GelRed® Nucleic Acid Stain (3X, Water)

Cat. # SCT121

pack size: 4L

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

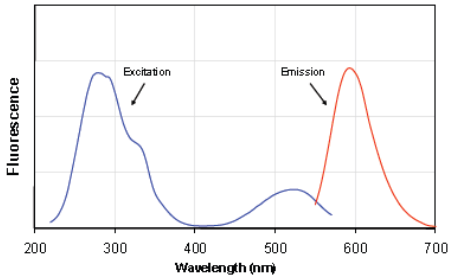

Figure 1. Excitation (left) and emission (right) spectra of GelRed® bound to dsDNA in TBE.

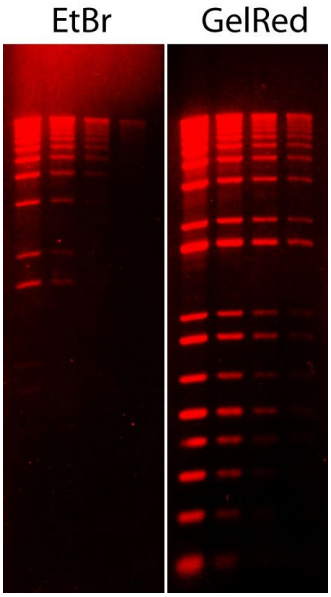

Figure 2. Comparison of ethidium bromide (EtBr) and GelRed® in precast gel staining using 1% agarose gel in TBE buffer. Two-fold serial dilutions of 1 kb Plus DNA Ladder were loaded in the amounts of 200 ng, 100 ng, 50 ng and 25 ng from left to right. Gels were imaged using 300 nm transilluminator and 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. Carefully place the gel in a suitable container such as a polypropylene staining tray. Add a sufficient amount of GelRed® 3X staining solution to submerge the gel.

Optional: NaCl can be added to GelRed® 3X staining solution at a final concentration of 0.1 M to improve sensitivity. Including NaCl in the staining solution enhances GelRed® staining but may promote dye precipitation if the staining solution is used repeatedly.

3. 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.
4. Destaining is not required, although the gel can be washed in water to reduce background if necessary.
5. 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.
6. Staining solution can be reused at least 2-3 times. Store staining at room temperature protected from light.

Precast Protocol for Agarose Gels

1. Dilute GelRed® 3X solution to 1X concentration using concentrated electrophoresis buffer of your choice (e.g., mix 20 mL of 3X GelRed® with 6 mL of 10X TBE buffer and 34 mL of H2O to make 60 mL of 1X GelRed in 1X TBE).
2. Add agarose powder to GelRed® 1X solution at the required concentration and heat to dissolve according to your standard protocol. Make sure agarose and GelRed® solution are thoroughly mixed.
3. Cast the gel and allow it to solidify. Unused gel solution may be stored and re-heated to cast additional gels. 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.
4. Load samples and run the gels using your standard protocol.
5. 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.

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