

## Nucleic Acids Staining in Gels with GelRed

GelRed is a fluorescent nucleic acid dye that could be used instead of ethidium bromide (EtBr), [dsSafe<sup>®</sup>](#), and others for staining dsDNA, ssDNA, or RNA in agarose gels or polyacrylamide (PAAG) gels. GelRed is more stable, more sensitive, and less toxic than EtBr (because it is cell membrane-impermeant) and doesn't require a destaining step. GelRed has been validated for Southern blotting.

GelRed and EtBr have virtually identical spectra (absorbance 300 nm, emission 605 nm), so one can directly replace EtBr with GelRed without changing the existing imaging system. GelRed is also compatible with downstream DNA manipulations such as restriction digest, sequencing, and cloning.

### Handling and disposal

Store 10,000× and working GelRed solutions at room temperature or at 4 °C in a place protected from light.

Storing at 4 °C may cause precipitation. If this occurs, heat the solution to 45–50 °C for two minutes and vortex.

GelRed is stable for at least one year from the date of receipt.

### Gel soaking

1. Perform electrophoresis of the sample(s) in agarose or polyacrylamide gel.
2. Measure out the required volume of 1× TAE or 1× TBE into a plastic container. To stain 20 mL of agarose gel, 35–50 mL of dye solution is sufficient.
3. Add dye concentrate to the buffer in a ratio of 1:10,000. Mix thoroughly.

*(Optional)* To increase sensitivity, the original reagent can be diluted ~3,300-fold to obtain a 3× staining solution. Adding 0.1 M NaCl to the staining solution also increases sensitivity but may cause dye precipitation when reusing the gel.

4. Place the gel in a plastic tray (the lid from a box of tips or a container for storing household products).  
*Important!* Avoid using a glass container, as the dye may be absorbed by its walls, leading to insufficient gel coloring.
5. Add enough dye solution to cover the gel completely.
6. Cover the gel and staining solution with aluminum foil or place them in a dark place to protect them from light.
7. Incubate the gel in the working dye solution for 30 minutes at room temperature. For polyacrylamide gels containing 3.5–10% acrylamide, the typical staining time is 30 minutes to 1 hour. Gels with higher acrylamide contents require longer staining times.

*Important!* The working solution can be reused no more than 2–3 times.

## Gel pre-staining

This method is only suitable for agarose gels, but not for PAAG.

*Important!* Note — this staining method can sometimes cause bands to warp or form smears. Use gel soaking in this case.

1. Dilute the concentrated dye in a ratio of 1:10,000 in agarose gel buffer (e. g., 1× TBE or 1× TAE). Add agarose powder to the dye solution. For example, if 20 mL of melted agarose is required, add 2 µL of 10,000× GelRed dye concentrate to 20 mL of 1× buffer, mix the solution well, and add the agarose to it.
2. Heat the agarose in the dye buffer to the boiling point and wait until it is completely dissolved. A microwave oven or other heating device can be used for heating.

*(Optional)* Alternatively, GelRed can be added directly to the melted agarose at a ratio of 1:10,000 (1 µL of dye concentrate per 10 mL of buffer).

3. Pour the mixture into a gel mold and wait for it to harden.

*Important!* As with gel pre-staining with ethidium bromide, the mobility of nucleic acid fragments in the gel may be slightly lower compared to their mobility in an unstained gel.

4. Load the samples and perform electrophoresis.

## Gel viewing and photographing

A standard transilluminator with a wavelength of 302 nm or 312 nm can be used to view stained gels.

When documenting, it is necessary to use a filter for ethidium bromide.

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## Troubleshooting

Problem	Solution
<b>Blurred stripes in the gel</b>	<ul style="list-style-type: none"> <li>• Blurred or smeared bands may be caused by excessive gel loading. Reduce the amount of nucleic acid loaded.</li> <li>• Use gel soaking instead of adding dye to the gel.</li> <li>• Use agarose gel with a lower percentage of content for better resolution of large fragments</li> <li>• Change the buffer. The TBE buffer has a greater buffer capacity than TAE.</li> </ul>
<b>Uneven migration of DNA in a pre-stained gel</b>	<ul style="list-style-type: none"> <li>• Reduce the amount of DNA loaded.</li> <li>• Reduce the amount of dye used, for example, use a 0.5× dilution when adding dye to the gel.</li> <li>• Use gel soaking instead of adding dye to the gel.</li> </ul>
<b>Weak fluorescence and decreased staining efficiency over time</b>	<p>Perhaps the dye precipitated out of the solution.</p> <ul style="list-style-type: none"> <li>• Heat the gelRed solution to 45–50 °C for two minutes and vortex to redissolve.</li> <li>• Store the dye at room temperature to prevent the dye from precipitating.</li> </ul>

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