

# Product Information

## GelRed Nucleic Acid Gel Stain, 10,000X in Water

**Catalog Number:** RGB-4103

**Packaging Size:** 0.5 mL

### Storage and Handling

GelRed is a very stable dye. We recommend that you store the 10,000X solution in water at room temperature. The solution may also be stored at a lower temperature such as 4°C. Dye precipitation may occur during prolonged low temperature storage. When this occurs, heat up the solution in a hot water bath at 45°C to 50°C for two minutes and/or vortex the solution. The 1X and 3X working solutions of the dye may also be stored at room temperature in a dark place for at least one year. 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 environmentally safe fluorescent nucleic acid dye designed to replace the highly toxic ethidium bromide (EB) for staining dsDNA, ssDNA or RNA 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 use a green fluorescent gel stain such as SYBR Green I, SYBR Safe 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 #: RGB-4105) which is spectrally similar to and is as sensitive as SYBR Green I in gel staining but is far more stable than the latter.

As 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. Post staining with GelRed is simple, requiring no destaining and no special buffer. Simply dilute the concentrated dye in 0.1 M NaCl or water and incubate the gel in the diluted dye solution for 30 minutes. The staining solution is perfectly stable at room temperature, permitting it to be used multiple times, and is substantially more sensitive than that using EB (Figure 2). Although the post-staining method is recommended, precast gels can also be tried with GelRed. However, some DNA samples, such as those derived from plasmid DNA digestion by certain restriction enzymes, may experience migration retardation or compromised resolution. Thus, both the post-stained and precast gels can be performed to determine which one may better meet your needs.

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

Note: GelRed is twice as sensitive to dsDNA than ssDNA or RNA.

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 water 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.

### Staining Protocols

#### 1. Staining DNA by Post Gel Staining

- 1.1 Run gels as usual according to your standard protocol.
- 1.2 Dilute the GelRed 10,000X stock reagent ~3,300 fold to make a 3X staining solution in H<sub>2</sub>O with 0.1 M NaCl (e.g., add 15µL of GelRed 10,000X stock reagent and 5 mL 1M NaCl to 45 mL H<sub>2</sub>O). While GelRed™ 1X staining solution can also be used for post gel staining, the sensitivity is generally less than with 3X staining solution. (Note: inspect the 10,000X vial carefully. If dye precipitation occurs, heat up or sonicate the vial.)  
  
Note: use of NaCl in the staining solution is optional. Including NaCl in the staining solution enhances the staining, but may promote dye precipitation if the staining solution is to be used repeatedly. Any staining solution to be reused is preferably stored at room temperature in a dark place to reduce possible dye precipitation problem.
- 1.3 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.
- 1.4 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.
- 1.5 View the stained gel with a standard transilluminator (302 or 312 nm) and photograph the gel using an ethidium bromide filter. Similarly, a SYBR or GelStar filter may also be used for photographing with equally good results.

#### 2. Staining DNA by Precasting GelRed Gels\*

- 2.1 Prepare agarose gel solution using your standard protocol.
- 2.2 Dilute the GelRed 10,000X stock reagent into the agarose gel solution at 1:10,000 (e.g., 5µL 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. (Note: inspect the 10,000X vial carefully. If dye precipitation occurs, heat up or sonicate the vial)

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.

- 2.3 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, 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.
- 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 photograph the gel using 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 gel and post-stain 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 nucleic acid loaded; lower running voltage; lower the amount of agarose in the gel; run a longer gel; increase the thickness of the gel; increase gel solidification time to ensure sharp well formation; improve your sample loading technique or select post gel staining as your protocol.

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

#### Toxicity

GelRed was subjected to a series of tests both by us and by three independent testing services to assess the dye's safety for routine handling and disposal. These tests include: 1) glove penetration test; 2) cell membrane permeability and cytotoxicity test; 3) Ames test; and 4) environmental safety tests. 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 appears to be completely cell membrane-impermeable, which may be a key factor responsible for the observed low toxicity. However, since these tests were not performed on human, we still advise that researchers exercise precautions when handling the dye or any other DNA-binding molecules by wearing protective gears. For detailed test results on GelRed, you may download a complete safety report at Biotium website.

#### Disposal

GelRed has successfully passed environmental safety tests in compliance with CCR Title 22 Hazardous Waste Characterization. As a result, GelRed is not classified as hazardous waste, thus can be safely disposed of down the drain or as regular trash, providing convenience and reducing cost in waste disposal. For detailed test results on GelRed, you may download a complete safety report at our website.

## Spectral Characteristics

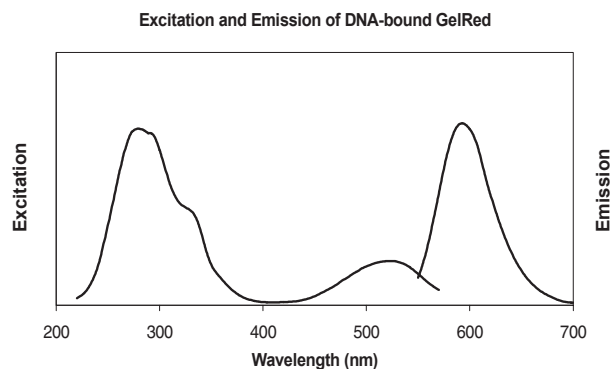


Figure 1. Excitation (left) and emission (right) spectra of GelRed bound to dsDNA in TBE buffer.

## Comparison with EB in Precast Gel Staining

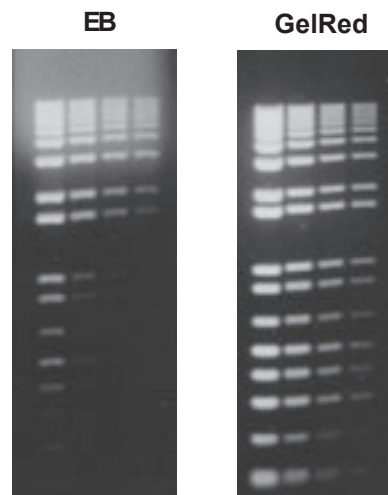


Figure 2. Comparison of ethidium bromide (EB) and GelRed in precast gel staining using 1% agarose gel in TBE buffer. Two-fold serial dilutions of 1 kb Plus DNA Ladder from Invitrogen were loaded onto each gel in 4 lanes in the amounts of 200 ng, 100 ng, 50 ng and 25 ng, respectively, from left to right. Gels were imaged using 300-nm transilluminator and photographed with an EB filter and Polaroid 667 black-and-white print films.

#### Related Products:

- GelRed Nucleic Acid Gel Stain at 10,000X in H<sub>2</sub>O, 10 mL
- GelRed Nucleic Acid Gel Stain at 10,000X in DMSO, 0.5 mL
- GelRed Nucleic Acid Gel Stain at 10,000X in DMSO, 10 mL
- GelRed Nucleic Acid Gel Stain, 3X in H<sub>2</sub>O, 4L: ready-to-use solution for post gel staining, or for precast gel staining after a 3-time dilution
- GelGreen Nucleic Acid Gel Stain at 10,000X in H<sub>2</sub>O, 0.5 mL
- GelGreen Nucleic Acid Gel Stain at 10,000X in H<sub>2</sub>O, 10 mL
- GelGreen Nucleic Acid Gel Stain at 10,000X 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.