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SafeGel red stain

Nucleic Acid Gel Stain – 10000X in water

	Cat#	M3193.0500	M3193.2000	M3193.5000	M3193.1010	Colour code of cap colour of tube
Component						
SafeGel red stain 10,000X solution in water		1x 500µL	2x 1mL	5x 1mL	10x 1mL	**amber**

Product description

SafeGel red stain is a third generation non-mutagenic highly fluorescent nucleic acid stain to replace the highly toxic and mutagenic Ethidium bromide for staining dsDNA, ssDNA or RNA in agarose gels or polyacrylamide gels. SafeGel red stain is far more sensitive than EB without requiring a destaining step. SafeGel red stain and EB have virtually the same spectra, so you can directly replace EB with SafeGel red stain without changing your existing imaging system.

SafeGel red stain can be used to stain dsDNA, ssDNA or RNA in agarose gel via either precast or post gel staining. SafeGel red stain can also be used to stain dsDNA, ssDNA or RNA in polyacrylamide gel via post gel staining, however SafeGel red stain is twice as sensitive to dsDNA than ssDNA or RNA.

SafeGel red stain is compatible with downstream DNA applications such as digestion with a restriction enzyme, Southern blotting techniques and cloning.

SafeGel red stain was subjected to AMES tests by third party service provider. These tests showed that SafeGel red stain is non-mutagenic in concentrations used for staining gels. Test results confirm that the dye is impenetrable to both latex gloves and cell membranes.

As nucleic acid binding dyes can affect DNA migration during electrophoresis, post-staining of gels is highly recommended. Post-staining with SafeGel red stain results in a high sensitivity. The precast protocol is not recommended for polyacrylamide gels.

Gel staining with SafeGel red stain is compatible with downstream DNA manipulation such as digestion with a restriction enzyme, Southern blotting techniques and cloning. SafeGel red stain may be removed from DNA by phenol/chloroform extraction and ethanol precipitation.

SafeGel red stain 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 SafeGel red stain).

We at Genaxxon recommend using SafeGel red stain for in-slot applications.
In case of in-slot application 500µL of the 10000X solution can be used for up to 250000 gel slots (about 25000 mini gels) (calculation base: 1µL of a 20X SafeGel red stain solution mixed with 4µL PCR reaction per slot). See Staining Protocol point 1 for details (page 5).

Features of SafeGel red stain

Safer than EB
SafeGel red stain was subjected to AMES tests by third party service provider.
These tests showed that SafeGel red stain is non-mutagenic in concentrations used for staining gels.
Test results confirm that the dye is impenetrable to both latex gloves and cell membranes.

Easy disposal
Save for direct disposal down the drain or in regular trash.
For details ask your local waste inspector.

Ultra-sensitive
Much more sensitive than EtBr with much lower background.

Extremely stable
Available as 10000X aqueous solution, stable at room temperature for long-term storage.
Can be microwaved but this is not recommended.

Simple to use
Very simple procedures for either precast and post gel staining

Perfectly compatible with a standard UV transilluminator
SafeGel red stain is spectrally compatible with existing instruments

Perfectly compatible with downstream applications
Compatible with downstream DNA manipulations such as digestion with a restriction enzyme, Southern blotting techniques and cloning.

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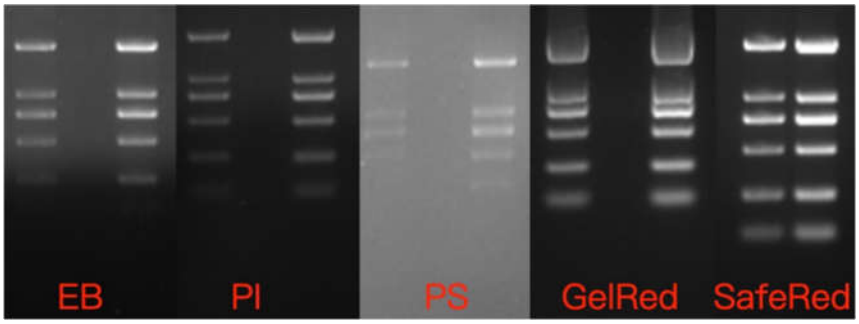
Figure 1: Comparison of different gel staining dyes



SafeGel red stain is significantly more sensitive than ethidium bromide (EB) for detecting low-level DNA, especially in the lower molecular weight area.
Gel was imaged using 300 nm transillumination and photographed with an EB filter and Polaroid 667 black-and-white print films.

Storage and Stability

SafeGel red stain is a very 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.

This product is for research use only!

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Frequently Asked Questions

Question	Answer
Can **SafeGel red stain** be used to stain ssDNA or RNA?	**SafeGel red stain** can be used to stain ssDNA and RNA, but it is twice as sensitive for dsDNA than for ssDNA or RNA.
Is **SafeGel red stain** 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 **SafeGel red stain** with no purification step.
Can **SafeGel red stain** be used for formaldehyde, polyacrylamide, DGGE, EMSA or PFGE (pulse-field) gels?	Yes. Customers have reported using **SafeGel red stain** in glyoxal and formaldehyde agarose gels for precast staining of RNA. Use the post-staining protocol for polyacrylamide. DGGE, EMSA, and PFGE gels.
Can **SafeGel red stain** be used in cesium chloride gradients?	Customer have reported using **SafeGel red stain** in cesium gradients. To extract **SafeGel red stain** 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.
Is **SafeGel red stain** compatible with Southern or northern blotting?	Yes. **SafeGel red stain** can be used for blotting. We recommend using the post-staining protocol for blotting applications.
What loading buffers are compatible with **SafeGel red stain**	We routinely use 6X loading buffer containing 15% glycerol, 7.5% Ficoll 400, 0.05% Bromophenol Blue. In internal testing 6X loading buffer containing 0.1% Orange G produced good results in precast and post-stained gels. SDS in loading buffer may contribute to band smearing in precast **SafeGel red stain** gels. If this occurs, we recommend using the post-staining protocol.
What emission filters are suitable for use with **SafeGel red stain**	Use the ethidium bromide filter for **SafeGel red stain**. Please review the emission spectra for **SafeGel red stain** for specific wavelengths.
Can I reuse **SafeGel red stain** pre-cast gel after electrophoresis?	Yes, it is possible, but we do not recommend reusing **SafeGel red stain** pre-cast gels as signal decreases with subsequent electrophoresis.
What is the lower detection limit of **SafeGel red stain**	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 **SafeGel red stain**?	**SafeGel red stain** most likely binds by a combination of intercalation and electrostatic interaction.
What is the chemical structure of **SafeGel red stain**?	The chemical structure of **SafeGel red stain** is proprietary.
Does **SafeGel red stain** migrate during electrophoresis?	**SafeGel red stain** 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 **SafeGel red stain** than with EtBr.
Does **SafeGel red stain** 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 **SafeGel red stain** 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 **SafeGel red stain** with success after accidentally leaving it in ambient light for more than one month.

Trouble shooting

Problem	Suggestion
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	**SafeGel red stain** 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 **SafeGel red stain** 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 **SafeGel red stain** solution to 45-50°C for two minutes and vortex to redissolve. 2.Store dye at room temperature to avoid precipitation.

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Protocol for preparing agarose gels for Agarose Gel Electrophoresis

Materials needed but not provided
Agarose, TAE (or TBE) buffer, 6x sample loading buffer, DNA ladder standard, electrophoresis chamber, power supply, gel casting tray and combs, staining tray, gloves, pipette and pipette tips

Pouring a standard agarose 1% agarose gel*

1. Weigh 1g of your agarose powder and add this 1g to 100mL 1X TAE or 1X TBE buffer into a glass bottle of at least 250mL.
2. Melt the agarose in a microwave for 1-3 minutes until the agarose is completely dissolved and the solution becomes clear (the solution is completely clear).
   NOTE: Caution HOT solution! Be careful!
   NOTE: Solution may boil out of the flask/bottle if the solution is boiled for too long periods!
3. **Optional for pre-stained gels (not recommended by Genaxxon bioscience)****
   Let agarose solution cool down to 55°C to 60°C, add 10µL SafeGel per 100mL gel.
   Gently stir the gel solution to mix SafeGel red stain.
4. Seal horizontal gel casting tray at both ends.
   Pour molten agarose (55°C to 60°C) onto the gel plate to a depth of 4-6mm.
   Insert comb (until its base is 1mm from the base of the gel).
   Allow cooling down to ambient temperature. This will take about 30 minutes at ambient temperature. Cooling can be speed up by placing the gel cast tray with the agarose in a fridge for 15 minutes. ***
   NOTE: Pour slowly to avoid bubbles, which will disrupt the gel.
   NOTE: Any bubbles can be pushed away from the well comb and the gel towards the sides/edges of the gel with a pipette tip.

* normally, agarose concentrations of 0.7% up to 2% were used, depending on the application and the size of the DNA to be separated.

** Genaxxon recommends „in-slot application" of a 20X SafeGel red stain-solution together with the DNA and, if needed the loading dye.

*** low melting agaroses need cooling down to +2°C to +8°C for at least 45 minutes to enable solidification.

Loading samples and running an agarose gel electrophoresis

1. Once solidified, remove the seals from the end of casting tray.
2. Place the casting tray into the electrophoresis apparatus.
3. Add 1/5 volume 6X Loading buffer to each sample and DNA ladder standard.
   NOTE: Loading buffer serves 2 purposes:
   a. It provides a visible dye that helps with gel loading and will also allow you to gauge how far the gel has already run.
   b. it contains a high percentage glycerol. So after adding it to your sample your sample will be heavier than water/buffer and will settle to the bottom of the slot and not diffusing into the buffer.
4. Fill electrophoresis apparatus with 1X TAE (or 1X TBE) buffer until the complete gel is covered by the buffer.
5. Carefully pipette 5µL of the DNA ladder standard into the first lane of the gel.
   NOTE: Place the very top of the pipette tip into the buffer just above the well. Very slowly and steadily, push the sample out and watch if the DNA ladder / your sample fills the slot.
6. Carefully pipette 5µL of each sample (already mixed with loading buffer) into other slots of the gel.
   NOTE: Take care that you mark which sample was loaded into which slot.
7. Run the gel at 80 – 150V until Bromophenol blue is near the end of the gel. Bromophenol blue runs at about 800bp.
   NOTE: The black cable is the negative pole. The red cable is the positive pole.
   NOTE: DNA is negatively charged and will run to towards the positive pole (electrode).
   NOTE: A typical run time is about 0.5 to 1 hour, depending on the gel size, the gel concentration, the buffer and the voltage applied.
8. Turn OFF power.
   Disconnect the electrodes/cables from the power source, and then carefully remove the gel from the casting tray.
9. Visualize or image the stained gel (or stained DNA bands) with a standard transilluminator (302 or 312nm), and photograph the gel using an appropriate filter (e.g., Ethidium bromide filter).

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Protocol for Staining agarose gels after agarose gel electrophoresis

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

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

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

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

   At Genaxxon this procedure shows the best results in sharpness of bands and is the most economical way to use SafeGel red stain!

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

   a. Run gels according to your standard protocol.

   b. Dilute the SafeGel red stain 10000X stock solution about 3,300 fold to 10,000 fold to prepare a 3X or 1X staining solution in water with 0.1M NaCl (e.g., add 5µL up to 15µL of SafeGel red stain stock solution and 5mL NaCl to 45mL water). While SafeGel red stain 1X staining solution can 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 SafeGel red stain filter may also be used for photographing with equally good results.

3. **Staining of DNA by Precasting SafeGel red stain Gels***

   a. Prepare agarose gel solution using your standard protocol.

   b. Dilute SafeGel red stain 10000X stock solution into the agarose gel solution at 1:10000 (e.g., 5µL stock solution added to 50mL of gel solution). Let agarose solution cool down to about 50-55°C before adding SafeGel red stain. 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).

   c. Cast the gel and allow it to solidify. To avoid mould formation, we recommend that the precast gels are 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. **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 SafeGel red stain Gels is not suitable for acrylamide gels. Use post gel staining for acrylamide gels.**