

# Product Information

## GelGreen Nucleic Acid Gel Stain, 10,000X in water

**Catalog Number:** RGB-4105

**Packaging Size:** 0.5 mL

### Storage and Handling:

GelGreen is a very stable dye. We recommend that you store the 10,000X solution in water at room temperature. The solution may also be stored at a lower temperature such as 4°C. Dye precipitation may occur during prolonged low temperature storage. When this occurs, heat up the solution in a hot water bath at 45°C to 50°C for two minutes and/or vortex the solution. The 1X and 3X working solutions of the dye may also be stored at room temperature in a dark place for at least one year. Exposure to light should be avoided during long-term storage. However, the dye can be handled under ambient light without any problem during staining experiment.

### Product Description:

GelGreen is a sensitive, stable and environmentally safe green fluorescent nucleic acid dye specifically designed for gel staining. GelGreen is far more sensitive than SYBR Safe (Figure 3). Unlike SYBR dyes, which are known to be unstable, GelGreen is very stable, both hydrolytically and thermally. Moreover, unlike the highly mutagenic EtBr and the reportedly mutation-enhancing SYBR Green I (Ohta et al Mutation Research 492, 91(2001)), GelGreen is shown to be nonmutagenic and noncytotoxic. A key reason for the observed low toxicity of GelGreen may be due to the dye's inability to cross cell membrane (Figure 2). GelGreen has sufficient 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).

As 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. Simply dilute the concentrated dye in 0.1M NaCl or water and incubate the gel in the diluted dye solution for 30 minutes. Although the post-staining method is recommended, precast gels can also be tried with GelGreen. However, some DNA samples, such as those derived from plasmid DNA digestion by certain restriction enzymes, may experience migration retardation or compromised resolution. Thus, both post-stained and precast procedures can be performed to determine which one may better meet your needs.

GelGreen can be used to stain either dsDNA or ssDNA or RNA in agarose gels. However, GelGreen is not recommended for staining DNA or RNA in polyacrylamide gels due to the dye's slow diffusion rate in the relatively tight polyacrylamide gel matrix.

**Note:** Although GelGreen can be used for staining single stranded nucleic acids, GelRed is more sensitive to ssDNA or RNA and is recommended for this application. GelRed is approximately 5 times more sensitive to single stranded nucleic acids than GelGreen.

Gel staining with GelGreen is compatible with downstream DNA manipulations such as digestion with a restriction enzyme, Southern blotting

techniques and clonings. GelGreen may be removed from DNA by ethanol precipitation.

GelGreen Nucleic Acid Gel Stain, 10,000X in water 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.

**Note:** GelGreen is not optimal for qPCR application, for which we recommend EvaGreen

### Staining Protocols

#### 1. Staining DNA by Post Gel Staining

- 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 with 0.1 M NaCl (e.g., add 15µL of GelGreen 10,000X stock reagent and 5 mL 1M NaCl to 45 mL H<sub>2</sub>O). While GelGreen1X staining solution can also be used for post gel staining, the sensitivity is generally less than with 3X staining solution.

**Note:** use of NaCl in the staining solution is optional. Including NaCl in the staining solution enhances the staining, but may promote dye precipitation if the staining solution is to be used repeatedly. Any staining solution to be reused is preferably stored at room temperature in a dark place to reduce possible dye precipitation problem.

- 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 View the stained gel with a 254 nm transilluminator, a Dark Reader or a similar transilluminator, or a laser-based gel scanner, and photograph the gel using any suitable imaging equipment. A long path green filter such as a SYBR filter or GelStar filter should be used for the photographing. (See figure 1 for GelGreen excitation and emission spectra).

#### 2. Staining DNA by Precasting GelGreen Gels

- 2.1 Prepare agarose gel solution using your standard protocol.
- 2.2 Dilute the GelGreen 10,000X stock reagent into the agarose gel solution at 1:10,000 (e.g., add 5µL of the GelGreen 10,000X stock reagent to 50 mL of the gel solution). Since GelGreen is generally thermally stable, the 10,000X stock reagent can be added while the gel solution is still hot, no need to wait for the gel solution to cool down prior to dye addition. Make sure that the dye is thoroughly mixed with the gel solution by swirling, stirring, or inversion.

Alternatively, the GelGreen stock reagent may be pre-combined with agarose powder and a buffer of your choice followed by microwaving or other heating procedures commonly used for preparing agarose gels. GelGreen is compatible with all commonly used electrophoresis buffers.

2.3 Cast the gels and allow it to solidify. Any leftover gel solution may be stored and re-heated later for additional gel casting. Since GelGreen is hydrolytically stable, GelGreen precast gels may be prepared in large quantities and stored for later use. To avoid mold formation, we recommend that the precast gels be stored in a refrigerator.

2.4 Load samples and run the gels using your standard protocol.

2.5 View the stained gel with a 254 nm transilluminator, a Dark Reader or a similar transilluminator, or a laser-based gel scanner, and photograph the gel using any suitable imaging equipment. A long path green filter such as a SYBR filter or GelStar filter should be used for the photographing. (See figure 1 for GelGreen excitation and emission spectra). (If you consistently see band smearing and/or poor band separation, run a post gel staining by following the protocol provided below to confirm if the problem is caused by the dye or other factors unrelated to the dye. If post gel staining is normal and the problem is not caused by the dye, try any of the followings: lower the amount of nucleic acid loaded; lower running voltage; lower the amount of agarose in the gel; run a longer gel; increase the thickness of the gel; increase gel solidification time to ensure sharp well formation; improve your sample loading technique or select post gel staining as your protocol. You may also try our GelRed which gives less DNA migration problem).

Note: Precasting GelGreen is not recommended for polyacrylamide gels. Use post gel staining for acrylamide gels.

#### Toxicity

GelGreen was subjected to a series of tests both by us and by three independent testing services to assess the dye's safety for routine handling and disposal. These tests include: 1) glove penetration test; 2) cell membrane permeability and cytotoxicity test; 3) Ames test; and 4) environmental safety tests. Test results confirm that the dye is impenetrable to both latex gloves and cell membranes. The dye is noncytotoxic and nonmutagenic at concentrations well above the working concentrations used in gel staining. GelGreen appears to be completely cell membrane-impermeable (Figure 2), which may be a key factor responsible for the observed low toxicity. However, since these tests were not performed on human, we still advise that researchers exercise precautions when handling the dye or any other DNA-binding molecules by wearing protective gears. For detailed test results on GelGreen, you may download a complete safety report at Biotium website.

#### Disposal

GelGreen has successfully passed environmental safety tests in compliance with CCR Title 22 Hazardous Waste Characterization. As a result, GelGreen is not classified as hazardous waste, thus can be safely disposed of down the drain or as regular trash, providing convenience and reducing cost in waste disposal. For detailed test results on GelGreen, you may download a complete safety report at Biotium website.

#### Spectral Characteristics

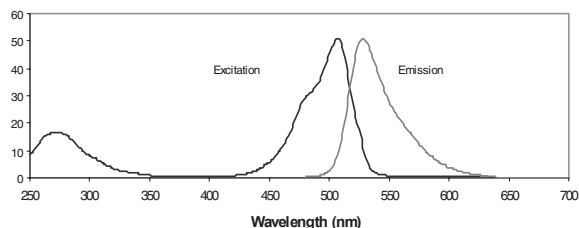


Figure 1. Excitation (left) and emission (right) spectra of GelGreen bound to dsDNA in TBE buffer.

#### Cell Membrane Permeability Comparison Between GelGreen™ and SYBR Dyes

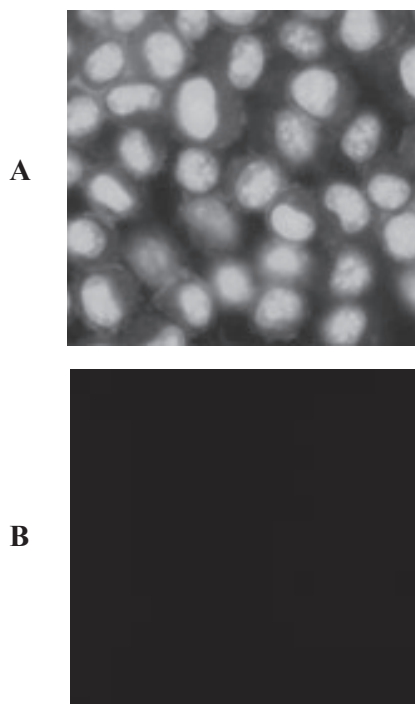


Figure 2. HeLa cells were incubated at 37°C with 1X of SYBR Green I, SYBR Safe, GelGreen, respectively. Staining of mitochondria and nuclear DNA was observed with SYBR Green I within 5 minutes of incubation. After 30 minutes of incubation, SYBR Green I stained cell nuclei with intense green fluorescence (panel A) while no cellular staining was visible with GelGreen™ (panel B) (only image from SYBR Green I is shown here). Images were taken using filters appropriate for each dye.

#### Comparison of GelGreen and SYBR Safe in post Gel Staining

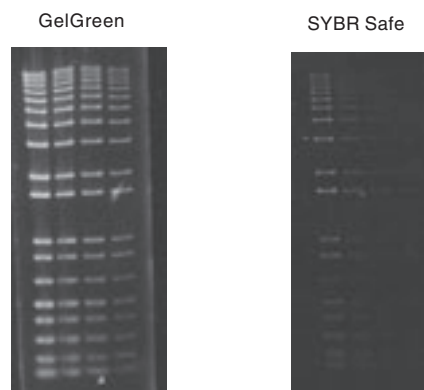


Figure 3. Comparison of GelGreen and SYBR Safe in post gel staining using 1% agarose gel in TBE buffer. Two fold serial dilutions of 1 kb Plus DNA Ladder from Invitrogen were loaded onto each gel in 4 lanes in the amounts of 200 ng, 100 ng, 50 ng and 25 ng, respectively, from left to right. Gels were imaged using 254-nm transilluminator and photographed with a SYBR filter and Polaroid 667 black-and-white print films.

#### Related Products:

- GelGreen Nucleic Acid Gel Stain at 10,000X in H<sub>2</sub>O, 10 mL
- GelGreen Nucleic Acid Gel Stain at 10,000X in DMSO, 0.5 mL
- GelRed Nucleic Acid Gel Stain at 10,000X in H<sub>2</sub>O, 0.5 mL
- GelRed Nucleic Acid Gel Stain at 10,000X in H<sub>2</sub>O, 10 mL
- GelRed Nucleic Acid Gel Stain at 10,000X in DMSO, 0.5 mL
- GelRed Nucleic Acid Gel Stain at 10,000X in DMSO, 10 mL
- GelRed Nucleic Acid Gel Stain, 3X in H<sub>2</sub>O, 4L: ready-to-use solution for post gel staining, or for precast gel staining after a 3-time dilution.

\* GelGreen™ and its uses are covered by pending US and international patents.

\*\* SYBR is a registered trademark of Molecular Probes, Inc. and GelStar is a registered trademark of FMC.