Revised: January 27, 2021

Product Information

GelGreen® Nucleic Acid Gel Stain, 10,000X

Catalog no.	Product	Size
41004	GelGreen® 10,000X in DMSO	0.5 mL
41005-T	GelGreen® 10,000X in water	0.1 mL
41005	GelGreen® 10,000X in water	0.5 mL
41005-1	GelGreen® 10,000X in water	10 mL

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. 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 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.biotium.com.

References

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

Spectral Characteristics

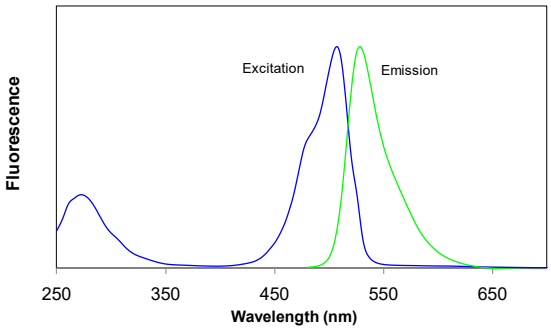

Figure 1. Excitation (left) and emission (right) spectra of GelGreen® bound to dsDNA in TBE buffer.

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. Biotium offers PAGE GelRed® which is especially formulated for staining the denser gel matrix in polyacrylamide gels.
- 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 (see Related Products).
- 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 the post-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 uL 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 254 nm transilluminator, or a gel reader with visible light excitation such as the Gel-Bright™ LED Gel Illuminator, 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. 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. GelGreen® can be adsorbed to activated charcoal (see Related Products) for disposal as chemical waste.

Quick Start Protocols

Although GelGreen® has undergone extensive safety testing, Biotium recommends 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. Destaining 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 Gels

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 254 nm transilluminator, the Gel-Bright™ LED Gel Illuminator (see related products), 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

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.
4. Image the stained gel with a 254 nm transilluminator, the Gel-Bright™ LED Gel Illuminator (see related products), 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.

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. Biotium offers a 1 kb ladder that has been optimized for use with GelGreen® (see related products below). 2. Perform post-staining instead of pre-casting. 3. Pour a lower percentage agarose gel for better resolution of large fragments. 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. 1. Reduce the amount of DNA loaded by one-half to one-third. 2. Reduce the amount of dye used, i.e. use 0.5X in precast gels. 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. 1. Heat GelGreen® solution to 45-50°C for two minutes and vortex to redissolve. 2. Store dye at room temperature to avoid precipitation.

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. Biotium's DNA Gel Extraction Kit (see Related Products), DNA gel extraction kits from Qiagen or Zymo, or phenol-chloroform extraction can be used to remove the dye from DNA.
Can GelGreen® be used for Comet Assay?	Yes, GelGreen® can be used for Comet assay.
Can GelGreen® be used in Capillary Gel Electrophoresis-Laser induced Fluorescence of double-stranded DNA fragments?	Yes, see Electrophoresis 34, 1555 (2013)
Is GelGreen® compatible with Southern or northern blotting?	GelGreen® has not been validated in blotting applications.
Why does my RNA or ssDNA appear yellowish-orange or pinkish with GelGreen®?	We and other users have often observed that GelGreen® stains ssDNA and RNA orange/ pink and dsDNA green. We have also seen that smaller dsDNA fragments can appear orange-pink, the color ranging from white-pink-orange. We are not sure about the underlying mechanism, possibly the structure of single-stranded nucleic acids favors an altered stacking interaction of GelGreen® monomers leading to the formation of J-aggregates that have red emission.
Can I reuse a GelGreen® precast gel after electrophoresis?	We do not recommend reusing GelGreen® precast gels as signal decreases with subsequent electrophoresis.
Can GelGreen® post-staining solution be reused?	Yes. However, if the sensitivity decreases, use a fresh solution of the dyes.
Can I make GelGreen® gels ahead of time and store them for later use?	You can store precast GelGreen® gels for up to a month. We recommend storing gels at room temperature in the dark. We no longer recommend storing gels at 4°C, because this can lead to dye precipitation and poor performance.
Can I re-melt gel with GelGreen® and cast again?	Yes, unused solidified agarose with GelGreen® can be remelted. If the unused agarose with dye is to be stored for more than a day or so, we recommend protecting it from light.
What is the stability of GelGreen® in molten agarose?	We do not recommend storing GelGreen® in molten agarose.
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 charcoal (see Related Products) 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.

Visit www.biotium.com for more FAQs and Tech Tips.

Related Products

Catalog number	Product
41028	Agarose LE, Ultrapure Molecular Biology Grade
41029	GelRed® Agarose LE
41030	GelGreen® Agarose LE
41008	PAGE GelRed® Nucleic Acid Gel Stain
41006	TBE Buffer, 5X (4L Cubitainer®)
99962-1	6X DNA Loading Buffer (Blue)
31022	Ready-to-Use 1 kb DNA Ladder
31032	Ready-to-Use 100 bp DNA Ladder
22007	Activated Charcoal Decontamination Bags
31030	DNA Gel Extraction Kit
41001	GelRed® Nucleic Acid Gel Stain, 3X in Water
41003	GelRed® Nucleic Acid Gel Stain, 10,000X in Water
41005	GelGreen® Nucleic Acid Gel Stain, 10,000X in Water
41020	DNAzure® Blue Nucleic Acid Gel Stain
41009	6X GelRed® Prestain Loading Buffer with Blue Tracking Dyes
41010	6X GelRed® Prestain Loading Buffer with Orange Tracking Dye
41011	GelRed® Prestain Plus 6X DNA Loading Dye
41024-4L	Water, Ultrapure Molecular Biology Grade (4L Cubitainer®)
31000-T	EvaGreen® Dye, 20X in water (trial size) 1 mL
31077-T	EvaGreen® Plus Dye, 20X in water (trial size) 1 mL
31020-T	Fast Plus EvaGreen® qPCR Master Mix (trial size, 100 rxn)
31066	AccuGreen™ High Sensitivity dsDNA Quantitation Kit for Qubit®
31069	AccuGreen™ Broad Range dsDNA Quantitation Kit for Qubit®
31028	AccuClear® Ultra High Sensitivity dsDNA Quantitation Kit
31073	AccuBlue® Broad Range RNA Quantitation Kit
31041	Forget-Me-Not™ EvaGreen® qPCR Master Mix (2-Color Tracking)
31043	Forget-Me-Not™ Universal Probe qPCR Master Mix
E90003	Gel-Bright™ LED Gel Illuminator

Please visit our website at www.biotium.com for information on our life science research products, including environmentally friendly EvaGreen® qPCR master mixes, fluorescent CF® dye antibody conjugates and reactive dyes, apoptosis reagents, fluorescent probes, and kits for cell biology research.

Materials from Biotium are sold for research use only, and are not intended for food, drug, household, or cosmetic use.