GelRed® Nucleic Acid Stain (10,000X, Water)

Cat. # SCT123

pack size: 0.5 mL

FOR RESEARCH USE ONLY.
NOT FOR USE IN DIAGNOSTIC PROCEDURES.
NOT FOR HUMAN OR ANIMAL CONSUMPTION.

Store at Room Temp

Data Sheet

Background

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).

The dye is noncytotoxic 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.

Storage

GelRed® is a very stable dye. Store 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. Protect From Light.

Spectral Properties

Absorbance: Standard Transilluminator (302 or 312 nm)
Emission: Ethidium Bromide, SYBR or GelStar Filter

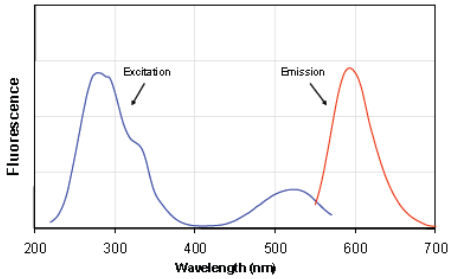

Figure 1. Excitation (left) and emission (right) spectra of GelRed® bound to dsDNA in TBE.

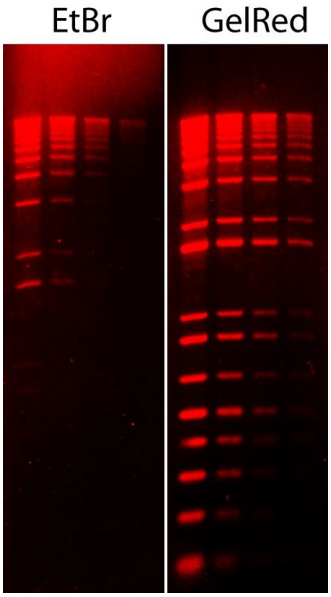

Figure 2. Comparison of ethidium bromide (EtBr) and GelRed® in precast gel staining using 1% agarose gel in TBE buffer. Two-fold serial dilutions of 1 kb Plus DNA Ladder were loaded in the amounts of 200 ng, 100 ng, 50 ng and 25 ng from left to right. Gels were imaged using 300 nm transilluminator and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

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Assay Protocol

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.

Note: the precast protocol is not recommended for acrylamide gels. Use the post-staining protocol for acrylamide 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.

Post Staining Protocol

1. Run gels as usual according to your standard protocol.
2. Dilute GelRed® 10,000X stock solution 3,300 fold to make a 3X staining solution in H2O. 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.

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.

Precast Protocol for Agarose Gels

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. 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.
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 for future use for up to a week. We recommend storing gels at room temperature in the dark. Storage of GelRed® precast gels at 4°C can cause dye precipitation and poor performance

Troubleshooting and FAQs

1. **Smeared DNA Bands.** Reduce the amount of DNA loaded by one-half to one-third. Perform post-staining instead of pre-casting. Pour a lower percentage agarose gel for better resolution of large fragments. Change the running buffer. TBE buffer has a higher buffering capacity than TAE. Loading buffers containing SDS may contribute to band smearing. If this occurs, use the post-staining protocol for applications requiring SDS-containing loading buffers.
2. **Weak Fluorescence.** The dye may have precipitated out of solution. Heat GelRed® solution to 45-50oC for two minutes and vortex to redissolve. Store dye at room temperature to avoid precipitation.

3. **Q. Can GelRed® be used to stain ssDNA or RNA? What is the detection limit?** A. GelRed® can be used to stain ssDNA and RNA, but it is twice as sensitive for dsDNA than for ssDNA or RNA. GelRed® is able to detect bands containing less than 0.1 ng DNA.
4. **Is GelRed® compatible with downstream applications such as DNA cloning, ligation and sequencing, COMET assays, southern/northern blotting?** A. Yes