Protocol

TD-P Revision 2.0

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GelGreen™ Nucleic Acid Gel Stain, 10,000X

Procedure for staining dsDNA, ssDNA or RNA in gels

Introduction

GelGreen™ is a sensitive, stable and environmentally safe green fluorescent nucleic acid dye designed to stain either dsDNA, ssDNA or RNA in agarose gels. 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™ is compatible with either a 254 nm UV transilluminator or a gel reader equipped with visible light excitation (such as blue LED light box, 488 nm laser-based gel scanner, or Dark Reader®).

A series of safety tests have confirmed that GelGreen™ is noncytotoxic, nonmutagenic and nonhazardous at concentrations well above the working concentrations used in gel staining. As a result, GelGreen™ can be safely disposed of down the drain or in regular trash, providing convenience and reducing cost in waste disposal. Unlike the highly mutagenic EtBr and the reportedly mutation-enhancing SYBR® Green, GelGreen™ is safe 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 nonhazardous waste.

Note: The GelGreen™ stock in water is a newer and improved product compared to the stock in DMSO. We recommend using **GelGreen™ 10,000X 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.

Materials

- GelGreen™ Nucleic Acid Gel Stain, 10,000X in DMSO (Catalog # G-720)
- GelGreen™ Nucleic Acid Gel Stain, 10,000X in water (Catalog # G-725)

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Performance Properties

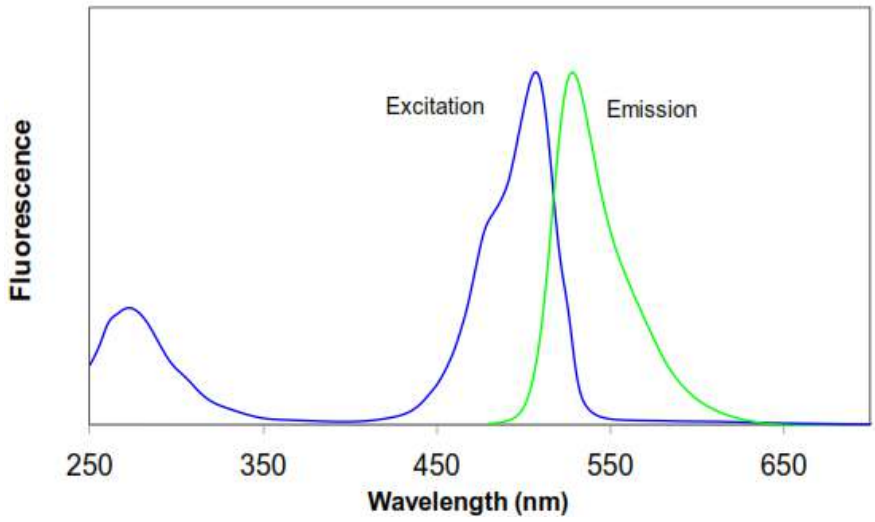

Figure 1. Excitation (left) and emission (right) spectra of GelGreen™ bound to dsDNA in TBE.

Method

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.

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 according to your standard protocol.

2. Dilute GelGreen™ 10,000X stock solution 3,300 fold to make a 3X staining solution in H_2O. Generally 50 ml staining solution is an adequate volume for one minigel.

   Note: Including 0.1M NaCl in the staining solution enhances sensitivity, but may promote dye precipitation if the gel stain is reused.

3. Place the gel in a suitable container such as a polypropylene staining tray. Add a sufficient amount of the 3X staining solution to submerge the gel.

4. Agitate the gel gently at room temperature for ~30 minutes.

   Note: Optimal staining time may vary somewhat depending on the thickness of the gel and the percentage of agarose.

   Note: Destaining is not required, but the gel can be washed in water to reduce background if necessary.

5. View the stained gel with a standard 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™ may be added while the gel solution is still hot.

3. Cast the gel and allow it to solidify.

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

5. View the stained gel with a standard 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. Unused agarose containing GelGreen™ can be remelted to cast more gels, but it may be necessary to add more dye for optimal signal. GelGreen gels may also be stored for later use, but may be prone to dye precipitation, in which case a post-stain should be performed. Precast gels containing GelGreen™ can be stored in the dark at 4°C.

Troubleshooting

Observation	Recommendation
Smeared DNA bands in precast gel	– 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.

	– 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.
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. – Reduce the amount of DNA loaded by one-half to one-third. – Reduce the amount of dye used, i.e. use 0.5X in precast gels. – 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. – Heat GelGreen™ solution to 45-50°C for two minutes and vortex to redissolve. – Store dye at room temperature to avoid precipitation.

Associated Products

GoldBio Catalog #	Product Name
A-201	Agarose LE (Molecular Biology Grade)
D010	1 kb DNA Ladder
D011	1 kb PLUS™ DNA Ladder
D001	100 bp DNA Ladder
P007	BLUEstain™ Protein ladder, 11-245 kDa
P008	BLUEstain™ 2 Protein ladder, 5-245 kDa
G-725	GelRed™ Nucleic Acid Stain Gel Stain, 10,000X in Water
E-670	EvaGreen® Dye, 20x in Water