

# Product Information

## GelGreen™ Nucleic Acid Gel Stain, 10,000X

### Catalog Numbers:

41004 (in DMSO)

41005 (in water)

**Packaging Sizes:** 0.5 mL (41004 and 41005), 0.1 mL (41005-T), 10 mL (41005-1)

### Storage and Handling

GelGreen™ is a very stable dye. Store 10,000X solution and dilute solutions of GelGreen™ at room temperature, protected from light. Dye precipitation may occur at lower temperatures, resulting in lower signal or the appearance of precipitate on the surface of the gel. If this occurs, heat the solution to 45-50°C for two minutes and vortex. GelGreen™ is stable for at least one year from the date it is received.

### Product Description

GelGreen™ is a sensitive, stable and environmentally safe green fluorescent nucleic acid dye specifically designed for gel staining. GelGreen™ has UV absorption between 250 nm and 300 nm and a strong absorption peak centered around 500 nm (Figure 1). Thus, GelGreen™ is compatible with either a 254 nm UV transilluminator or a gel reader equipped with visible light excitation (such as a 488 nm laser-based gel scanner or a Dark Reader). GelGreen™ is far more sensitive than SYBR® Safe (Figure 2). Unlike SYBR® dyes, which are known to be unstable, GelGreen™ is very stable, both hydrolytically and thermally.

GelGreen™ was subjected to a series of tests at Biotium and by three independent testing services to assess the dye's safety for routine handling and disposal. Test results confirm that the dye is impenetrable to both latex gloves and cell membranes (Figure 3). Unlike the highly mutagenic EtBr and the reportedly mutation-enhancing SYBR® Green I (1), GelGreen™ is noncytotoxic and nonmutagenic at concentrations well above the working concentrations used in gel staining, because of the dye's inability to cross cell membranes. GelGreen™ successfully passed environmental safety tests in compliance with CCR Title 22 Hazardous Waste Characterization, under which GelGreen™ is classified as non-hazardous waste. A complete safety report is available at [www.biotium.com](http://www.biotium.com).

GelGreen™ Nucleic Acid Gel Stain, 10,000X in DMSO is a concentrated GelGreen™ solution that can be diluted 10,000 times for use in precast gel staining or ~3,300 times for use in post gel staining according to the procedures described below. One vial (0.5 mL) of 10,000X solution can be used to prepare at least 100 precast minigels or post-stain at least 100 minigels.

Gel staining with GelGreen™ is compatible with downstream applications such as gel extraction and cloning. GelGreen™ is efficiently removed from DNA by phenol/chloroform extraction and ethanol precipitation. GelGreen™ is not designed for qPCR applications, for which we recommend EvaGreen™ dye (cat# 31000).

### Spectral Characteristics

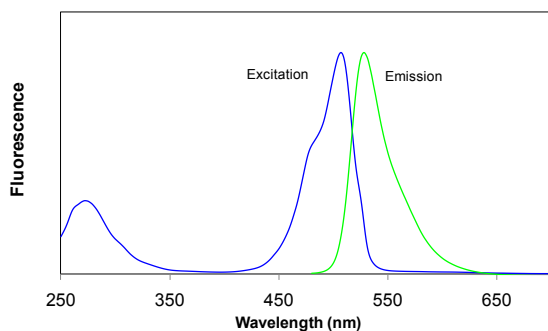


Figure 1. Excitation (left) and emission (right) spectra of GelGreen™ bound to dsDNA in TBE buffer.

### GelGreen™ Nucleic Acid Gel Stain

### Staining Protocols

Because nucleic acid binding dyes can affect DNA migration during electrophoresis, post-staining of gels is highly recommended. Post-staining with GelGreen™ results in superior sensitivity and eliminates the possibility of dye interference with DNA migration. Post-staining with GelGreen™ is simple, requiring no destaining and no special buffer. GelGreen™ also can be included in agarose gels using the precast method. While the precast protocol is more convenient, some DNA samples may experience migration retardation or compromised resolution in the presence of GelGreen™. Thus, the post-staining and precast protocols should be compared to determine which one better meets your needs.

Although GelGreen™ has undergone extensive safety testing, Biotium recommends following universal safety precautions when working in the laboratory.

#### 1. Post-staining Protocol

- 1.1 Run gels as usual according to your standard protocol.
- 1.2 Dilute the GelGreen™ 10,000X stock reagent ~3,300 fold to make a 3X staining solution in H<sub>2</sub>O. Note: including 0.1 M NaCl in the staining solution enhances sensitivity, but may promote dye precipitation if the gel stain is reused.
- 1.3 Carefully place the gel in a suitable polypropylene container. Gently add a sufficient amount of the 3X staining solution to submerge the gel.
- 1.4 Agitate the gel gently at room temperature for ~30 minutes.
- 1.5 Image the stained gel with a 254 nm transilluminator, a Dark Reader® or a similar transilluminator, or a laser-based gel scanner using a long path green filter such as a SYBR® filter or GelStar® filter.
- 1.6 Staining solution can be reused at least 2-3 times. Store staining solution at room temperature protected from light.

#### 2. Pre-cast protocol

- 2.1 Prepare molten agarose gel solution using your standard protocol.
- 2.2 Dilute the GelGreen™ 10,000X stock reagent into the molten agarose gel solution at 1:10,000 and mix thoroughly. GelGreen™ can be added while the gel solution is still hot.
- 2.3 Cast the gel and allow it to solidify. Any leftover gel solution may be stored and re-heated later for additional gel casting. GelGreen™ gels may be stored for later use for up to a month. We recommend storing gels at room temperature in the dark. Storing GelGreen™ precast gels at 4°C can result in dye precipitation and poor performance.
- 2.4 Load samples and run the gels using your standard protocol.
- 2.5 Image the stained gel with a 254 nm transilluminator, a Dark Reader® or a similar transilluminator, or a laser-based gel scanner using a long path green filter such as a SYBR® filter or GelStar® filter.

Note: The pre-cast protocol is not recommended for polyacrylamide gels. Use the post staining protocol for acrylamide gels.

### References

1. Ohta et al. (2001) Mutation Research 492, 91.

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## Troubleshooting

Problem	Suggestion
Smeared DNA bands in precast gel	<ol style="list-style-type: none"> <li>1. Reduce the amount of DNA loaded by one-half to one-third. Blown out or smeared bands can be caused by overloading. This is frequently observed with DNA ladders. Biotium offers a 1 kb ladder that has been optimized for use with GelGreen (see related products below).</li> <li>2. Perform post-staining instead of pre-casting.</li> <li>3. Pour a lower percentage agarose gel for better resolution of large fragments.</li> <li>4. Change the running buffer. TBE buffer has a higher buffering capacity than TAE.</li> </ol>
Discrepant DNA migration in pre-cast gel	<p>GelGreen is designed to be larger than other dyes to prevent it from entering cells, thus rendering the dye safer. The migration of DNA may be affected depending on the dye:DNA ratio.</p> <ol style="list-style-type: none"> <li>1. Reduce the amount of DNA loaded by one-half to one-third.</li> <li>2. Reduce the amount of dye used, i.e. use 0.5X in precast gels.</li> <li>3. Post-stain gel in 3X GelGreen to avoid any interference the dye may have on migration during electrophoresis.</li> </ol>
Weak fluorescence, decreased dye performance over time, or film of dye remains on gel after post-staining	<p>The dye may have precipitated out of solution.</p> <ol style="list-style-type: none"> <li>1. Heat GelGreen solution to 45-50°C for two minutes and vortex to redissolve.</li> <li>2. Store dye at room temperature to avoid precipitation.</li> </ol>

## Frequently Asked Questions

Question	Answer
Can GelGreen be used to stain ssDNA or RNA?	GelGreen can be used to stain ssDNA and RNA, but we recommend GelRed for this application because it is five times more sensitive for single stranded nucleic acids than GelGreen.
Is GelGreen compatible with downstream applications such as cloning, ligation and sequencing?	Yes. We recommend Qiagen or ZymoClean gel extraction kits or phenol-chloroform extraction to remove the dye from DNA.
Is GelGreen compatible with Southern or northern blotting?	GelGreen™ has not been validated in blotting applications.
Can I reuse a GelGreen precast gel after electrophoresis?	We do not recommend reusing GelGreen precast gels as signal decreases with subsequent electrophoresis.
How should I dispose of GelGreen?	GelGreen has passed the EPA regulated Title 22 test. Some facilities have approved the disposal of GelGreen directly down the drain. However, because regulations vary, please contact your safety office for local disposal guidelines. GelGreen can be adsorbed to activated carbon (also known as activated charcoal) for disposal as chemical waste.
What is the lower detection limit of GelGreen?	Some users have reported being able to detect less than 0.1 ng DNA. However, the limit of detection will depend on instrument capability and exposure settings.
Does GelGreen need to be used in the dark?	You can use the dye in room light, however we recommend storing the dye in the dark.
Is there a difference between 10,000X GelGreen in DMSO and water?	The GelGreen stock in water is a newer and improved product compared to the stock in DMSO. We recommend using GelGreen in water to avoid the potential hazards of handling DMSO, a solvent that can be absorbed through the skin. We continue to offer GelGreen in DMSO because some users do not wish to alter their established laboratory protocols.

## Comparison of GelGreen™ and SYBR® Safe in Post Gel Staining

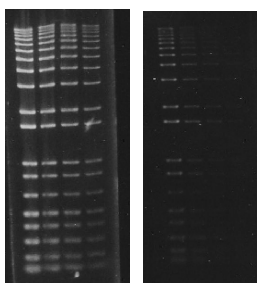


Figure 2. Comparison of GelGreen and SYBR® Safe in post gel staining using 1% agarose gel in TBE buffer. Two fold serial dilutions of 1 kb Plus DNA Ladder from Invitrogen were loaded onto each gel in 4 lanes in the amounts of 200 ng, 100 ng, 50 ng and 25 ng, respectively, from left to right. Gels were imaged using 254-nm transilluminator and photographed with a SYBR filter and Polaroid 667 black-and-white print films.

### Related Products:

Catalog No.	Product
31021	1 KB DNA Ladder (100ng/uL), 30 ug/300 ul
31022	Ready-to-Use 1 KB DNA Ladder, 150 applications (1.5 ml)
41001	GelRed™ Nucleic Acid Gel Stain, 3X in H <sub>2</sub> O, 4L
41002	GelRed™ Nucleic Acid Gel Stain, 10,000X in DMSO, 0.5 mL
41003	GelRed™ Nucleic Acid Gel Stain, 10,000X in H <sub>2</sub> O, 0.5 mL
31000-T	EvaGreen® Dye, 20X in water (trial size) 1 mL
31014-T	Fast Plus EvaGreen® qPCR Master Mix, high Rox (trial size, 100 rxn)
31015-T	Fast Plus EvaGreen® qPCR Master Mix, low Rox (trial size, 100 rxn)
31020-T	Fast Plus EvaGreen® qPCR Master Mix, no Rox (trial size, 100 rxn)

## GelGreen™ Nucleic Acid Gel Stain

## Cell Membrane Permeability of GelGreen™ and SYBR® Dyes

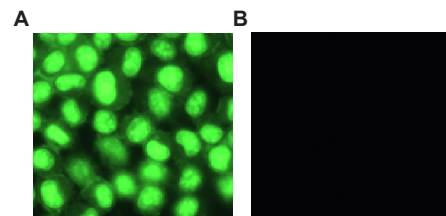


Figure 3. HeLa cells were incubated at 37°C with 1X of SYBR® Green I, SYBR® Safe, GelGreen™, respectively. Staining of mitochondria and nuclear DNA was observed with SYBR® Green I and SYBR® Safe within 5 minutes of incubation (not shown). After 30 minutes of incubation, SYBR® Green I stained cell nuclei with intense green fluorescence (panel A) while no cellular staining was visible with GelGreen™ (panel B). Images were taken using filters appropriate for each dye.

Please visit our website at [www.biotium.com](http://www.biotium.com) for information on our life science research products, including environmentally friendly EvaGreen® qPCR dye and qPCR master mixes, Cheetah™ chemically-modified hot-start Taq polymerase, Lumitein™ fluorescent gel stain, fluorescent CF™ dye antibody conjugates and reactive dyes, apoptosis reagents, fluorescent probes, and kits for cell biology research.

GelGreen and its uses are covered by US patent numbers 7960498, 7803943, and 8232050. SYBR is a registered trademark of Molecular Probes/Invitrogen; GelStar is registered trademark of FMC corporation; Dark Reader is a registered trademark of Clare Chemical Research. Materials from Biotium are sold for research use only, and are not intended for food, drug, household, or cosmetic use.