SafeView/SafeWhite Safety Test

Ethidium Bromide, a strong carcinogen, is widely used in nucleic acid electrophoresis. It has been always a great personal and environmental concern when it is used in laboratories. In recent years, great efforts have been made to develop and search for alternative nucleic staining dyes that are safe to lab personnel and the environment. Safeview™ products (SafeView and SafeWhite) represent unique alternative DNA/RNA staining dyes that not only address the laboratory and environmental safety concerns, but also revolutionise the way DNA/RNA electrophoresis is done.

With our new product SafeWhite™, nucleic samples (DNA/RNA) become visible simply by mixing samples with the dyes, without the need of adding dyes to both running buffer and gel matrix. This feature significantly reduces the risk of chemical contamination for both laboratory personnel and the environment. We have presented here the side-by-side comparison of toxicity results between our Safeview™ products vs. EtBr.

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 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.

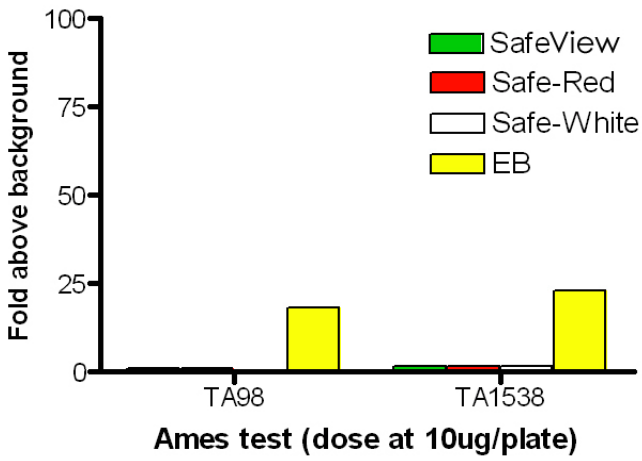
Test Articles and Vehicle Description

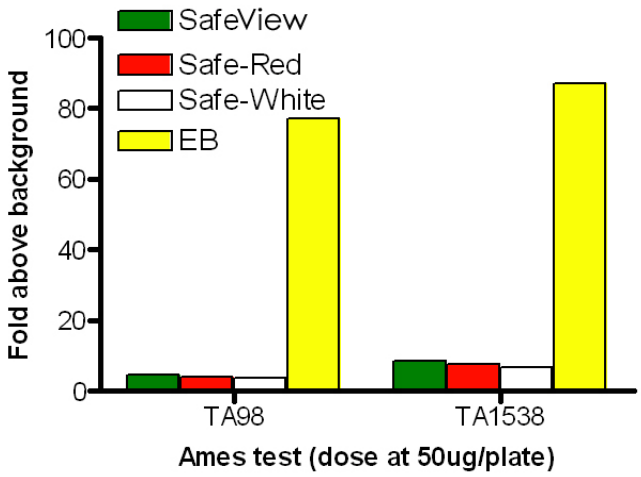
Safeview™ along with Ethidium bromide (EB) as a reference was tested under the same conditions. DMSO was used for dissolving each dye to give the following stock concentrations: 0 (control), 1, 2.5, 5, 10, 25, 50, 75, 100, 250 and 500 µg/mL.

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 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. 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.





SafeView™ stain Mammalian Genotoxicity Analysis

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

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

Conclusion

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