GelGreen® Nucleic Acid Stain (10,000X, DMSO)

Cat. # SCT124

pack size: 0.5 mL

FOR RESEARCH USE ONLY.
NOT FOR USE IN DIAGNOSTIC PROCEDURES.
NOT FOR HUMAN OR ANIMAL CONSUMPTION.

Store at Room Temp

Data Sheet

Background

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.

The dye is noncytotoxic and non-mutagenic at concentrations well above the working concentrations used in gel staining. GelGreen® successfully passed environmental safety tests in compliance with CCR Title 22 Hazardous Waste Characterization, under which GelGreen® is not classified as hazardous waste.

Storage

GelGreen® is a very stable dye. Store 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. Protect From Light.

Spectral Properties

Absorbance: Standard Transilluminator (254 nm) such as a Dark Reader®
Emission: Long path green filter such as a SYBR® filter or GelStar® filter.

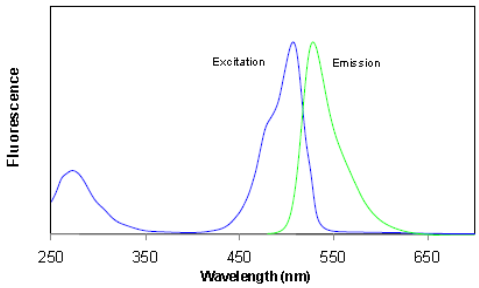

Figure 1. Excitation (left) and emission (right) spectra of GelGreen® bound to dsDNA in TBE.

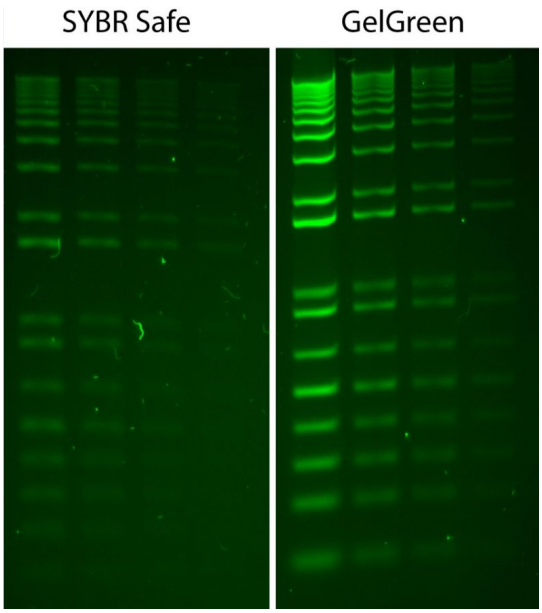

Figure 2. Comparison of ethidium bromide (EtBr) and GelGreen® in precast gel staining using 1% agarose gel in TBE buffer. Two-fold serial dilutions of 1 kb Plus DNA Ladder were loaded in the amounts of 200 ng, 100 ng, 50 ng and 25 ng from left to right. Gels were imaged using a 254 nm transilluminator and a Dark Reader.

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Assay Protocol

Because high affinity 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. Agarose gels can be precast with GelGreen®, however, GelGreen® may affect the migration or resolution of some DNA samples in precast gels.

Note: the precast protocol is not recommended for acrylamide gels. Use the post-staining protocol for acrylamide gels.

GelGreen® can be used to stain dsDNA, ssDNA or RNA, however GelGreen® is twice as sensitive for dsDNA than ssDNA or RNA. Gel staining with GelGreen® is compatible with downstream applications such as sequencing and cloning. GelGreen® is efficiently removed from DNA by phenol/chloroform extraction and ethanol precipitation.

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 H2O.

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 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.
6. Staining solution can be reused at least 2-3 times. Store staining solution at room temperature protected from light.

Precast Protocol for Agarose 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 gel solution is still hot.
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 4oC can result in dye precipitation and poor performance.
4. Load samples and run the gels using your standard protocol.
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.

Troubleshooting and FAQs

1. **Smeared DNA Bands.** Reduce the amount of DNA loaded by one-half to one-third. Perform post-staining instead of pre-casting. Pour a lower percentage agarose gel for better resolution of large fragments. Change the running buffer. TBE buffer has a higher buffering capacity than TAE. Loading buffers containing SDS may contribute to band smearing. If this occurs, use the post-staining protocol for applications requiring SDS-containing loading buffers.
2. **Weak Fluorescence.** The dye may have precipitated out of solution. Heat GelGreen® solution to 45-50oC for two minutes and vortex to redissolve. Store dye at room temperature to avoid precipitation.

3. **Q. Can GelGreen® be used to stain ssDNA or RNA? What is the detection limit?** A. GelGreen® can be used to stain ssDNA and RNA, but it is twice as sensitive for dsDNA than for ssDNA or RNA. GelGreen® is able to detect bands containing less than 0.1 ng DNA.
4. **Is GelGreen® compatible with downstream applications such as DNA cloning, ligation and sequencing, COMET assays, southern/northern blotting?** A. Yes