Technical Info





ROTI[®]GelStain Background and Safety Tests

ROTI®GelStain Molecular Data

Ready-to-use staining mixture prepared from a variety of reagents

Dye component: orange-red to brown powder

UPAC: 1,1'3,3',5,5'6,6'-Octamethyl-2,2'-spiro (2,3-dihydro-1H-benzimidazole)

CAS [99643-38-6] • Formula C₂₁H₂₈N₄ • MW: 336.47

DNA-binding properties: Benzimidazoles typically bind to the minor groove of helical nucleic acid. No intercalation takes place.

Application: Very well suited for staining of single and double stranded nucleic acids in agarose gels via addition to the agarose (in-gel staining), to the running buffer, or via post-run staining. Less recommended for use via addition to loading buffers. Not suitable for staining of PAA gels.

Light spectrum (bound to DNA): Excitation maximum at 290-320 nm Excitation also possible at 420 nm Emission maximum at 515 nm

ROTI[®]GelStain Safety Parameters

Ethidium bromide is a widely used staining reagent in nucleic acid electrophoresis and is putatively strong carcinogenic. It has been always a great concern for personnel and environment when using it in the laboratories. In recent years, great efforts have been made to establish alternative nucleic staining dyes that are safe to both, lab personnel and environment. ROTI[®]GelStain represents an alternative DNA/RNA staining dye that addresses the laboratory and environmental safety concerns, and, additionally, makes life in electrophoresis labs significantly more easy.

LD50

Mouse, subcutaneous: 250 mg/kg Target organ: Blood





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Mammalian genotoxicity analysis

Marker	Cell Type / Test	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

Ames Test System

The test employed two *Salmonella typhimurium* 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.

Ames Test Procedure

ROTI®GelStain 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 μ g/ml.

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 μ g/ 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 μ g/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 an 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.



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Results of Ames Tests

Two test results are shown exemplarily.

At a dose of 1 µg/plate, ethidium bromide is significantly positive in Ames test (here approx. 19x and 23x above background), whereas ROTI[®]GelStain still is completely negative.



At a dose of 50 µg/plate, ROTI[®]GelStain is just considered Ames test-positive (here approx. 4x and 8x above background), while ethidium bromide is more or less beyond measures.



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