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GelRed(TM)

Nucleic Acid Gel Stain – 10000X in water

Product	Cat#	Package size
GelRed(TM) 10000X solution in water	M3199.0500	500µL
GelRed(TM) 10000X solution in water	M3199.2000	2mL
GelRed(TM) 10000X solution in water	M3199.5000	5mL
GelRed(TM) 10000X solution in water	M3199.1010	10mL

Product description

GelRed™ is an sensitive, stable and environmentally safe fluorescent nucleic acid dye designed to replace the toxic and possibly mutagenic ethidium bromide (EB) for staining dsDNA, ssDNA or RNA in agarose gels or polyacrylamide gels. GelRed is far more sensitive than EB without requiring a destaining step. GelRed™ and EB have virtually the same spectra, so you can directly replace EB with GelRed without changing your existing imaging system.
GelRed™ can be used to stain dsDNA, ssDNA or RNA in agarose gel via either precast or post gel staining. GelRed can also be used to stain dsDNA, ssDNA or RNA in polyacrylamide gel via post gel staining. GelRed™ is also compatible with downstream DNA manipulations such as digestion with a restriction enzyme, Southern blotting techniques and cloning.

GelRed™ was subjected to a series of tests at Biotium and by three independent testing services to assess the dye's safety for routine handling and disposal. Test results confirm that the dye is impenetrable to both latex gloves and cell membranes. The dye is noncytotoxic and nonmutagenic at concentrations well above the working concentrations used in gel staining. GelRed™ successfully passed environmental safety tests in compliance with CCR Title Hazardous Waste Characterization, under which GelRed™ is not classified as hazardous waste. A complete safety report is available at www.biotium.com.

GelRed™ is supplied as 10,000X solution in water or for your convenience, as a ready-to-use 3X solution in water that can be directly used for post gel staining.

As 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 also be used to stain dsDNA, ssDNA or RNA however GelRed™ is twice as sensitive to dsDNA than ssDNA or RNA.

Gel staining with GelRed™ is compatible with downstream DNA manipulation such as digestion with a restriction enzyme, Southern blotting techniques and cloning. GelRed™ may be removed from DNA by ethanol precipitation.

GelRed™ Nucleic Acid Gel Stain – 10000X in water is a concentrated solution that can be diluted 10,000 times for use in precast gel staining or 3,300 times for use in post gel staining according to the procedures described.

500µL of 10000X solution can be used to stain at least 100 precast gels or post-stain at least 100 mini gels (if the agarose is supplemented with GelRed™.

We at Genaxxon recommend to use GelRed™ for in-slot applications.
In this case 500µL of the 10000X solution can be used for up to 250000 gel slots (about 25000 mini gels) (calculation base: 1µL of a 20X GelRed™ solution mixed with 4µL PCR reaction per slot).

Storage and Handling

GelRed™ is a very stable dye. Store 10,000X solution and dilute solutions of GelRed™ at room temperature, protected from light.

Dye precipitation may occur at lower temperatures (winter times), resulting in lower signal or the appearance of precipitate on the surface of the gel. If this occurs, heat the solution to 45-50°C for two minutes and vortex.

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Staining Protocols

1. **In-slot staining protocol (recommended by Genaxxon)**

   a. Prepare agarose gel as usual without adding any dye.

   b. Prepare diluted GelRed™ solution (final concentration of 20X).
   Add 3µL of the 10000X GelRed™ solution to 97µL H2O resulting in a 300X GelRed™ solution.
   Add 8µL of the 300X GelRed™ solution to 100µL 6X Loading buffer plus 12µL H2O resulting in a 20X GelRed™ solution.

   Add 1µL to 2µL of the 20X GelRed® solution to 4µL sample and apply directly into the slot of the agarose gel!

   At Genaxxon this procedure show the best results in sharpness of bands and is the most economic way to use GelRed™!

2. **Staining DNA by Post Gel Staining**

   a. Run gels according to your standard protocol.

   b. Dilute the GelRed™ 10000X stock solution about 3,300 fold to prepare a 3X staining solution in water with 0.1M NaCl (e.g. add 15µL of GelRed™ stock solution and 5mL NaCl to 45mL water). While GelRed™ 1X staining solution can also be used for post gel staining, the sensitivity is generally less than with 3X staining solution (**NOTE:** inspect the 10000X vial carefully. If dye precipitation occurs, heat up or sonicate the vial).
   NOTE: Use of NaCl in the staining solution is optional. NaCl in the solution enhances the staining, but may promote dye precipitation if the staining solution is to be used repeatedly. Any staining solution to be re-used is preferably stored at room temperature in a dark place to reduce possible dye precipitation problems.

   c. Carefully place the gel in a suitable container such as a polypropylene container. Gently add sufficient amount of the 3X staining solution to submerge the gel.

   d. Agitate gel gently at room temperature for about 30 minutes. 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 up to 1 hour with gels of higher acrylamide content requiring longer staining time. The staining solution can be re-used at least 2-3 times. The unused staining solution can be stored at room temperature in a dark place.

   e. View the stained gel with a standard transilluminator (302nm o 312nm) and photograph the gel using an ethidium bromide filter. Similarly, a SYBR™ or GelStar(R) filter may also be used for photographing with equally good results.

3. **Staining of DNA by Precasting** GelRed™ **Gels***

   a. Prepare agarose gel solution using your standard protocol.

   b. Dilute GelRed™ 10000X stock solution into the agarose gel solution at 1:10000 (e.g. 5µL stock solution added to 50mL of gel solution). Since GelRed™ is generally thermally stable the 10000X stock solution can be added while the gel solution is still hot. Make sure that the dye is thoroughly mixed with the gel solution by swirling, stirring, or inversion.
   NOTE: inspect the 10000X vial carefully. If dye precipitation occurs, heat up or sonicate the vial).

   Alternatively the stock solution may be pre-combined with agarose powder and electrophoresis buffer of your choice followed by microwaving or other heating procedures commonly used for preparing agarose gels. GelRed™ is compatible with all commonly used electrophoresis buffers.

   c. Cast the gel and allow it to solidify. Any leftover gel solution may be stored and re-heated later for additional gel casting. Since GelRed™ is hydrolytically stable, GelRed™ precast gels may be prepared in large quantities and stored for later use. To avoid mould formation, we recommend that the precast gels be stored in a refrigerator.

   d. Load samples and run the gels using your standard protocol.
   NOTE: Use only 1/3 or less of your normal DNA-marker amount to avoid overexposure (no bands of marker visibly separated).

   e. View the stained gel with a standard transilluminator (302nm o 312nm) and photograph the gel using an ethidium bromide filter. Similarly, a SYBR™ or GelStar® filter may also be used for photographing with equally good results.
   NOTE: If you consistently see band smearing and/or poor band separation, run a gel and post-stain by following the protocol provided (point 1) to confirm if the problem is caused by the dye or other factors unrelated to the dye. If post gel staining is normal and the problem is not caused by the dye, try any of the followings:

   - Lower the amount of nucleic acid loaded.
   - Lower running voltage
   - Lower the amount of agarose in the gel
   - Run a longer gel
   - Increase the thickness of the gel
   - Increase the solidification time to ensure sharp well formation
   - Improve your loading technique or select post gel staining

*** Precasting GelRed™ gel is not suitable for acrylamide gels. Use post gel staining for acrylamide gels.**

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Features of GelRed™

Safer than EB
Shown by the Ames test and other tests to be nonmutagenic and noncytotoxic at working concentrations.
Easy disposal
Passed environmental safety tests for direct disposal down the drain or in regular trash.
For details ask your local waste inspector.
Ultra-sensitive
Much more sensitive than EtBr and SYBR Safe
Extremely stable
Available as 10000X aqueous solution, stable at room temperature for long-term storage and microwavable.
Simple to use
Very simple procedures for either precast and post gel staining
Perfectly compatible with a standard UV transilluminator
GelRed™ replaces EtBr with no optical setting change
Perfectly compatible with downstream applications
Compatible with downstream DNA manipulations such as digestion with a restriction enzyme, Southern blotting techniques and cloning.

The Most Sensitive and Stable Precast Gel Stain

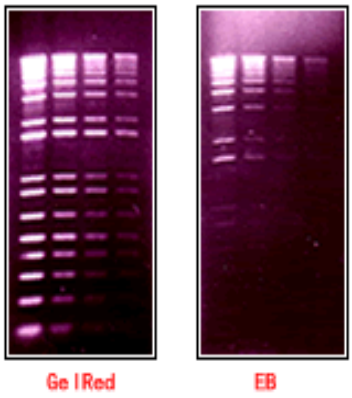

Figure 1. GelRed™ is significantly more sensitive than ethidium bromide (EB) for detecting low-level DNA, especially in the lower molecular weight area. Shown left are two-fold serial dilutions of 1 Kb Plus DNA Ladder from Invitrogen electrophoresed on 1% agarose gels precasted with GelRed™ or EB in 1x TBE. The total amount of DNA loaded per lane was: 200ng, 100ng, 50ng and 25ng from left to right. Gels were imaged using 300 nm transillumination and photographed with an EB filter and Polaroid 667 black-and-white print films.

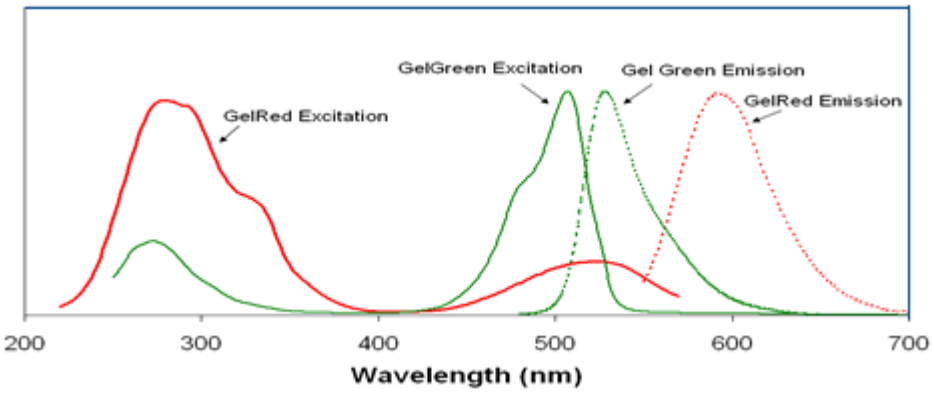

Figure 2. Excitation and emission spectra of GelRed™ and GelGreen™ in the presence of DNA in PBS buffer.

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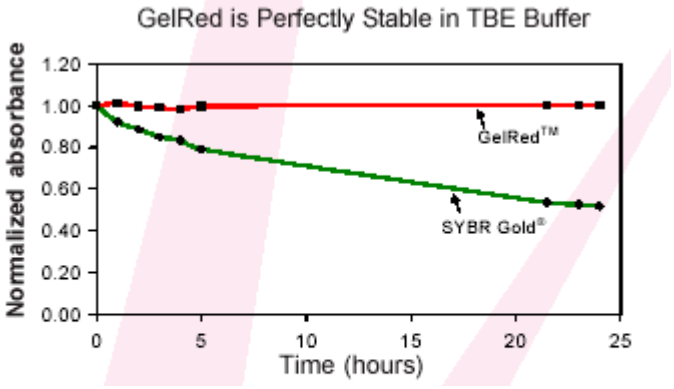

Figure 3: Normalized absorbances of GelRed™ and SYBR™ Gold 1X TBE gel staining solutions at 500nm and 488nm respectively over time at room termperature. The starting absorbance values for GelRed™ and SYBR™ Gold were 0.029 and 0.051, respectively.

Note: *GelRed™ and its uses are covered by pending US and international patents.
**SYBR is trademark of Molecular Probes, Inc.

GelRed™ is avery stable dye. We recommend that the 10000X solutions are stored at RT in a dark place. The solution may also be stored at a lower temperature such as +2°C to +8°C. Dye precipitation may occur during prolonged low temperature storage. When this occurs, heat up the solution in a hot water batch at 45°C to 50°C for two minutes and vortex the solution.

The 1X or 3X post column staining solutions may also be stored at room temperature in a dark place for at least one year. Exposure to light should be avoided during long-term storage. However, the dye can be handled under ambient light without any problem during staining experiments.

Toxicity

GelRed™ was subjected to a series of tests by three independent testing services to assess the dye's safety for routine handling and disposal. These tests include: 1. Glove penetration test. 2. Cell membrane permeability and cytotoxicity test. 3. Ames test. 4. Environmental safety tests. Test results confirm that the dye is impenetrable to both latex gloves and cell membranes. The dye is noncytotocix and nonmutagenic at concentrations well above the working concentrations used in gel staining. GelRed™ appears to be completely cell membrane-impermeable, which may be a key factor responsible for the observed low toxicity. However, since these tests were not performed on human, we still advise that researchers exercise precautions when handling the dye or any other DNA-binding molecules by wearing protective gears.

Disposal

GelRed™ has successfully passed environmental safety tests in compliance with CCR Title 22 Hazardous Waste Characterization. As a result, GelRed™ is not classified as hazardous waste, thus can be safely disposed of down the drain or as regular trash, providing convenience and reducing cost in waste disposal.
For details ask your local waste inspector.

This product is for research use only.

Trouble shooting

Problem	Suggestion

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Smeared DNA bands in gel	1.Reduce the amount of DNA loaded by one-half to one-third. 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	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 on-half to one-third. 2.Reduce the amount of dye used, i.e. use 0.5X in pre-cast gels. 3.Post-stain the 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. 1.Heat GelRed™ solution to 45-50°C for two minutes and vortex to redissolve. 2.Store dye at room temperature to avoid precipitation.

Frequently Asked Questions

Question	Answer
Can GelRed be used to stain ssDNA or RNA?	GelRed™ can be used to stain ssDNA and RNA, but it is twice as sensitive for dsDNA than for ssDNA or RNA.
Is GelRed™ compatible with downstream applications such as cloning, ligation and sequencing?	Yes. We recommend gel extraction kits, Exo-Sap protocol or phenol-chloroform extraction to remove the dye from DNA. Some users have reported performing PCR on DNA in the presence of GelRed™ with not purification step.
Can GelRed™ be used for formaldehyde, polyacrylamide, DGGE, EMSA or PFGE (pulse-field) gels?	Yes. Customers have reported using GelRed™ in glyoxal and formaldehyde agarose gels for precast staining of RNA. Use the post-staining protocol for polyacrylamide. DGGE, EMSA, and PFGE gels..
Can GelRed™ be used in COMET assay?	Yes. GelRed™ can be used in COMET assay by post-staining.
Can GelRed™ be used in cesium chloride gradients?	Customer have reported using GelRed™ in cesium gradients. To extract GelRed™ from DNA after cesium banding, we recommend add SDS to a final concentration of 0.1% before butanol extraction. Alternatively, chloroform can be used instead of butanol for extraction.
Is GelRed™ compatible with Southern or northern blotting?	Yes. GelRed™ can be used for blotting. We recommend using the post-staining protocol for blotting applications.
What loading buffers are compatible with GelRed™	We routinely use 6X loading buffer containing 15% glycerol, 7.5% Ficoll 400, 0.05% Bromophenol Blue. In internal testing 6X loading buffer containg 0.1% Orange G produced good results in precast and post-stained gels. SDS in loading buffer may contribute to band smearing in precast GelRed™ gels. If this occurs, we recommend using the post-staining protocol.
What emission filters are suitable for use with GelRed™	Use the ethidium bromide filter for GelRed™, SYBR or GelStar filters also can be used for gel imaging with equally good results. Please review the emission spectra for GelRed™ for specific wavelengths.

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Frequently Asked Questions (continued)

Can I reuse GelRed™ pre-cast gel after electrophoresis?	Yes, it is possible, but we do not recommend reusing GelRed™ pre-cast gels as signal decreases with subsequent electrophoresis.
How should I dispose of GelRed?	GelRed™ has passed EPA regulated Title 22 test. Some facilities have approved the disposal of GelRed™ directly down the drain. However, because regulations vary, please contact your safety office for local disposal guidelines. GelRed™ can be adsorbed to activated carbon (also known as activated charcoal) for disposal as chemicals waste.
What is the lower detection limit of GelRed™?	Some users have reported being able to detect less than 0.1ng DNA. However, the limit of detection will depend on instrument capability and exposure settings.
What is the binding mechanism of GelRed™?	GelRed™ most likely binds by a combination of intercalation and electrostatic interaction.
What is the chemical structure of GelRed™?	The chemical structure of GelRed™ is proprietary.
Does GelRed™ migrate during electrophoresis?	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 homogenously with GelRed™ than with EtBr.
Does GelRed™ need to be used in the dark?	You can use the dye in room light, however we recommend storing the dye in the dark.
I accidentally left my GelRed™ in the light. Will it still work?	While we recommend that you protect the dye from light during long term storage, we have had customers report using GelRed™ with success after accidentally leaving it in ambient light for one month.
Is there a difference between 10,000X GelRed™ in DMSO and water?	The GelRed™ stock in water is a newer and improved product compared to the stock in DMSO. We recommend using GelRed™ in water to avoid the potential hazards of handling DMSO, a solvent that can be absorbed through the skin.

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