Product Information

Super GelRed® Nucleic Acid Gel Stain, 10,000×

Catalog Number: S2001 (in water) S2002 (in DMSO)

Product Size: 0.5 mL

Ethidium bromide (EB) has been the stain of choice for nucleic acid gel staining for decades. The dye is inexpensive, sufficiently sensitive and very stable. However, EB is also a known powerful mutagen. It poses a major health hazard to the user, and efforts in decontamination and waste disposal ultimately make the dye expensive to use. To overcome the toxicity problem of EB, scientists at US Everbright Inc. developed Super GelRed® and Super GelGreen® nucleic acid gel stains as superior alternatives. Extensive tests demonstrate that both dyes have significantly improved safety profiles over EB. Super GelRed® and Super GelGreen® passed 1) glove penetration test; 2) cell membrane permeability and cytotoxicity test; 3) Ames test; and 4) environmental safety tests. All these data showed Super GelRed® and Super GelGreen® are non-toxic, nonmutagenic and can be safely released into the environment when the concentration at 3× or lower.

Although Super GelRed® has undergone extensive safety testing, US Everbright Inc. recommends following universal safety precautions when working in the laboratory.

Storage and Handling

Super GelRed® is a very stable dye. Store 10,000× solution and dilute solutions of Super GelRed® at room temperature. 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 ℃ for two minutes and vortex. Super GelRed® is stable for at least ten years from the date it is received.

Description

Super 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. Super GelRed® and EtBr have virtually the same spectra (Figure 1), so you can directly replace EtBr with Super GelRed® without changing your existing imaging system. In addition, Super GelRed® is far more sensitive than EtBr (Figure 2).

Spectral Properties

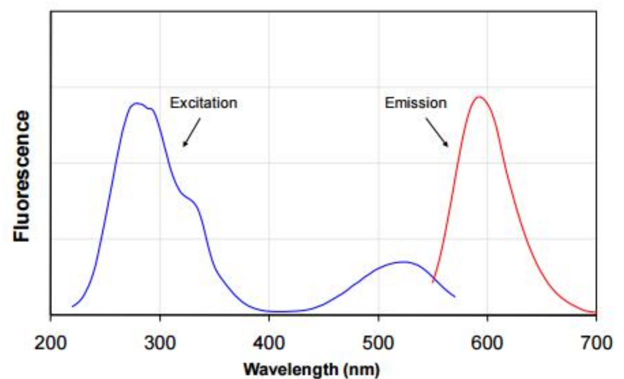

Figure 1. Excitation (left) and emission (right) spectra of Super GelRed® bound to dsDNA in TBE.

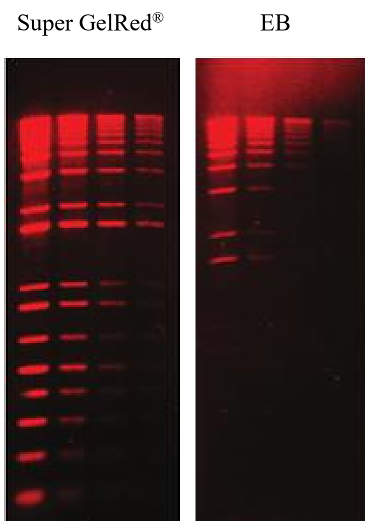

Figure 2. Comparison of ethidium bromide (EtBr) and Super GelRed® in precast gel staining using 1% agarose gel in TBE buffer. Two-fold serial dilutions of 1kb DNA Ladder were loaded in the amounts of 200 ng, 100 ng, 50 ng and 25 ng from left to right. Gels were imaged using 300 nm transilluminator

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and photographed with an EtBr filter.

Protocol

Because high affinity nucleic acid binding dyes can affect DNA migration during electrophoresis, post-staining of gels is highly recommended. Post-staining with Super GelRed® results in superior sensitivity and eliminates the possibility of dye interference with DNA migration. Agarose gels can be precast with Super GelRed®, however, Super GelRed® may affect the migration or resolution of some DNA samples in precast gels.

Super GelRed® can be used to stain dsDNA, ssDNA or RNA, however Super GelRed® is twice as sensitive for dsDNA than ssDNA or RNA. Gel staining with Super GelRed® is compatible with downstream applications such as sequencing and cloning. Super GelRed® is not recommended for polyacrylamide gels, We highly recommend to use Super Page GelRed (S2005 US Everbright Inc.) for polyacrylamide gels. Super 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 Super GelRed® 10,000× stock solution 3,300 fold to make a 3× staining solution in H_2O containing 0.1 M NaCl. Generally 50 mL staining solution is an adequate volume for one minigel. (i.e., add 15 µL Super GelRed® 10,000× to 50mL H_2O containing 0.1 M NaCl.)
3. Place the gel in a suitable container such as a polypropylene staining tray. Add a sufficient amount of the 3× 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.

Highly recommended:

In order to save time, the 3× staining solution can be pre-heated to a suitable temperature, and then placed the gel, and incubated for ~10 minutes to obtain a desired effect. The heating method can be water bath or microwave heating method, the specific steps are as follows:

a). Water bath heating method: Put the 3× staining solution into a 70-75℃ water bath to preheat it. Then put the gel into it and incubate for 5-10 minutes. During the dyeing process, you can gently shake it several times to accelerate the binding of dye and nucleic acid.

b). Microwave heating method: put the container (involving gel and the 3× staining solution) into the microwave and heat it for 30s, and then take out the container and agitate the gel gently at room temperature for 30 s to 1 min. After the temperature of the staining solution drops, the microwave heating is performed for 15 s. then shake the gel, repeat 4-5 times (note the time of microwave heating cannot be too long to prevent the gel melt).

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 solution at room temperature.

Precast Protocol for Agarose Gels

1. Prepare molten agarose gel solution using your standard protocol.
2. Dilute the Super GelRed® 10,000× stock reagent into the molten agarose gel solution at 1:10,000 and mix thoroughly. (i.e., add 5µL Super GelRed® 10,000× to 50mL gel solution) Super 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 Super 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 Super GelRed® in molten form (i.e., at 50 ℃) for more than a few days. Precast gels containing Super GelRed® can be stored for future use for up to a week. We recommend storing gels at room temperature. Storage of Super GelRed® precast gels at 4℃ can cause dye precipitation and poor performance.

Notes

1. Due to the high sensitivity of Super GelRed® and eliminating

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the possibility of dye interference with DNA migaration in precast, it is recommend to reduce the amount of DNA loaded. We recommend to load 50-200 ng/lane (small lanes of 8-lanes) for known concentrations of sample. For samples with unknown concentration, Try 1/3 or 1/5 of the commonly used sample volume. Appropriately increase or decrease the sample volume according to the size of the gel hole.

2. For better results, it is recommended to replace TAE with 1×TBE buffer, because the borate-containing reagents are more conductive. The voltage during electrophoresis should not be too high. Generally, TBE should not exceed 120V. TAE should not exceed 100 V.

Troubleshooting

Problem	Suggestion
Smeared DNA bands in precast gel	1. Reduce the amount of DNA loaded by one-third to one-fifth. Super GelRed® is much more sensitive than EtBr. Blown out or smeared bands can be caused by overloading. This is frequently observed with DNA ladders. 2. Perform post-staining instead of pre-casting. 3. Pour a lower percentage agarose gel for better resolution of large fragments. 4. Change the running buffer. TBE buffer has a higher buffering capacity than TAE. 5. 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	Super 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. 1. Reduce the amount of DNA loaded by one-third to one-fifth. 2. Post-stain gel in 3× Super 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. 1. Heat Super GelRed® solution to 45-50℃ for two minutes and vortex to redissolve. 2. Store dye at room temperature to avoid precipitation.

Frequently Asked Questions	Answers
Can Super GelRed® be used to stain ssDNA or RNA?	Super GelRed® can be used to stain ssDNA and RNA, but it is twice as sensitive for dsDNA than for ssDNA or RNA.
What emission filters are suitable for use with Super GelRed®?	Use the ethidium bromide filter for Super GelRed®. SYBR or GelStar filters also can be used for gel imaging with equally good results. Please review the emission spectra for Super GelRed® for specific wavelengths.
Can I reuse a Super GelRed® precast gel after electrophoresis?	We do not recommend reusing Super GelRed® precast gels as signal decreases with subsequent electrophoresis.
How should I dispose of Super GelRed®?	Some facilities have approved the disposal of Super GelRed® directly down the drain. However, because regulations vary, please contact your safety office for local disposal guidelines. Super Super GelRed® can be adsorbed to activated carbon (also known as activated charcoal) for disposal as chemical waste.
What is the lower detection limit of Super	Some users have reported being able to detect bands containing less than 0.1 ng

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GelRed®?	DNA. However, the limit of detection will depend on instrument capability and exposure settings.
What is the chemical structure of Super GelRed®?	The chemical structure of Super GelRed® is proprietary.
What is the binding mechanism of Super GelRed®?	Super GelRed® has been shown to bind DNA exclusively by electrostatic attraction.
Does Super GelRed® migrate during electrophoresis?	Super GelRed® does not migrate through the gel as easily as EtBr. It is not necessary to add dye to the running buffer, and the gel will be stained more homogeneously with Super GelRed® than with EtBr.
Does Super GelRed® need to be used in the dark?	Super GelRed® is very stable. You can use the dye in room light.
Is there a difference between 10,000× Super GelRed® in DMSO and water?	The Super GelRed® stock in water is a newer and improved product compared to the stock in DMSO. We recommend using Super GelRed® in water to avoid the potential hazards of handling DMSO, a solvent that can be absorbed through the skin. We continue to offer Super GelRed® in DMSO because some users do not wish to alter their established laboratory protocols.

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