What is the difference between GelRed® and GelGreen®? Which one should I choose?

The main difference between GelRed® and GelGreen® is their fluorescence excitation and emission wavelengths. GelRed® has red fluorescence, similar to ethidium bromide. GelGreen® has green fluorescence, similar to SYBR® Green or SYBR® Safe. Both dyes are compatible with standard UV transilluminators. GelGreen® is also compatible with blue light transilluminators, which allow users to avoid exposing themselves and their DNA samples to ultraviolet radiation.

GelRed® and GelGreen® have higher sensitivity for double stranded nucleic acids compared to single stranded nucleic acids, but GelRed® is more sensitive for staining single stranded nucleic acids than GelGreen®. GelRed® is about twice as sensitive for double stranded nucleic acids compared to single-stranded nucleic acids, and about five times more sensitive than GelGreen® for staining single stranded nucleic acids.

For more information about these products, please visit our DNA stains technology page.

View the GelRed® Product Line

View the GelGreen® Product Line

Why do you offer GelRed® and GelGreen® in DMSO or water?

The water formulation is a newer and improved product compared to the stock in DMSO. We recommend using dyes in water to avoid the potential hazards of handling DMSO, which can be absorbed through the skin. We continue to offer dyes in DMSO because some users do not wish to alter their established laboratory protocols. Based on internal testing, both formulations perform similarly.

How do I use GelRed® and GelGreen®?

GelRed® and GelGreen® can be added to agarose during gel casting at a final concentration of 1X, or they can be used for post-electrophoresis gel staining at a final concentration of 3X in water. For detailed protocols for use, please download the GelRed® Product Information Sheet or GelGreen® Product Information Sheet.

Why am I seeing smeared or smiling DNA band(s) or discrepant DNA migration?

Many customers use GelRed® or GelGreen® precast gels for convenience. However, because GelRed® and GelGreen® are high affinity dyes designed to be larger dyes to improve their safety, they can affect the migration of DNA in precast gels. Some samples, such as restriction digested DNA may migrate abnormally in GelRed® or GelGreen® precast gels.

Tip #1: Load less DNA

Smearing and smiling in GelRed® or GelGreen® precast gels most often caused by overloading of DNA. If you see band migration shifts or smearing and smiling, try reducing the amount of DNA loaded. The recommended loading amount for ladders and samples of known concentration is 50-200 ng/lane. For samples of unknown concentration, try loading one half or one third of the usual amount of DNA. This usually solves band migration problems.

Tip #2: Try the post-staining protocol

To avoid any interference the dye may have on DNA migration, we recommend using the post-staining protocol. If your application requires loading more than the recommended amount of DNA, use the post-staining protocol. While we recommend post-staining gels for 30 minutes, you may be able see bands in as little as five minutes, depending on how much DNA is present. Post-staining solutions can be reused. See the GelRed® Product Information Sheet or GelGreen® Product Information Sheet for detailed protocols.

Other tips to improve agarose gel resolution:

- If you see DNA migration issues or smearing after post-staining with GelRed® or GelGreen®, then the problem is not caused by the nucleic acid dye. Avoid overfilling gel wells to prevent smearing of DNA down the surface of the gel.
- Pour a lower percentage agarose gel. Higher molecular weight DNA separates better with a lower percentage gel.
- Change the running buffer. TBE buffer has a higher buffering capacity than TAE buffer.

Why do I see weak fluorescence, decreased dye performance over time, or a film of dye remaining on the gel after post-staining?

There are a few possibilities:

1. The dye may have precipitated out of solution.

- Heat the GelRed® or GelGreen® solution to 45-50°C for two minutes and vortex to dissolve.
- Store dye at room temperature to avoid precipitation.

2. If you are seeing high background staining of the gel, the agarose that you are using may be of low quality. Contaminants in the agarose may bind to the dye, resulting in increased background.

What DNA ladders and loading buffers can I use with GelRed® and GelGreen®?

We have tested a variety of DNA ladders from different suppliers with GelRed® and GelGreen® with good results. Similarly, any common DNA loading buffers can be used. Band migration problems or smearing or smiling DNA bands are most commonly caused by overloading of ladder. If you are experiencing band migration problems with your ladder, please try reducing the amount of ladder you load. We recommend loading 50-200 ng ladder per lane for GelRed® or GelGreen® precast gels. See the FAQ for smearing or smiling DNA bands for more tips.

Biotium also offers 1 kb and 100 bp DNA ladders in TE buffer or ready-to-use format in loading buffer. Ladders are supplied with a separate tube of 6X DNA Loading Buffer to use with your samples.

Do GelRed® and GelGreen® need to be used in the dark?

GelRed® and GelGreen® are stable dyes. You can use the dyes in room light. However, we recommend storage of the dyes in the dark between uses. We have had a customer report using GelRed® with success after accidentally leaving it in ambient light for one month.

Can GelRed® and GelGreen® be used to stain ssDNA or RNA?

GelRed® and GelGreen® can be used to stain both ssDNA and RNA, but GelRed® is about 5 times more sensitive for single-stranded nucleic acids than GelGreen®. Titration assays using a fluorescence microplate reader showed that the fluorescence signal of GelRed® bound to ssDNA and RNA is about half that of GelRed® bound to dsDNA.

Why does my RNA or ssDNA appear yellowish-orange or pinkish with GelGreen®?

We and other users have often observed that GelGreen® stains ssDNA and RNA orange/ pink and dsDNA green. We have also seen that smaller dsDNA fragments

can appear orange-pink, the color ranging from white-pink-orange. We are not sure about the underlying mechanism, possibly the structure of single-stranded nucleic acids favors an altered stacking interaction of GelGreen® monomers leading to the formation of J-aggregates that have red emission.

Can GelRed® be used in formaldehyde based RNA gels?

Yes, customers have reported using GelRed® in glyoxal and formaldehyde agarose gels for precast staining of RNA.

Can GelRed® be used to detect ssDNA in a precast gel?

Yes, see Ana. Biochem. doi: http://dx.doi.org/10.1016/j.ab.2012.09.003 .

Can GelRed®/GelGreen® be used to stain DNA in acrylamide gels?

Yes, use the post-staining protocol for polyacrylamide gels. 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.,

Biotium also offers PAGE GelRed® a non-toxic, non-mutagenic dye specifically designed for staining DNA in polyacrylamide gels.

Can GelRed® be used for polyacrylamide, DGGE, EMSA or PFGE (pulse-field) gels?

Yes. Please use the post-staining procedure for DGGE and EMSA gels. For PFGE gels, the pre-cast or post-staining protocol may be used.

Is GelRed® compatible with alkaline gel running buffer (30mM NaOH, 1mM EDTA)?

Yes, GelRed® is compatible with alkaline running buffer.

Can GelRed®/GelGreen® be used for Comet Assay?

Yes, GelRed® and GelGreen® can be used for Comet assay.

Can GelRed® be used in Loop-mediated Isothermal Amplification Assay?

Yes, see Virol J. 2012, 9, 110.

Can GelGreen® be used in Capillary Gel Electrophoresis-Laser induced Fluorescence of double-stranded DNA fragments?

Yes, see Electrophoresis, doi: 10.1002/elps.201200624 .

Can GelRed® be used for cesium chloride gradient purification of DNA?

Customers have reported using GelRed® in cesium gradients. To extract GelRed® from DNA after cesium banding, we recommend adding SDS to a final concentration of 0.1% before butanol extraction. Alternatively, chloroform can be used instead of butanol for extraction.

What is the lower detection limit of GelRed®/GelGreen®?

GelRed® and GelGreen® are ultra-sensitive dyes. Some users have reported being able to detect bands containing less than 0.1 ng DNA. However, the sensitivity of the staining will depend on the instrument capability and exposure settings.

What is the binding mechanism of GelRed®/GelGreen®?

GelRed® has been shown to bind DNA exclusively by intercalation (https://link.springer.com/article/10.1007/s00249-014-0995-4). GelGreen® most likely binds by a combination of intercalation and electrostatic interaction.

In which direction does GelRed®/GelGreen® migrate?

GelRed® and GelGreen® do not migrate through the gel as easily as ethidium bromide. It is not necessary to add additional dye to the running buffer, and the gel will be stained more homogenously than a gel stained with ethidium bromide.

Are GelRed® and GelGreen® compatible with downstream applications such as cloning, ligation and sequencing?

Yes. We recommend Qiagen or Zymoclean™ gel extraction kits or phenol-chloroform extraction to remove the dye from DNA. Some users have reported performing PCR on DNA in the presence of GelRed® with no purification step, for example by incubating GelRed®-stained gel slices in TE buffer to extract DNA by passive diffusion for use in PCR, or by using a few microliters of molten agarose from GelRed®-stained gel slices containing DNA for PCR.

What purification protocols are recommended to remove GelRed®/GelGreen® after staining?

Customers report good results using ZymoClean™ Gel DNA Recovery Kit from Zymo Research, GenElute™ Agarose Spin Column from Sigma, PureLink® Quick Gel Extraction kit from Life Technologies, illustra GFX PCR DNA and Gel Band Purification kit from GE Healthcare, High Pure PCR Product Purification Kit from Roche Applied Sciences, and GenJET gel extraction kit from Thermo Scientific.

Can GelRed®/GelGreen® be used for Southern or Northern blotting?

GelRed® has been validated for Southern blotting. We recommend using the post-staining protocol for blotting applications.

How much GelRed®/GelGreen® do I need to use? How many gels can I run with a vial of GelRed®/GelGreen®?

GelRed® and GelGreen® can be added to agarose during gel casting at a final concentration of 1X, or used for post-electrophoresis gel staining at a final concentration of 3X in water. Dilute the 10,000X stock 10,000-fold for 1X precast gels (for example, 5 uL for a 50 mL gel), or 3,333-fold for a 3X post staining solution (15 uL for a 50 mL solution).

A 0.5 mL vial of GelRed® or GelGreen® is can be used to prepare 100 minigels (50 mL each) using the precast protocol, or for post-electrophoresis staining of 33 minigels in 50 mL staining solution per gel. Post-staining solution also can be re-used for staining two or more gels. For detailed protocols for use, please download the GelRed® Product Information Sheet or GelGreen® Product Information Sheet.

Does post-staining require a de-staining step?

No, but de-staining with water can be performed if background is high.

Can GelRed®/GelGreen® post-staining solution be reused?

Yes. However, if the sensitivity decreases, use a fresh solution of the dyes.

How much GelRed® or GelGreen® should I add to my 6X DNA loading buffer?

We don't recommend adding GelRed® or GelGreen® directly to loading buffer, because this can result in inaccurate band migration. Biotium offers 6X GelRed®

Prestain Loading Buffers designed for this application, although we do not recommended them for applications where precise DNA band sizing is required. For the most accurate determination of DNA band sizes, we recommend using GelRed® post-staining (see the GelRed® protocol for details).

What instruments can be used to detect GelRed® and GelGreen®?

GelRed® is compatible with a standard UV transilluminator (302 or 312 nm). GelGreen® has sufficient absorption between 250-300 nm and a strong absorption peak at around 500 nm. GelGreen® is compatible with a 254 nm UV transilluminator or a gel reader with visible light excitation such as a Dark Reader or a 488 nm laser gel scanner.

What emission filters are suitable for use with GelRed® and GelGreen®?

Use the ethidium bromide filter for GelRed®; use a SYBR® Green or yellow filter for GelGreen®. Alternatively, a long-pass yellow filter can be used with both GelRed® and GelGreen®. Please review the emission spectra for GelRed® and GelGreen® for more specific wavelengths.

Can I make GelRed®/GelGreen® gels ahead of time and store them for later use?

You can store precast GelRed® gels for up to a week, and GelGreen® gels for up to a month. We recommend storing gels at room temperature in the dark. We no longer recommend storing gels at 4°C, because this can lead to dye precipitation and poor performance.

Can I reuse a GelRed®/GelGreen® precast gel after running samples?

No. We do not recommend that GelRed®/GelGreen® gels be reused after electrophoresis because the staining intensity can be decreased with sequential electrophoresis.

Can I re-melt gel with GelRed®/GelGreen® and cast again?

Yes, unused solidified agarose with GelRed® or GelGreen® can be remelted. If the unused agarose with dye is to be stored for more than a day or so, we recommend protecting it from light.

What is the stability of GelRed®/GelGreen® in molten agarose?

GelRed® is more stable than GelGreen®. We do not recommend storing GelRed® in molten agarose for more than a few days.

How safe is GelRed®/GelGreen®?

In AMES and related tests, GelRed® and GelGreen® were shown to be much safer alternatives to EtBr and SYBR dyes. Nevertheless, please exercise safe laboratory practices when using these reagents.

Please visit our website www.biotium.com to download a comprehensive safety report.

How should I dispose of GelRed® and GelGreen®?

GelRed® and GelGreen® is classified as non-hazardous for drain disposal under California Code of Regulations Title 22. Some facilities have approved the disposal of GelRed® and GelGreen® directly down the drain. However, because regulations vary, please contact your safety office for local disposal guidelines. Please review the GelRed®/GelGreen® safety report for more detailed information.

My product was accidentally left out at room temperature or exposed to light. Is it ruined?

Most of our products are stable at room temperature for many days, so in all likelihood the product will still work just fine. To be on the safe side, we recommend performing a small scale positive control experiment to confirm that the product still works for your application before processing a large number of samples or precious samples.

One exception that we are aware of is GelGreen™, which is more sensitive to light exposure than most of our other fluorescent dyes. If GelGreen™ is exposed to ambient light for a prolonged period of time (days to weeks), its color will change from dark orange to brick red. If this occurs, the GelGreen will no longer work for gel staining.