Revised: April 30, 2013

Product Information

6X GelRed™ Prestain Loading Buffers

Catalog Numbers

41009 6X GelRed Prestain Loading Buffer with Blue Tracking Dyes
41010 6X GelRed Prestain Loading Buffer with Orange Tracking Dye

Unit Size: 1 mL

Storage and Handling

Store at 4°C, protected from light. Product is stable for at least 6 months from date of receipt when stored as recommended. The dye can be handled under ambient light during sample preparation and electrophoresis without affecting product performance.

Product Description

GelRed™ is a sensitive, stable and environmentally safe fluorescent nucleic acid dyes designed to replace the highly toxic ethidium bromide (EtBr). 6X GelRed Prestain Loading Buffers are gel loading buffers containing density agents, tracking dyes, and GelRed dye. The 6X prestain loading buffer is added to samples in place of gel loading buffer, and eliminates the need to add fluorescent DNA dye to the agarose gel during casting. See frequently asked questions, next page, for a comparison of prestain, precast, and post-stain protocols for GelRed.

Prestain buffer with blue tracking dyes (cat. no. 41009) contains two blue electrophoresis tracking dyes that run at approximately 1.5 kb and 200 bp in a 1% agarose gel. Prestain buffer with orange tracking dye (cat. no. 41010) contains an orange electrophoresis tracking dye that runs at approximately 50 bp in a 1% agarose gel.

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, which cannot be used in DNA loading buffer to prestain DNA. GelRed is compatible with downstream applications such as sequencing and cloning. GelRed is efficiently removed from DNA by commercial gel extraction kits or by phenol/chloroform extraction and ethanol precipitation.

GelRed Safety

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 the dye is not classified as hazardous waste. A complete safety report is available at www.biotium.com.

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

Product Protocol

Note: The optimal loading amount of DNA is 50-200 ng DNA per lane. For samples of unknown DNA concentration, we recommend loading 1/2 or less the volume you would normally run on an ethidium bromide precast gel. Loading more than the recommended amount of DNA may result in smearing or smiling of bands or shifted band migration (see Figure 2). If you need to run more than the recommended amount of DNA per lane, or if highly accurate sizing of DNA fragments is required, we recommend using catalog number 41001 or 41003 (GelRed 3X in water or 10,000X in water) to stain gels after electrophoresis using the post-staining procedure. See Table 1 for a comparison of GelRed staining methods.

1. Prepare agarose gel according to your standard protocol. Do not add ethidium bromide, GelRed, or any other fluorescent DNA dye to the agarose or buffer.
2. Briefly vortex 6X GelRed Prestain Loading Buffer. Add 6X buffer to DNA samples at a volume ratio of 1:5 (for example, mix 10 uL sample + 2 uL 6X loading buffer). For best results, the 10 uL sample should contain between 50-100 ng DNA for ladder, or 10-20 ng DNA per band.
3. Load samples and run gels according to your standard protocol.
4. Visualize bands using a UV transilluminator or other gel documentation system. Gels can be imaged using an ethidium bromide emission filter. SYBR® Green or GelStar™ filters also can be used for gel imaging with equally good results.

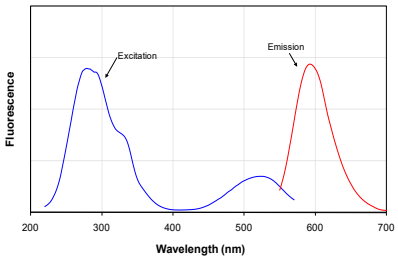

Figure 1. Excitation and emission spectra of GelRed dye in the presence of dsDNA.

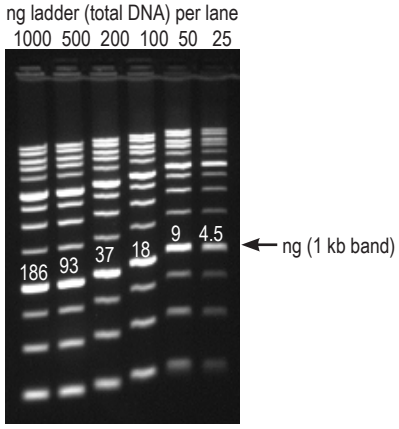

Figure 2. Varying amounts of Biotium's 1 kb ladder (cat. no. 31021) were prestained using 6X GelRed Loading Dye and run on a 1% TBE agarose gel in 1X TBE at 100V for 90 minutes. Gel was imaged using a GelDoc-It imaging system from UVP with an ethidium bromide filter. Total ladder amount loaded is listed above each lane; mass of the 1 kb band is shown above the band. See product protocol for DNA loading recommendations.

Table 1. Comparison of GelRed™ staining methods

Method	Catalog No.	Procedure	Recommended for	Advantages	Disadvantages
Prestain	41009 41010	GelRed loading buffer is added directly to the sample	• Rapid, routine analysis of less than 200 ng DNA per well.	• Rapid results	• GelRed can alter band migration • Overloading of DNA causes band smearing or smiling
Precast	41003 GelRed, 10,000X in water -or- 41001 GelRed, 3X in water	GelRed is added to molten agarose at 1X concentration before casting the gel			
Post-stain		No fluorescent dye is added during electrophoresis. The gel is stained in 3X GelRed solution after electrophoresis.	• Highly accurate band sizing • If more than 200 ng DNA per well must be loaded • Staining restriction enzyme digested DNA	• Sharpest bands and highly accurate sizing • Staining solution can be re-used • Addition of sodium chloride to staining solution enhances sensitivity	• 30 minute staining step required after electrophoresis • Note: some customers have reported good results after only five minutes of staining when using fresh dye solution

Frequently Asked Questions	Answers
What is the difference between GelRed and GelGreen?	GelRed is spectrally similar to ethidium bromide, and can be imaged using UV excitation. GelGreen is spectrally similar to SYBR Green or SYBR Safe, and can be used with either UV or blue light excitation.
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. Gel extraction or phenol-chloroform extraction can be used to remove dye from DNA. Biotium's gel extraction kit (catalog no. 31030) has been validated for removal of GelRed and GelGreen from DNA. Gel extraction kits from other suppliers also have be used with GelRed and GelGreen.
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, EMSA, and PFGE gels.
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 catalog no. 41003 (10,000X GelRed in water) and following the post-staining protocol for blotting applications.
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. If required, GelRed can be adsorbed by activated charcoal bags (catalog no. 22007) 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 most likely binds by a combination of intercalation and electrostatic interaction.
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.
Can I reuse a GelRed precast gel after electrophoresis?	We do not recommend reusing GelRed precast gels as signal decreases with subsequent electrophoresis.
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 10,000X GelRed in water?	GelRed in water is the newer and improved product. We recommend using GelRed, 10,000X in water (catalog no. 41003) to avoid 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

Cat. No.	Description
41001	GelRed™ Nucleic Acid Gel Stain, 3X in H_2O, 4L
41003	GelRed™ Nucleic Acid Gel Stain, 10,000X in H_2O, 0.5 mL
41005	GelGreen™ Nucleic Acid Gel Stain, 10,000X in H_2O, 0.5 mL
31030	DNA Gel Extraction Kit, 50 or 250 columns
31021	1 KB DNA Ladder (100 ng/uL), 30 ug/300 ul
31022	Ready-to-Use 1 KB DNA Ladder, 150 applications (1.5 ml)
31031	100 bp DNA Ladder (100 ng/uL), 30 ug/300 ul
31032	Ready-to-Use 100 bp DNA Ladder, 150 applications (1.5 ml)
41006	TBE Buffer, 5X, 4L