Data Invention Biotech

SafeRed Nucleic Acid Gel Stain

Description:

SafeRed® Nucleic Acid Gel Stain is a kind of new generation of fluorescent nucleic acid gel stain designed to replace the highly toxic ethidium bromide (EtBr). The Ames test confirmed that DuGreen are nonmutagenic at concentrations well above their working concentrations used for gel staining. DuGreenNucleic Acid Gel Stain is highly sensitive than EtBr either as precast gel stains or post gel stains.

SafeRed® and EB have virtually the same spectra, so you can directly replace EB with SafeRed® without changing your existing imaging system. SafeRed® cannot be sufficiently excited with a 488nm argon laser or similar visible light. SafeRed® can also be used to stain dsDNA, ssDNA or RNA in polyacrylamide gel via post gel staining. Precast polyacrylamide gel staining with SafeRed® is not recommended because of relatively high background fluorescence.

SafeRed® Nucleic Acid Gel Stain 10,000X in DMSO is a concentrated DuGreen® solution that can be diluted 10,000 times for use in precast gel staining for ~3,300 times for use in post gel staining according to the procedures described below. One vial (0.5ml) of 10,000X solution can be used to prepare at 100 precast minigels or post-stain at least 100 minigels.

Gel staining with SafeRed® is compatible with downstream applications such as gel extraction and cloning. SafeRed® is efficiently removed from DNA by phenol/chloroform extraction and ethanol precipitation.

Features

Ø Safety: Nonmutagenic and noncytotoxic

Ø Easy disposal: Safe to dispose in the drain

Ø Compatibility: Spectrally compatible with existing instruments

Ø Sensitivity: Higher signal but lower background

Ø Stability: can be stored at RT and microwavable

1.Post-staining Protocol

1.1 Run gels as usual according to your standard protocol.

1.2 Dilute the SafeRed® 10,000X stock reagent~3,300 fold to make a 3X staining solution in H2O with 0.1M Nacl(e.g, add 15ul of SafeRed® 10,000X stock reagent and 5ml 1M Nacl to 45ml H2O). Note: including 0.1M NaCl in the staining solution enhances sensitivity, but may promote dye precipitation if the gel stain is reused.

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

1.4 Agitate the gel gently at room temperature for~30 minutes.

1.5 Image the stained gel with a standard transilluminator (302 or 312nm), and photograph the gel using an ethidium bromide filter.

1.6 Staining solution can be reused at least 2~3times. Store staining solution at room temperature protected from light.

2. Pre-cast Protocol

2.1 Prepare molten agarose gel solution using your standard protocol.

2.2 Dilute the SafeRed® 10,000X stock reagent into the molten agarose gel solution at 1:10,000 (e.g.,5ul of SafeRed® 10,000Xstock reagent added to 50ml of the gel solution)and mix thoroughly. DuGreen can be added while the gel solution is still hot.

2.3 Cast the gel and allow it to solidify. Any leftover gel solution may be stored and reheated later for additional gel casting. SafeRed® precast gels may be stored at 4°C for later use.

2.4 Load samples and run the gels using your standard protocol.

2.5 Image the stained gel with a standard transilluminator(302 or 312nm), and photograph the gel using an Ethidium bromide filter.

Note: The pre-cast protocol is not recommended for polyacrylamide gels. Although the post-staining method is recommended, precast gels may also be tried with SafeRed®. However, some DNA samples, such as those derived from plasmid DNA digestion by certain restriction enzymes, may experience migration retardation or compromised resolution. Thus, both the post-stained and precast gels can be performed to determine which one may better meet your needs.

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SafeRed® and DuGreen® troubleshooting

1. Why am I seeing smeared or smiling DNA band(s) or discrepant DNA migration? SafeRed and DuGreen cannot penetrate live cell membranes then go into the DNA double helix structure like EB because they are larger molecules (big molecules than EB). They are high affinity dyes designed to be larger dyes to improve their safety. So they may affect the migration of DNA in precast gels. Specially, such as restriction digested DNA may migrate abnormally in precast gels.

Please try the following methods to reduce the smeared or smiling DNA band(s) or discrepant DNA migration:

1) **Load less DNA** Smearing and smiling in SafeRed or DuGreen 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.. Blown out or smeared bands can be caused by overloading. This is frequently observed with DNA ladders.

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.

3) Pour a lower percentage agarose gel for better resolution of large fragments. Higher molecular weight DNA separates better with a lower percentage gel.

4) Change the running buffer. TBE buffer has a higher buffering capacity than TAE.

5) If you see DNA migration issues or smearing after post-staining with SafeRed or DuGreen, 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.

1) 2. Why do I see weak fluorescence, decreased dye performance over time, or a film of dye remaining on the gel after post-staining?The dye may have precipitated out of solution.

2) Heat SafeRed solution to 45-50°C for two minutes and vortex to dissolve.

3) Store dye at room temperature to avoid precipitation. The SafeRed and DuGreen are stable in room temperature more than one year.