

Tech Tip: Eight things to keep in mind for optimal gel staining with GelRed® and GelGreen®

The most common use of GelRed® and GelGreen® nucleic acid stains is in pre-cast agarose gels, where the dyes are added to molten agarose during gel preparation. However, due to their larger size designed to improve safety, band migration of DNA in pre-cast gels may be affected. Some samples, such as restriction digested DNA can migrate abnormally in GelRed or GelGreen precast gels.

GelRed and GelGreen are larger dyes than ethidium bromide, a design feature related to their safety

This is why overloading DNA results in smearing, smiling, or curvy bands, and aberrant DNA migration. The recommended loading amount for samples of known concentration or commercial ladders/ DNA markers is 50-200 ng/ lane. For samples of unknown concentration, loading 1/2 – 1/4 the usual amount of DNA usually solves migration issues.

GelRed and GelGreen are ultra-sensitive dyes, more sensitive than ethidium bromide

Low DNA amounts (0.1 ng or lower) can be reliably detected. Loading high DNA amounts is not required, and can result in issues with DNA migration. Loading lower DNA amounts gives better separation and sharper bands, saving on precious samples, DNA ladder and conserving costs.

GelRed and GelGreen do not migrate through the gel as easily as ethidium bromide, giving more homogenous staining throughout the gel

Loss of staining, or faint staining of low molecular weight DNA bands is not an issue as GelRed and GelGreen do not migrate as fast as ethidium bromide through the gel. It is not necessary to add additional dye to the running buffer.

Using a lower percentage gel can improve band resolution with GelRed and GelGreen

As GelRed and GelGreen are larger dyes, high molecular weight DNA separates better on lower percentage agarose gels.

Buffers with higher buffering capacity can improve band resolution

Try TBE if you are using TAE.

Avoid storing pre-cast gels with GelRed or GelGreen at 4°C can cause dye precipitation and poor performance.

Precast gels containing GelRed or GelGreen can be stored for future use for up to a week, or month respectively. We recommend storing gels at room temperature, in the dark, as storage at 4°C

Store stock solutions of GelRed and GelGreen at room temperature to avoid dye precipitation

Weak fluorescence, decreased dye performance over time, or a film of dye remaining on the gel after post-staining, are indications that the dyes may have precipitated out of solution. Warm the dye solutions to ~50°C, and mix well by vortexing to re-dissolve precipitates.

Post-staining eliminates ANY possibility of dye interference with DNA migration

Because high affinity nucleic acid binding dyes like GelRed and GelGreen can affect DNA migration during electrophoresis, post-staining of gels is highly recommended. Post-staining is also recommended if (i) loading higher than the recommended amount of DNA is needed, (ii) using loading buffers containing SDS, which can contribute to band smearing, or (iii) if band migration issues in precast gels with GelRed or GelGreen are persistent. Post-staining can take 5-30 min, depending on how much DNA is present, and the solution can be reused.