

## PRODUCT INFORMATION

### GelRed™ Nucleic Acid Gel Stain, 10,000X

#### Catalog Numbers:

41002 (in DMSO)  
41003 (in water)

**Packaging Sizes:** 0.1 mL (41003-T), 0.5 mL (41002 and 41003), 10 mL (41002-1 and 41003-1)

#### 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™ 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 noncytotoxic and nonmutagenic 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](http://www.biotium.com).

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

#### Spectral Properties

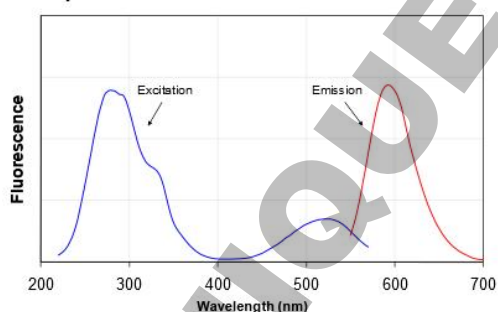


Figure 1. Excitation (left) and emission (right) spectra of GelRed™ bound to dsDNA in TBE.

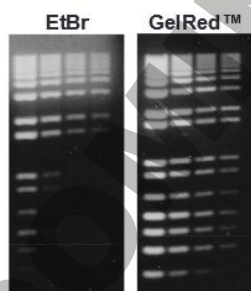


Figure 2. Comparison of ethidium bromide (EtBr) and GelRed™ in precast gel staining using 1% agarose gel in TBE buffer. Two-fold serial dilutions of 1 kb Plus DNA Ladder (Invitrogen) were loaded in the amounts of 200 ng, 100 ng, 50 ng and 25 ng from left to right. Gels were imaged using 300 nm transilluminator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

#### Staining Protocols

Because high affinity nucleic acid binding dyes can affect DNA migration during electrophoresis, post-staining of gels is highly recommended. Post-staining with GelRed™ results in superior sensitivity and eliminates the possibility of dye interference with DNA migration. Agarose gels can be precast with GelRed™, however, GelRed™ may affect the migration or resolution of some DNA samples in precast gels. The precast protocol is not recommended for polyacrylamide gels.

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

#### 1. Post-Staining Protocol

- 1.1 Run gels according to your standard protocol.
- 1.2 Dilute GelRed™ 10,000X stock solution 3,300 fold to make a 3X staining solution in H<sub>2</sub>O. 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.
- 1.3 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.
- 1.4 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.
- 1.5 Destaining is not required, but the gel can be washed in water to reduce background if necessary.
- 1.6 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.
- 1.7 Staining solution can be reused at least 2-3 times. Store staining solution at room temperature protected from light.

#### 2. Precast Protocol for Agarose Gels

- 2.1 Prepare molten agarose gel solution using your standard protocol.  
**Note: the precast protocol is not recommended for polyacrylamide gels. Polyacrylamide gels can be stained using the post-stain protocol.**
- 2.2 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.
- 2.3 Cast the gel and allow it to solidify.
- 2.4 Load samples and run the gels using your standard protocol.
- 2.5 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.
- 2.6 Unused agarose containing GelRed™ can be remelted to cast more gels, but it may be necessary to add more dye for optimal signal. We do not recommend storing agarose containing GelRed™ in molten form (i.e., at 50°C) for more than a few days. Precast gels containing GelRed™ can be stored at 4°C for future use.

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## Troubleshooting

Problem	Suggestion
Smear DNA bands in precast gel	<ol style="list-style-type: none"> <li>1. Reduce the amount of DNA loaded by one-half to one-third. 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).</li> <li>2. Perform post-staining instead of pre-casting.</li> <li>3. Pour a lower percentage agarose gel for better resolution of large fragments.</li> <li>4. Change the running buffer. TBE buffer has a higher buffering capacity than TAE.</li> <li>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.</li> </ol>
Discrepant DNA migration in pre-cast gel	<p>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.</p> <ol style="list-style-type: none"> <li>1. Reduce the amount of DNA loaded by one-half to one-third.</li> <li>2. Reduce the amount of dye used, i.e. use 0.5X in precast gels.</li> <li>3. Post-stain gel in 3X GelRed to avoid any interference the dye may have on migration during electrophoresis.</li> </ol>
Weak fluorescence, decreased dye performance over time, or film of dye remains on gel after post-staining	<p>The dye may have precipitated out of solution.</p> <ol style="list-style-type: none"> <li>1. Heat GelRed solution to 45-50°C for two minutes and vortex to redissolve.</li> <li>2. Store dye at room temperature to avoid precipitation.</li> </ol>

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. We recommend Qiagen or Zymoclean gel extraction kits or phenol-chloroform extraction 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 precast 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 add 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 (Plant Cell Report doi:10.1007/s00299-011-1150-7). We recommend using the post-staining protocol for blotting applications.
What loading buffers are compatible with GelRed?	We routinely use 6X loading buffer containing 15% glycerol, 7.5% Ficoll 400, 0.05% Bromophenol Blue, and 0.1% Patent Blue VF. In internal testing, 6X loading buffer containing 0.1% Orange G produced good results in precast and post-stained GelRed gels. SDS in loading buffer may contribute to band smearing in precast GelRed gels. If this occurs, we recommend using the post-staining protocol.
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.
How should I dispose of GelRed?	GelRed has passed the EPA regulated Title 22 test. Some facilities have approved the disposal of GelRed directly down the drain. However, because regulations vary, please contact your safety office for local disposal guidelines. GelRed can be adsorbed to activated carbon (also known as activated charcoal) for disposal as chemical waste.
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 binding mechanism of GelRed?	GelRed has been shown to bind DNA exclusively by intercalation ( <a href="http://link.springer.com/article/10.1007/s00249-014-0995-4">http://link.springer.com/article/10.1007/s00249-014-0995-4</a> ).
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.

### Related Products:

Catalog No.	Product
31021	1 KB DNA Ladder (100ng/ul), 30 ug/300 ul
31022	Ready-to-Use 1 KB DNA Ladder, 150 applications (1.5 ml)
41001	GelRed™ Nucleic Acid Gel Stain, 3X in H <sub>2</sub> O, 4L
41005	GelGreen™ Nucleic Acid Gel Stain at 10,000X in H <sub>2</sub> O, 0.5 mL
31000-T	EvaGreen® Dye, 20X in water (trial size) 1 mL
31020-T	Fast Plus EvaGreen® qPCR Master Mix (trial size, 100 rxn)

Please visit our website at [www.biotium.com](http://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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