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Biotium

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Revised: August 8, 2006

Product Information

GelRed™ Nucleic Acid Gel Stain, 10,000X in DMSO

Catalog Number: 41002

Packaging Size: 0.5 mL

Storage and Handling

GelRed™ is a very stable dye. The 10,000X solution in DMSO can be safely stored at room temperature for at least one year from the time of receiving the material. The 1X and 3X working solutions of the dye may be stored at room temperature in a dark place for at least one year. Although not necessary, GelRed™ solutions may also be stored at a lower temperature. Exposure to light should be avoided during long-term storage. However, the dye can be handled under ambient light without any problem during staining experiment.

Product Description

GelRed™ is a sensitive, stable and relatively safe fluorescent nucleic acid dye designed to replace the highly toxic ethidium bromide (EB) for staining dsDNA or ssDNA in agarose gels or polyacrylamide gels. GelRed™ is far more sensitive than EB without requiring a destaining step. GelRed™ and EB have virtually the same spectra (**Figure 1**), so you can directly replace EB with GelRed™ without changing your existing imaging system. If you have been using a green fluorescent gel stain such as SYBR® Green I or GelStar® with a UV transilluminator for viewing gels, you may replace the dye and continue to use the existing SYBR® filter or GelStar® filter for photographing. However, GelRed™ can not be sufficiently excited with a 488 nm argon laser or similar visible light and therefore is not recommended for use with a gel reader equipped with such visible light. In such cases, we recommend that you use our GelGreen™(Cat# 41004), which is spectrally similar to and is as sensitive as SYBR® Green I in gel staining but is far more stable than the latter.

GelRed™ can be used for either precast agarose gel staining or post agarose gel staining. In general, post gel staining gives better sensitivity than precast gel staining, and eliminates any possibility of dye interference with DNA migration. Post staining with GelRed™ is simple, requiring no destaining and no special buffer. Simply dilute the concentrated dye in 0.1 M NaCl and incubate the gel in the diluted dye solution for 30 minutes, followed by viewing the gel. The staining solution is perfectly stable at room temperature (**Figure 2**), permitting it to be used multiple times. On the other hand, precast gel staining is both simpler and more economical than post gel staining because it does not need an extra staining step and uses less dye. Precast agarose gel staining using GelRed™ is substantially more sensitive than that using EB (**Figure 3**). GelRed™ typically has minimal effect on DNA migration (**Figure 4**). However, in some rare cases, some DNA samples derived from plasmid DNA digestion by certain restriction enzymes may experience somewhat more migration retardation or compromised resolution. Thus, we highly recommend that you try both precast and post gel staining procedures to determine which one may better meet your needs.

GelRed™ can also be used to stain dsDNA or ssDNA in polyacrylamide gel via post gel staining. Precast polyacrylamide gel staining with GelRed™ is not recommended because of relatively high background fluorescence.

In addition to its remarkable sensitivity and stability, toxicity test by an independent laboratory has shown that GelRed™ is nonmutagenic and noncytotoxic at concentrations used for gel staining. A key aspect of GelRed™ safety is that the dye appears to be completely cell membrane-impermeable, compared to SYBR® Green I, which enters cells rapidly (**Figure 5**). For more details on the safety of GelRed™, please go to Biotium website to download the complete safety report.

Gel staining with GelRed™ is compatible with downstream DNA manipulations such as digestion with a restriction enzyme, Southern blotting techniques and clonings. GelRed™ may be removed from DNA by ethanol precipitation.

GelRed™ Nucleic Acid Gel Stain, 10,000X in DMSO is a concentrated GelRed™ solution that can be diluted **10,000** times for use in precast gel staining or ~**3,300** times for use in post gel staining according to the procedures described below. One vial (0.5 mL) of 10,000X solution can be used to prepare at least 100 precast minigels or post-stain at least 100 minigels.

GelRed™ is also available in the following formats: GelRed™ 10,000X in H_2O, 0.5mL (cat# 41003); GelRed™ 10,000X in DMF, 0.5mL (cat# 41000); and GelRed™ 3X in H_2O, 4L (cat# 41001).

Staining Protocols

1. Staining DNA by Precasting GelRed™ Gels*

1.1 Prepare agarose gel solution using your standard protocol.

1.2 Dilute the GelRed™ 10,000X stock reagent into the agarose gel solution at 1:10,000 (e.g., 5 µL of the GelRed™ 10,000X stock reagent added to 50 mL of the gel solution). Since GelRed™ is generally thermally stable, the 10,000X stock reagent can be added while the gel solution is still hot (no need to wait for the gel solution to cool down prior to dye addition). Make sure that the dye is thoroughly mixed with the gel solution by swirling, stirring, or inversion.

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Alternatively, the GelRed™ stock reagent may be pre-combined with agarose powder and a buffer of your choice followed by microwaving or other heating procedures commonly used for preparing agarose gels. GelRed™ is compatible with all commonly used electrophoresis buffers.

1.3 Cast the gel and allow it to solidify. Any leftover gel solution may be stored and re-heated later for additional gel casting. Since GelRed™ is hydrolytically stable (See **Figure 2**), GelRed™ precast gels may be prepared in large quantities and stored for later use. To avoid mold formation, we recommend that the precast gels be stored in a refrigerator.

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

1.5 View the stained gel using a standard transilluminator (302 or 312 nm) and photograph the gel using Polaroid 667 films and an ethidium bromide filter. Since the fluorescence is in the red wavelength

region, a SYBR® or GelStar® filter can also be used for photographing with equally good results (See figure 1 for GelRed™ excitation and emission spectra). (*If you consistently see band smearing and/or poor band separation, run a post gel staining by following the protocol provided below to confirm if the problem is caused by the dye or other factors unrelated to the dye. If post gel staining is normal and the problem is not caused by the dye, try any of the followings: lower the amount of agarose in the gel; lower the amount of nucleic acid loaded; run a longer gel; increase the thickness of the gel; improv your sample loading technique or select post gel staining as your protocol. In general,* GelRed™ *or GelGreen™ affects DNA migration much less than SYBR® Green I does as shown in* ***Figure 3***).

**Precasting GelRed™ gel is not suitable for acrylamide gels. Use post gel staining for acrylamide gels.*

2. Staining DNA by Post Gel Staining

2.1 Run gels as usual according to your standard protocol.

2.2 Dilute the GelRed™ 10,000X stock reagent ~3,300 fold to make a 3X staining solution in H_2O with 0.1 M NaCl (e.g., add 15 µL of GelRed™ 10,000X stock reagent and 5 mL 1M NaCl to 45 mL H_2O). While GelRed™ 1X staining solution can also be used for post gel staining, the sensitivity is generally less than with 3X staining solution..

2.3 Carefully place the gel in a suitable container such as a polypropylene container. Gently add a sufficient amount of the 3X staining solution to submerge the gel.

2.4 Agitate the gel gently at room temperature for ~30 minutes. 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 min to 1 hour with gels of higher acrylamide content requiring longer staining time. The staining solution can be reused at least 2-3 times. The unused staining solution can be stored at room temperature in a dark place.

2.5 View the stained gel with a standard transilluminator (302 or 312 nm) and photograph the gel using Polaroid 667 films and an ethidium bromide filter. Similarly, a SYBR® or GelStar® filter may also be used for photographing with equally good results.

Toxicity

Ames test performed by an independent lab, Litron Laboratories (Rochester, NY), showed that GelRed™ is not mutagenic at concentrations used for gel staining and is only weakly mutagenic following metabolic activation at 18.5 mg/mL, which is more than 3 times higher than the 3X concentration used for GelRed™ post gel staining. Tests also showed that GelRed™ is noncytotoxic. GelRed™ appears to be completely cell membrane-impermeable(**Figure 5**), which may be a key factor reponsible for the observed low toxicity. However, since these tests were not performed on human, we still advise that researchers excercise precautions when handling the dye or any other DNA-binding molecules by wearing protective gears. For more information on the Ames test result, you may download a complete report at Biotium website.

Disposal

Whether GelRed™ waste solution can be directly poured into the drain may depend on local regulations despite its nonmutagenicity and noncytotoxicity. Alternatively, GelRed™ solution may be disposed of using one of the following methods: 1) Add 25~50 mL bleach (regular household bleach) to each gallon (~4L) of the waste staining solution and let the mixture react for at least 8 hours before pouring the solution to a sink (Practically, you may simply accumulate your GelRed™ waste solution in a jar containing appropriate amount of bleach); 2) Pour each 10 liters of GelRed™ waste solution through ~1g of activated charcoal. The filtrate may directly go to the drain while the charcoal may be treated as regular solid waste. For pre-cast gels, you can simply let the gels dry out first and then let the dried waste go in regular trash.

Spectral Characteristics

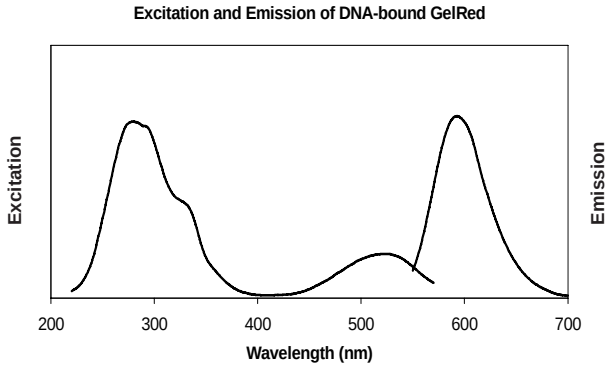

Figure 1. Excitation (left) and emission (right) spectra of GelRed™ bound to dsDNA in TBE buffer.

Stability in Staining Buffer

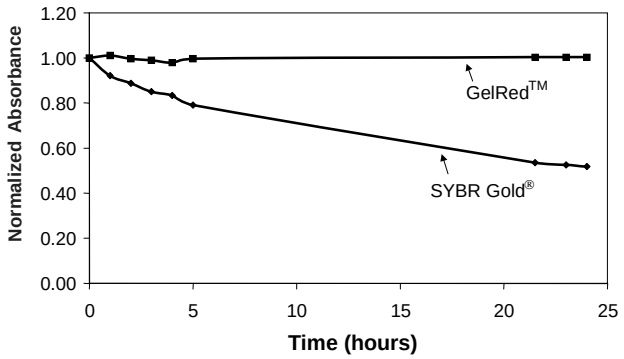

Figure 2. Normalized absorbances of GelRed™ and SYBR® Gold 1X TBE gel-staining solutions at 500 and 488 nm respectively over time at room temperature. The starting absorbance values for GelRed™ and SYBR® Gold were 0.029 and 0.051, respectively. GelRed™ 1X or 3X working solution has been shown to be stable at room temperature for at least one year.

Comparison with EB in Precast Gel Staining

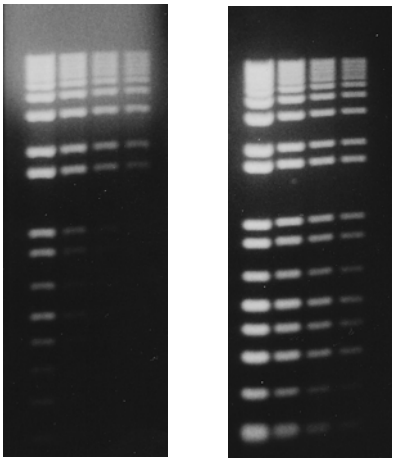

Figure 3. Comparison of GelRed™ and ethidium bromide (EB) in precast gel staining using 1% agaose gel in TBE buffer. Two-fold serial dilution of 1 kb Plus DNA Ladder from Invitrogen were loaded onto each gel in 4 lanes in the amounts of 200 ng, 100 ng and 50 ng, respectively, from left to right. Gels were imaged using 300-nm transillumination and photographed with an EB filter and Polaroid 667 black-and-white print films.

Effect of GelRed™ in DNA Migration in Agarose Gels

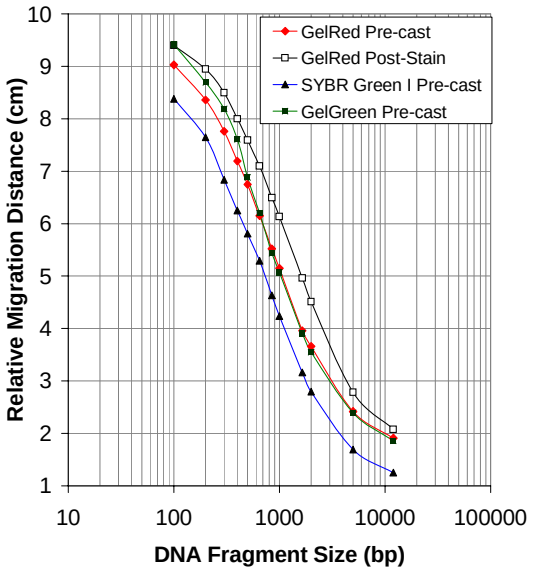

Figure 4. Plot of relative migration distance of dsDNA in various 1% agarose precast gels vs DNA size. Precast gels containing each of the 3 dyes, GelRed™, GelGreen™ and SYBR® Green I, each at 1x concentration were compared. The curve representing DNA migration distance in the gel post-stained with GelRed vs DNA size was used as a reference. The data shows that GelRed™ and GelGreen™ have less effect on DNA migratioin than SYBR Green I does.

Cell Memberane Permeability Comparison Between GelRed™ and SYBR® Green I

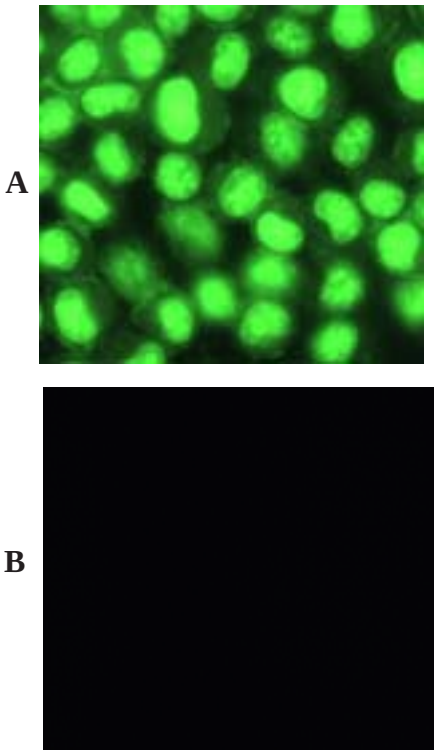

Figure 5. HeLa cells were incubated with either GelRed™ or SYBR® Green I at 1.2 µM dye concentration. Staining of mitochondria and nuclear DNA were observed with SYBR® Green I within 5 minutes of incubation. After 30 minutes of incubation, SYBR® Green stained cell nuclei with intense green fluorescence (panel A) while no cellular staining was visible with GelRed™ (panel B). Images were taken using filters appropriate for each dye.

Related Products:

GelRed™ Nucleic Acid Gel Stain at 10,000 in H_2O, 0.5 mL
GelRed™ Nucleic Acid Gel Stain at 10,000 in DMF, 0.5 mL
GelRed™ Nucleic Acid Gel Stain, 3X in H_2O: ready-to-use solution for post gel staining, or for precast gel staining after a 3-time dilution
GelGreen™ Nucleic Acid Gel Stain at 10,000 in DMSO, 0.5 mL: for gel readers equipped with visible light excitation.

* GelRed™ and its uses are covered by pending US and international patents.
** SYBR® is a registered trademark of Molecular Probes, Inc. and GelStar® is a registered trademark of FMC.