Revised: January 15, 2021

Product Information

GelRed® Nucleic Acid Gel Stain, 10,000X

Catalog no.	Product	Size
41002	GelRed® 10,000X in DMSO	0.5 mL
41002-1	GelRed® 10,000X in DMSO	10 mL
41003-T	GelRed® 10,000X in water	0.1 mL
41003	GelRed® 10,000X in water	0.5 mL
41003-1	GelRed® 10,000X in water	10 mL

Storage and Handling

GelRed® is a very stable dye. Store 10,000X solution and dilute solutions of GelRed® 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. GelRed® is stable for at least one year from the date it is received.

Product Description

GelRed® is a sensitive, stable and environmentally safe fluorescent nucleic acid dye designed to replace the highly toxic ethidium bromide (EtBr) for staining dsDNA, ssDNA or RNA in agarose gels or polyacrylamide gels. GelRed® has been shown to bind DNA exclusively by intercalation (1). GelRed® and EtBr have virtually the same spectra (Figure 1), so you can directly replace EtBr with GelRed® without changing your existing imaging system. In addition, GelRed® is far more sensitive than EtBr (Figure 2).

GelRed® 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 is impenetrable to both latex gloves and cell membranes. The dye is non-cytotoxic and non-mutagenic at concentrations well above the working concentrations used in gel staining. GelRed® successfully passed environmental safety tests in compliance with CCR Title 22 Hazardous Waste Characterization, under which GelRed® is not classified as hazardous waste. A complete safety report is available at www.biotium.com.

Although GelRed® has undergone extensive safety testing, Biotium recommends following universal safety precautions when working in the laboratory.

References

1. Eur. Biophys. J. 44, 1(2015)

Spectral Properties

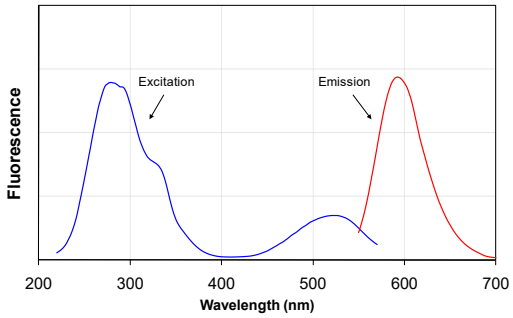

Figure 1. Excitation (left) and emission (right) spectra of GelRed® bound to dsDNA in TBE.

Considerations for Staining

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

- GelRed® can be used as precast in agarose gels or post-staining protocols. Post-staining with GelRed® 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 also offers PAGE GelRed® which is especially formulated for staining the denser gel matrix in polyacrylamide gels.
- GelRed® can be used with any commonly used loading buffer in precast and post-stained GelRed® 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 GelRed® gels. If this occurs, we recommend using the post-staining protocol. It is not necessary to add GelRed® 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.
- GelRed® can be imaged with a UV transilluminator and the EtBr filter. SYBR® or GelStar® filters also can be used for gel imaging with equally good results. See the emission spectra for GelRed® for specific wavelengths (Figure 1).
- While some facilities have approved the disposal of GelRed® directly down the drain, please contact your safety office for local disposal guidelines. GelRed® can be adsorbed to activated charcoal (see Related Products) for disposal as chemical waste.

Quick Start Protocols

Post-Staining Protocol

1. Run gels as usual according to your standard protocol.
2. Dilute GelRed® to 3X concentration using electrophoresis buffer.
3. Carefully place the gel in a suitable container such as a polypropylene staining tray. Add a sufficient amount of GelRed® 3X staining solution to submerge the gel.
4. Agitate the gel gently at room temperature for ~30 minutes.
5. Destaining is not required, although the gel can be washed in water to reduce background if necessary.
6. Image gels using an EtBr filter

Pre-cast Protocol for Agarose Gels

1. Dilute GelRed® to 1X concentration using concentrated electrophoresis buffer.
2. Add agarose powder and heat to dissolve. Make sure agarose and GelRed® solution are thoroughly mixed.
3. Cast the gel.
4. Load samples and run the gels using your standard protocol.
5. Image gels using an EtBr filter

Detailed Staining Protocols

Post-Staining Protocol

1. Run gels according to your standard protocol.
2. Dilute GelRed® 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 0.1 M 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. For polyacrylamide gels containing 3.5-10% acrylamide, typical staining time is 30 minutes to 1 hour with gels of higher acrylamide content requiring longer staining time. We also offer PAGE GelRed®, specifically designed for staining PAGE gels (see related products).
5. Destaining is not required, but the gel can be washed in water to reduce background if necessary.
6. View the stained gel with a standard transilluminator (302 or 312 nm) and image the gel using an ethidium bromide filter. SYBR® or GelStar® filters also may be used for gel imaging with equally good results.
7. Staining solution can be reused at least 2-3 times. Store staining solution at room temperature protected from light.

Pre-cast Protocol for Agarose Gels

Note: The precast protocol is not recommended for polyacrylamide gels. Polyacrylamide gels can be stained using the post-stain protocol. We also offer PAGE GelRed®, specifically designed for staining PAGE gels (see related products).

1. Prepare molten agarose gel solution using your standard protocol.
2. Dilute the GelRed® 10,000X stock reagent into the molten agarose gel solution at 1:10,000 and mix thoroughly. GelRed® can 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 using a standard transilluminator (302 or 312 nm) and image the gel using an ethidium bromide filter. SYBR® or GelStar® filters also can be used for gel imaging with equally good results.

Storing GelRed® gels

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

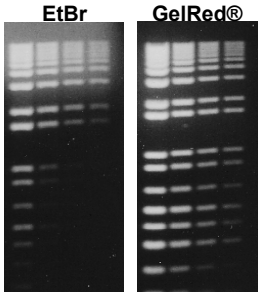

Figure 2. Comparison of ethidium bromide (EtBr) and GelRed® in precast gel staining using 1% agarose gel in TBE buffer. Two-fold serial dilutions of 1 kb Plus DNA Ladder (Invitrogen) were loaded in the amounts of 200 ng, 100 ng, 50 ng and 25 ng from left to right. Gels were imaged using 300 nm transilluminator and photographed with an EtBr filter and Polaroid 667 black-and-white print film.

Troubleshooting

Problem	Suggestion
Smeared DNA bands in precast gel	1. Reduce the amount of DNA loaded by 1/2 to 1/3. GelRed® is much more sensitive than EtBr. 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 GelRed® (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. 5. Loading buffers containing SDS may contribute to band smearing. If this occurs, use the post-staining protocol for applications requiring SDS-containing loading buffers.
Discrepant DNA migration in pre-cast gel	GelRed® 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 1/2 to 1/3. 2. Reduce the amount of dye used, i.e. use 0.5X in precast gels. 3. Post-stain gel in 3X GelRed® 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 GelRed® solution to 45-50°C for two minutes and vortex to redissolve. 2. Store dye at room temperature to avoid precipitation.

Frequently Asked Questions	Answers
Can GelRed® be used to stain ssDNA or RNA?	GelRed® can be used to stain ssDNA and RNA, but it is twice as sensitive for dsDNA than for ssDNA or RNA.
Is GelRed® compatible with downstream applications such as cloning, ligation and sequencing?	Yes. Biotium's DNA Gel Extraction Kit (see Related Products), gel extraction kits from Qiagen or Zymo, or phenol-chloroform extraction can be used to remove the dye from DNA. Some users have reported performing PCR on DNA in the presence of GelRed® with no purification step, for example by incubating GelRed®-stained gel slices in TE buffer to extract DNA by passive diffusion for use in PCR, or by using a few microliters of molten agarose from GelRed®-stained gel slices containing DNA for PCR.
Can GelRed® be used for formaldehyde, polyacrylamide, DGGE, EMSA or PFGE (pulse-field) gels?	Yes. Customers have reported using GelRed® in glyoxal and formaldehyde agarose gels for pre-cast staining of RNA. Use the post-staining protocol for polyacrylamide, DGGE, and EMSA gels. For PFGE gels, the pre-cast or post-staining protocol may be used.
Can GelRed® be used for COMET assay?	Yes, GelRed® can be used for COMET assay by post-staining.
Can GelRed® be used in cesium chloride gradients?	Customers have reported using GelRed® in cesium gradients. To extract GelRed® from DNA after cesium banding, we recommend adding SDS to a final concentration of 0.1% before butanol extraction. Alternatively, chloroform can be used instead of butanol for extraction.
Is GelRed® compatible with Southern or northern blotting?	GelRed® has been validated for Southern blotting (see Plant Cell Rep. 31,167 (2012)). We recommend using the post-staining protocol for blotting applications.
Is GelRed® compatible with alkaline gel running buffer (30mM NaOH, 1mM EDTA)?	Yes, GelRed® is compatible with alkaline running buffer.
What emission filters are suitable for use with GelRed®?	Use the ethidium bromide filter for GelRed®. SYBR® or GelStar® filters also can be used for gel imaging with equally good results. Please review the emission spectra for GelRed® for specific wavelengths.
Can I reuse a GelRed® precast gel after electrophoresis?	We do not recommend reusing GelRed® precast gels as signal decreases with subsequent electrophoresis.
Can I make GelRed® gels ahead of time and store them for later use?	You can store precast GelRed® gels for up to a week, and 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 GelRed® post-staining solution be reused?	Yes. However, if the sensitivity decreases, use a fresh solution of the dyes.
Can I re-melt gel with GelRed® and cast again?	Yes, unused solidified agarose with GelRed® or 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 GelRed® in molten agarose?	We do not recommend storing GelRed® in molten agarose for more than a few days.
What is the lower detection limit of GelRed®?	Some users have reported being able to detect bands containing less than 0.1 ng DNA. However, the limit of detection will depend on instrument capability and exposure settings.
What is the chemical structure of GelRed®?	The chemical structure of GelRed® is proprietary.
Does GelRed® migrate during electrophoresis?	GelRed® does not migrate through the gel as easily as EtBr. It is not necessary to add dye to the running buffer, and the gel will be stained more homogeneously with GelRed® than with EtBr.
Does GelRed® need to be used in the dark?	GelRed® is very stable. You can use the dye in room light, however we recommend storing the dye in the dark.
I accidentally left my GelRed® in the light. Will it still work?	While we recommend that you protect the dye from light during long-term storage, we have had a customer report using GelRed® with success after accidentally leaving it in ambient light for one month.
Is there a difference between 10,000X GelRed® in DMSO and water?	The GelRed® stock in water is a newer and improved product compared to the stock in DMSO. We recommend using GelRed® in water to avoid the potential hazards of handling DMSO, a solvent that can be absorbed through the skin. We continue to offer GelRed® 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.

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