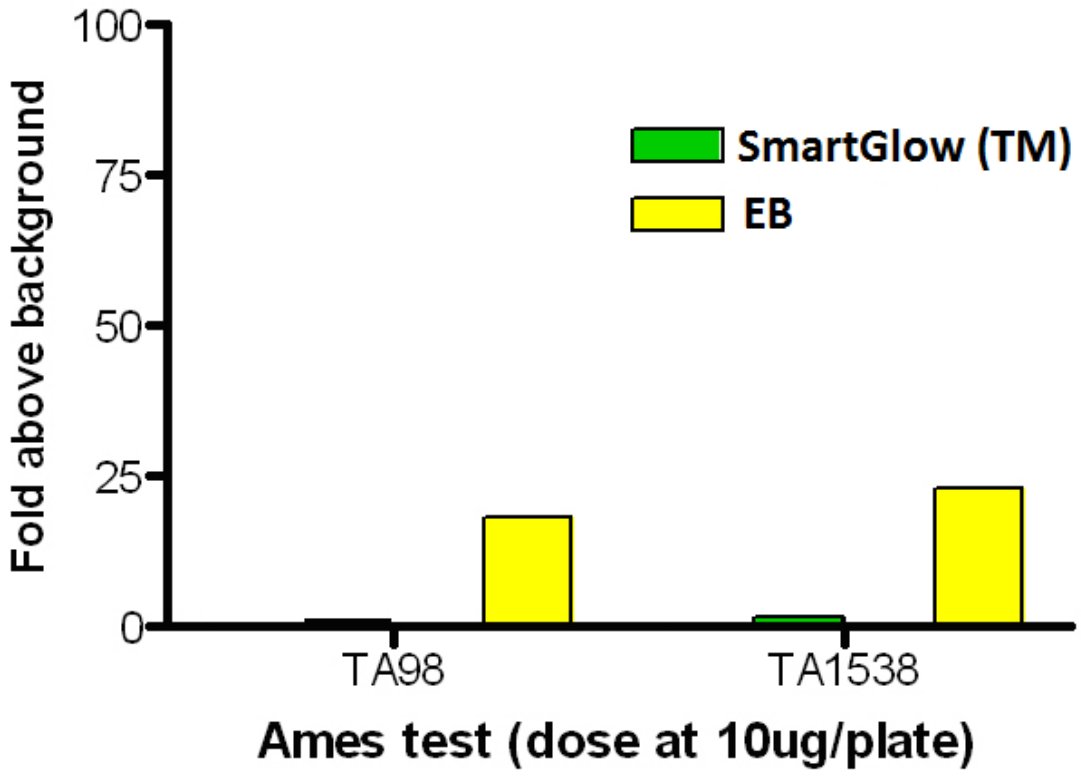
The test employed two Salmonella strains, TA98 and TA1538, both of which carry mutation(s) in the operon encoding for histidine biosynthesis. When these bacteria are exposed to mutagenic agents under certain conditions, reverse mutation from amino acid (histidine) auxotrophy to prototrophy occurs, giving 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.

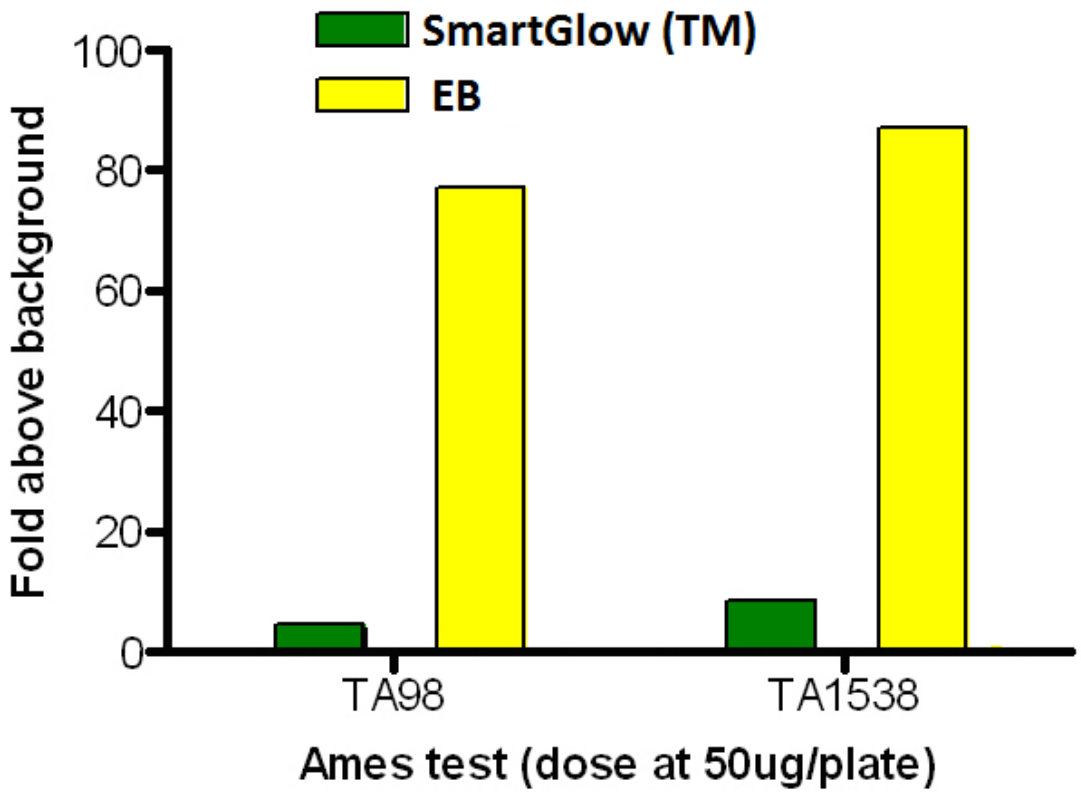
Test Articles and Vehicle Description

SmartGlow™ along with EB as a reference was 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 TA1538), 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. 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. For strain TA1538, an increase in revertants of more than threefold over background indicates a positive result, whereas an increase in revertants of more than two fold over background indicates a positive result for mutagenicity in this test for strain TA98.





SmartGlow™ Mouse Spermatogonial Chromosomal Aberration Test

The purpose of the mammalian spermatogonial chromosomal aberration test is to identify chemicals that cause structural aberrations in cultured mammalian cells. In this assay, the numerical chromosome changes such as polyploidy and duplication is measured. The test employed Mouse Spermatocytes. The cells were seeded in 96-well plates and treated with SmartGlow™ for 24 hours (without S9) and for 4 hours (with S9). All cultures were harvested 24 hours from the initiation of treatment. The chromosomal aberrations assay was conducted after 24 hours of the initial treatment. The test dose used were SmartGlow™ at concentrations of 0.500, 1.00, 2.00, 4.00, 6.00, 8.00, and 10.0 µg/mL. Structural chromosomal aberrations were evaluated and no significant increase in the number of cells with structural aberrations, polyploidy, or endoreduplication was observed for both assays with, and without S9. SmartGlow™ was considered negative for inducing structural chromosomal aberrations with and without metabolic activation.

SmartGlow™ Mouse Marrow Chromophilous Erythrocyte Micronuleus Test

The test employed Mouse marrow chromophilous erythrocytes. The test detects small, extra nuclei in the cell cytoplasm that are indicative of chromosome fragments or whole chromosomes excluded from nuclei at cell division due to chemically induced damage. The cells were seeded in 96-well plates and treated with SmartGlow™ for 24 hours (without S9) and for 4 hours (with S9). Cytochalasin B, a cytokinesis blocker, was then added after 24 hours and the cells were incubated for an additional 24 hours. After the incubations, the cells are fixed and scored for micronuclei. The test dose used were SmartGlow™ at concentrations of 0.500, 1.00, 2.00, 4.00, 6.00, 8.00, and 10.0 µg/mL. No micronuclei were identified for both assays with, and without S9. SmartGlow™ was considered negative for inducing chromosomal damages with and without metabolic activation.

In Vitro Test	**Cell Type**	**Result with S9 Activation**	**Result without S9 Activation**
Chromosomal aberrations	Mouse spermatocyte aberrations test	Negative	Negative
Mutation	Mouse marrow chromophilous erythrocyte micronucleus test	Negative	Negative

The in vitro mutation tests were performed by SBS Biotech Inc. and Ames tests were performed by Keygen Biotech Inc.

Conclusion

SmartGlow™ Products are a safe alternative to EB for nucleic acid electrophoresis applications.