# GelGreen®

## Nucleic Acid Stain (10,000X)

RG-1550-01

For research use only

| Cat. No. | Vial Size | Conc. |
|---|---|---|
| RG-1550-01 | 200 µl | 10,000X |

## PRODUCT DATA SHEET

### 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 a blueBox™ transilluminator or a gel reader equipped with visible light excitation (such as a 488 nm laser-based gel scanner. GelGreen® is far more sensitive than SYBR® Safe. 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 by three independent testing services to assess the dye's safety for routine handling and disposal. Test results confirm that the dye does not penetrate latex gloves and cell membranes. 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.minipcr.com.

### REFERENCES

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

### SPECTRAL CHARACTERISTICS

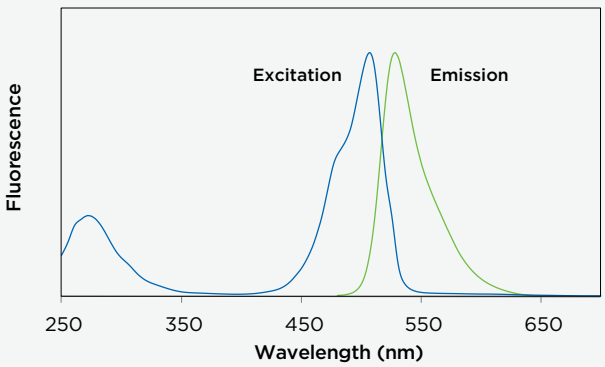


Figure 1. Excitation (left and emission (right) spectra of GelGreen® bound to dsDNA in TBE buffer.

### CONSIDERATIONS FOR STAINING

The following are general considerations for staining gels with GelGreen®. See the staining protocols on page 2 for step by step directions.

• GelGreen® can be used as precast in agarose

gels or post-staining protocols. Post-staining with GelGreen® is recommended and results in superior sensitivity and eliminates the possibility of dye interference with DNA migration.

- The precast protocol is not recommended for acrylamide gels. For acrylamide gels we recommend post-staining.
- 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.
- GelGreen® can be used with any commonly used loading buffer in precast and post-stained GelGreen® gels. We have had good results with 6X loading buffer containing 15% glycerol, 7.5% Ficoll® 400, 0.05% Bromophenol Blue. As a tracking dye we have also used 0.1% Patent Blue VF or 0.1% Orange G with good results.
  SDS in loading buffer may contribute to band smearing in precast GelGreen® gels. If this occurs, we recommend using thepost-staining protocol. It is not necessary to add GelGreen® to the loading or the running buffer.
- Recommended loading in precast gels is 50-200 ng DNA or ladder per lane, or 2-5 µL PCR product. If the DNA concentration is unknown, run 1/2 to 1/3 the amount you would load on an EtBr gel. If you need to load more DNA, use the post-staining protocol.
- GelGreen® can be imaged with a gel reader with visible light excitation such as the blueBox S or blueBox Pro transilluminators, or a similar transilluminator, or a laser-based gel scanner using a long path green filter such as a SYBR® filter or GelStar® filter. See the emission spectra for GelGreen® for specific wavelengths (Figure 1).
- While some facilities have approved the disposal of GelGreen® directly down the drain, please contact your safety office for local disposal guidelines.

## QUICK START PROTOCOLS

Although GelGreen® has undergone extensive safety testing, we recommend following universal safety precautions when working in the laboratory.

## POST-STAINING PROTOCOL

1. Run gels as usual according to your standard protocol.
2. Carefully place the gel in a suitable container such as a polypropylene staining tray. Add a sufficient amount of GelGreen® 3X staining solution to submerge the gel.
3. Agitate the gel gently at room temperature for ~30 minutes.
4. Destining is not required, although the gel can be washed in water to reduce background if necessary.
5. Image the gels with a blue light transilluminator (recommended) or a UV transilluminator.

## PRE-CAST PROTOCOL FOR AGAROSE GELS1.

1. Dilute GelGreen® to 1X concentration using concentrated electrophoresis buffer.
2. Add agarose powder and heat to dissolve. Make sure agarose and GelGreen® solution are thoroughly mixed.
3. Cast the gel.
4. Load samples and run the gels using your standard protocol.
5. Image the gels with a blue light transilluminator (recommended) or a UV transilluminator.

## DETAILED STAINING PROTOCOLS

### Post-staining Protocol

1. Run gels as usual according to your standard protocol.
2. Dilute the GelGreen® 10,000X stock reagent -3,300 fold to make a 3X staining solution in $H_2O$. **Note:** Including 0.1 M NaCl in the staining solution enhances sensitivity, but may promote dye precipitation if the gel stain is reused.
3. Carefully place the gel in a suitable polypropylene container. Gently add a sufficient amount of the 3X staining solution to submerge the gel.
4. Agitate the gel gently at room temperature for ~30 minutes.
5. Image the stained gel with a similar transilluminator, or a laser-based gel scanner using a long path green filter such as a SYBR® filter or GelStar® filter.
6. Staining solution can be reused at least 2-3 times. Store staining solution at room temperature protected from light.

## PRECAST PROTOCOL

**Note:** The precast protocol is not recommended for polyacrylamide gels. Use the post staining protocol for acrylamide gels.

1. Prepare molten agarose gel solution using your standard protocol.
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 solution is still hot.3. Cast the gel and allow it to solidify. Load samples and run the gels using your standard protocol.
3. Image the stained gel with a blue light transilluminator, or a laser-based gel scanner using a long path green filter such as a SYBR® filter or GelStar® filter.

## STORING GELGREEN® GELS

Leftover gel solution with GelGreen® may be stored at room temperature, protected from light, and re-heated later for additional gel casting. GelGreen® precast gels may be stored for later use for up to a month at room temperature in the dark. Storing GelGreen® precast gels at 4°C can result in dye precipitation and poor performance.

## TROUBLESHOOTING

| Problem | Suggestion |
|---|---|
| Smeared DNA bands in precast gel | 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.<br>2. Perform post-staining instead of pre-casting.<br>3. Pour a lower percentage agarose gel for better resolution of large fragments.<br>4. Change the running buffer. TBE buffer has a higher buffering capacity than TAE. |
| Discrepant DNA migration in pre-cast gel | 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.<br>1. Reduce the amount of DNA loaded by one-half to one-third.<br>2. Reduce the amount of dye used, i.e. use 0.5X in precast gels.<br>3. Post-stain gel in 3X GelGreen® to avoid any interference the dye may have on migration during electrophoresis. |
| Weak fluorescence, decreased dye performance over time, or film of dye remains on gel after post-staining | The dye may have precipitated out of solution.<br>1. Heat GelGreen® solution to 45-50°C for two minutes and vortex to redissolve.<br>2. Store dye at room temperature to avoid precipitation. |