Protocol

TD-P Revision 2.0

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GelRed™ Nucleic Acid Gel Stain, 10,000X

Procedure for staining dsDNA, ssDNA or RNA in gels

Introduction

GelRed™ is an ultra-sensitive, extremely stable and environmentally safe fluorescent nucleic acid dye designed to replace the highly toxic ethidium bromide (EtBr) for agarose gels or polyacrylamide gels. GelRed™ is far more sensitive than EtBr without requiring a destaining step and since GelRed™ and EtBr have virtually the same spectra, you can easily replace EtBr with GelRed™ without changing your existing imaging system. GelRed™ can be used to stain dsDNA, ssDNA or RNA in agarose gels via either precast or post gel staining or can be used to stain polyacrylamide gels via post gel staining. GelRed™ is also compatible with downstream DNA manipulations such as restriction digest, sequencing, and cloning.

A series of safety tests have confirmed that GelRed™ is noncytotoxic, nonmutagenic and nonhazardous at concentrations well above the working concentrations used in gel staining. As a result, GelRed™ can be safely disposed of down the drain or in regular trash, providing convenience and reducing cost in waste disposal.

Note: The GelRed™ stock in water is a newer and improved product compared to the stock in DMSO. We recommend using **GelRed™ 10,000X in Water** to avoid the potential hazards of 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.

Performance Properties

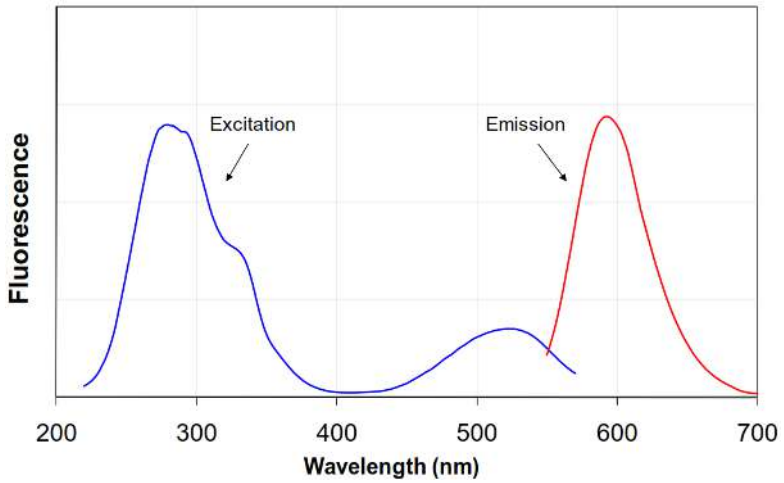

Figure 1. Excitation (left) and emission (right) spectra of GelRed™ bound to dsDNA in TBE.

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Materials

- GelRed™ Nucleic Acid Gel Stain, 10,000X in DMSO (Catalog # G-720)
- GelRed™ Nucleic Acid Gel Stain, 10,000X in water (Catalog # G-725)

Method

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. The precast protocol is not recommended for polyacrylamide gels.

GelRed™ can be used to stain dsDNA, ssDNA or RNA. GelRed is twice as sensitive for dsDNA than it is for 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 according to your standard protocol.

2. Dilute GelRed™ 10,000X stock solution 3,300 fold to make a 3X staining solution in H_2O. Generally 50 ml staining solution is an adequate volume for one minigel.

   Note: Including 0.1M 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.

   Note: Destaining is not required, but 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 EtBr 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 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™ may 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 with a standard transilluminator (302 or 312 nm) and image the gel using an EtBr filter. SYBR® or GelStar® filters also may 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 at 4°C for future use.

Troubleshooting

Observation	Recommendation
Smeared DNA bands in precast gel	– Reduce the amount of DNA loaded by one-half to one-third (<400 ng). GelRed™ is much more sensitive than EtBr. Blown out or smeared bands can be caused by overloading. This is frequently observed with DNA ladders. – 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.
Discrepant DNA migration in pre-cast gel	– GelRed™ is designed to be larger than other dyes to prevent it from entering cells, thus rendering the dye safer. The migration of DNA may be affected depending on the dye:DNA ratio. – Reduce the amount of DNA loaded by one-half to one-third. – Reduce the amount of dye used, i.e. use 0.5X in precast gels. – Post-stain gel in 3X GelRed to avoid any interference the dye may have on migration during electrophoresis.
Weak fluorescence, decreased dye performance over time, or film of dye remains on gel after post-staining	– The dye may have precipitated out of solution. – Heat GelRed™ solution to 45-50°C for two minutes and vortex to redissolve. – Store dye at room temperature to avoid precipitation.

Associated Products

GoldBio Catalog #	Product Name
A-201	Agarose LE (Molecular Biology Grade)
D010	1 kb DNA Ladder
D011	1 kb PLUS™ DNA Ladder
D001	100 bp DNA Ladder
P007	BLUEstain™ Protein ladder, 11-245 kDa
P008	BLUEstain™ 2 Protein ladder, 5-245 kDa
G-745	GelGreen™ Nucleic Acid Stain Gel Stain, 10,000X in Water
E-670	EvaGreen® Dye, 20x in Water