Xpert Green DNA Stains
Safety Report

Introduction:

For decades, ethidium bromide (EtBr) has been the stain of choice for the visualization of DNA and RNA in agarose and polyacrylamide gels. It has become popular because it is relatively cheap and has sufficient sensitivity for most applications. However, EtBr is a powerful mutagen and carcinogen and as such hazardous for users, other laboratory personnel and environment. Thus, usage of EtBr requires decontamination methods and correct waste disposal, making it costly to use.

Xpert Green DNA Stains are new and safe alternatives to ethidium bromide for the visualization of DNA (double-stranded and single-stranded DNA) and RNA in agarose and polyacrylamide gels. Extensive testing by independent laboratories, demonstrate that Xpert Green DNA Stains have greatly improved safety profiles compared to EtBr. The results of these tests are summarized in Table 1, and details regarding results and experimental set-up are described further in this report.

Table 1. Summary of Xpert Green DNA Stains Test Results

	#GS01 Xpert Green DNA Stain (20.000x)	#GS02 Xpert Green DNA Stain - Direct
Genotoxicity	Non-mutagenic	Non-mutagenic
Cytotoxicity	Non-cytotoxic	Non-cytotoxic
Aquatic Toxicity	Non-toxic to aquatic life	Non-toxic to aquatic life
Latex Glove penetration	Impenetrable	Impenetrable
Cell membrane penetration	Impenetrable	Impenetrable
Reactivity Test	Non-reactive	Non-reactive
Corrositivity Test	Non-corrosive	Non-corrosive
Ignitability Test	Non-flammable	Non-flammable

Conclusion:

Xpert Green DNA stains are non-mutagenic, even at much higher concentrations than normal working concentrations, as determined by the Ames-test. Furthermore, genotoxicity analysis shows negative results for both the mouse marrow chromophilous erythrocyte micronucleus test and mouse spermatocyte chromosomal aberration test. Results also confirm that dyes are impenetrable to latex gloves and cell membranes. Moreover, these products have passed successfully environmental safety tests and are not classified as hazardous waste and thus can be conveniently and safely disposed of as regular waste, reducing overall costs of waste disposal.

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1. Ames Test

Introduction

The Ames test is a widely employed method to assess the mutagenic potential of chemical compounds. A positive result is a strong indication that the chemical is also carcinogenic, as cancer is often linked to mutations. The Ames test uses several Salmonella strains, which carry mutations in the operon coding for histidine synthesis so that cells cannot synthesize histidine and require histidine in the medium for growth. Under certain conditions, upon exposure to mutagenic agents, reverse mutation from histidine auxotrophy to prototrophy occurs, allowing cells to grow in histidine-free medium. Tester strains also carry mutations making the cell wall more permeable and in the excision repair system making the test more sensitive. Rat liver extract (S9 fraction containing a mixture of metabolic enzymes) can be added to simulate metabolism, as some compounds that are not mutagenic themselves, *in vivo*, give rise to mutagenic metabolic products. In order to test the mutagenic toxicity of Xpert Green DNA stains, the Ames test was carried out with Salmonella strains TA98 and TA1537. For this, dyes, along with EtBr and SYBR® Green as references, were dissolved using DMSO at final concentrations ranging from 1 to 500µg/ml and tested under identical conditions.

Procedure

One hundred microliters of each dye at each concentration to be tested (or control) were added to a culture tube containing 0.1ml of an overnight culture of Salmonella (TA98 or TA1537) and 2.0ml of Top agar, and supplemented with either 0.5ml of S9 mixture or 0.5ml of PBS. After vortexing, tube contents were poured onto Vogel-Bonner media plates and incubated at 37°C, according to normal protocol. The following quantities of each compound were used: 0, 0.1, 0.25, 0.5, 1, 2.5, 5, 7.5, 10, 25, and 50µg/plate. After 2 days, colonies were counted using a New Brunswick Biotran III automatic colony counter, and compared with appropriate positive and negative mutagentic control substances.

Results

In the absence of S9 metabolic activation, the Ames test carried out with both Salmonella strain TA98 (+1 frameshift indicator strain) and strain TA1537 (-1 frameshift indicator strain) demonstrated that nor the Xpert Green DNA stains nor EtBr were mutagenic over the whole dose range tested (0.1µg/plate (40ng/ml) - 50µg/plate (18.5µg/ml)), with on average less than 10 colonies per plate and without any dose-response relationship. SYBR® Green I also showed weak dose-dependent mutagenic response up to a concentration of 1µg/plate, however, it became cytotoxic at higher concentrations (similar to reported in Singer *et al.* (1999) *Mutat. Res*. **439**: 37)

In the presence of S9, using TA98 and TA1537, EtBr demonstrated to be highly mutagenic, with a clear dose-response relationship, whereas neither Xpert Green DNA Stain nor Xpert Green DNA Stain Direct, like SYBR® Green, showed any mutagenic effects over the whole dose range tested.

Conclusion

Both stains, Xpert Green DNA Stain and Xpert Green DNA Stain Direct, are non-mutagenic for up to a concentration of at least 18.5µg/ml, whereas Ethidium Bromide is not (which is consistent with earlier reports: McCann *et al*. (1975) *Proc. Natl. Acad. Sci. USA* **72**: 5135). This means that normal working concentrations of both Xpert Green DNA Stain and Xpert Green DNA Stain Direct are well within the safety range.

2. Mammalian Genotoxicity Analysis

Introduction

In Vitro Mammalian Chromosomal Aberration tests identify agents that cause structural chromosomal aberrations by exposing cultured mammalian somatic cells to test substances. Cells are treated with a metaphase-blocker, harvested, stained and analyzed via microscopy for the presence of chromosomal aberrations. *In Vitro* Mammalian Cell Gene Mutation tests measure mutation at TK, HPRT and XPRT genes in commonly used cells lines such as CHO.

Procedure

Mammalian Genotoxicity Analysis was done by carrying out *in vitro* mammalian chromosomal aberration and cell gene mutation tests using appropriate positive and negative controls.

Results

Table 2. Genotoxicity Analysis

Test	Stain	Result (with S9 activation)	Result (without S9 activation)
Mouse spermatocyte aberration test (chromosomal aberration)	Xpert Green DNA Stain	Negative	Negative
	Xpert Green DNA Stain Direct	Negative	Negative
Mouse marrow chromophilous erythrocyte micronucleus test (mutation)	Xpert Green DNA Stain	Negative	Negative
	Xpert Green DNA Stain Direct	Negative	Negative

Conclusion

Both Xpert Green DNA Stain and Xpert Green DNA Stain Direct show negative results for both the *in vitro* mammalian chromosomal aberration test and the *in vitro* mammalian cell gene mutation test, and thus assessed as non-genotoxic.

3. Latex Glove Penetration Test

Introduction
Since latex gloves are the most worn gloves by researchers, it is important to demonstrate that, Xpert Green DNA Stains do not diffuse through the latex material.

Procedure
A finger of a latex glove containing 1x TAE Buffer was dialyzed against 1x TAE Buffer containing either 5x Xpert Green DNA Stain or Xpert Green DNA Stain Direct for 48 hours. The solution in the finger was subsequently analyzed for dye presence by fluorescence analysis using the fluorescence of the dye (1x concentrated) as reference and in the presence of salmon sperm dsDNA to increase detection sensitivity.

Results

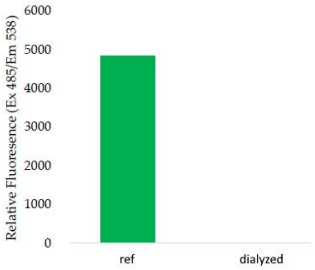

Fig 1. Relative fluorescence of solution dialyzed in latex against 5x Xpert Green DNA stain.

The data indicate that the fluorescence intensities of the dialyzed solutions are negligible; hence results show that both Xpert Green DNA stain and Xpert Green DNA stain direct (data not shown) are impenetrable to latex gloves.

Conclusion
Handling Xpert Green DNA stains with latex gloves is safe. There is no need for the usage of nitrile gloves.

4. Cell Membrane Penetration Test

Introduction
The aim of this test is to determine if Xpert Green DNA stains can cross cell membranes and subsequently stain nuclear DNA.

Procedure
HeLa cells were incubated at 37°C with working concentrations (1X) of Xpert Green DNA stains. SYBR® Green I was used as reference, as this dye is known to stain nuclear DNA within minutes.

Results

Fig 2. Images taken after 30 minutes of incubation with A) SYBR® Green I, B) Xpert Green DNA Stain, C) Xpert Green DNA Stain Direct

Whereas SYBR® Green can readily penetrate cells and then bind to and stain nuclear DNA, Xpert Green DNA stains cannot.

Conclusion
Xpert Green DNA stain and Xpert Green DNA stain Direct cannot penetrate living cell membranes.

5. *Pimephales promelas* Toxicity Test

Introduction
The aim of this test is to determine if the aquatic toxicity of Xpert Green DNA stains.

Procedure
Procedure was carried out according to the method published by the California Department of Fish & Game (1988) Acute Procedures; EPA/600/4-85/013 (1985) Acute Manual, and regulatory guidelines: CCR Title 22 Hazardous Waste Characterization.

Results
For each Xpert Green DNA stain, twenty Fathead minnows (*Pimephales promelas*) were subjected (in two replicates of 10 fish each) during a period of 4 months to several concentrations of each stain and outcome was compared with the lab control, Average length/weight of the fish was 34mm/0.34g and dye concentrations were 250, 500 and 750µg/ml.

Table 3. *Pimephales promelas* toxicity test results

Compound	Concentation (µg/ml)	Survival rate (%)
Lab Control	-	95
Xpert Green DNA stain	250	100
Xpert Green DNA stain	500	700
Xpert Green DNA stain	750	100
Xpert Green DNA stain Direct	250	100
Xpert Green DNA stain Direct	500	100
Xpert Green DNA stain Direct	750	100

In order to be qualified as “not hazardous to aquatic life”, compounds must result in a survival rate of at least 50% at a concentration of 500mg/L (LC50>500mg/L). Both Xpert Green DNA stain and Xpert Green DNA stain direct pass this requirement.

Conclusion
Both Xpert Green DNA stain and Xpert Green DNA stain direct are classified as non-hazardous to aquatic life under CCR Title 22 Regulation and as such can be safely disposed of into the environment.

6. Enviromental Safety Tests

Introduction
Corrosivity, reactivity and ignitability of Xpert Green DNA stains were determined for the purpose of assessing the environmental safety and safety concerns associated with transport, handling and storage of these solutions.

Procedure

Procedures were carried out with stains at 3x working concentration according to EPA or ASTM guidelines, as outlined in table 4.

Results

Table 4. Environmental Safety Test results

Test Method (guideline code)	Xpert Green DNA Stain (3X)	Xpert Green DNA Stain Direct (3X)
Reactive Sulfide (SW-846 CH.7)	Non detected	Non detected
Reactive Cyanide (SW-846 CH.7)	Non detected	Non detected
Flash point (ASTM D-93)	>150 deg. F (>66ºC)	>150 deg. F (>66ºC)

Conclusion
Xpert Green DNA stain and Xpert Green DNA stain at 3x working concentration are classified as non-corrosive/non-hazardous.