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SYBR® Green I Nucleic Acid Gel Stain

Introduction

SYBR® Green I Stain is a highly sensitive fluorescent stain for detecting nucleic acids in agarose and polyacrylamide gels. The dye exhibits a preferential affinity for DNA and its fluorescent signal is greatly enhanced when bound to DNA (more than an order of magnitude greater than the fluorescent enhancement of bound ethidium bromide). The detection limit using SYBR® Green I Stain is as low as 60 pg per band of ds DNA using 300 nm transillumination. With 254 nm epi-illumination, as little as 20 pg of ds DNA can be detected. This is approximately 25 to 100 times more sensitive than ethidium bromide staining. The stain can also detect single stranded DNA and RNA, although the sensitivity is lower. The detection limit for oligonucleotides stained with SYBR® Green I Stain is 1 ng-2 ng with 300 nm transillumination.

The exceptional sensitivity of SYBR® Green I Stain makes it useful for the detection of low-cycle number and low target number DNA amplification products, the detection and restriction analysis of low-copy number DNA and RNA vectors, and the detection of products of nuclease protection and bandshift assays. SYBR® Green I Stain's superior sensitivity may allow it to replace radioisotopes in some applications.

Spectral Characteristics

SYBR® Green I Stain is maximally excited at 494 nm and has secondary excitation peaks at 284 nm and 382 nm. The emission of DNA stained with SYBR® Green I Stain is centered at 521 nm. The fluorescent characteristics of SYBR® Green I Stain make it compatible with UV trans- and epi-illuminators, blue-light transilluminators such as the Clare Chemical's Dark Reader® transilluminator, and argon ion lasers such as those used with the Molecular Dynamics FluorImager™ SI. Nucleic acids stained with SYBR® Green I Stain can also be detected by a hand-held ultraviolet lamp with some loss in sensitivity.

Storage

SYBR® Green I Stain is supplied in an anhydrous DMSO solution and is shipped at ambient temperature. As a fluorescent stain, it needs to be protected from light and stored at -20°C. Since DMSO is hygroscopic, the stain should be stored desiccated. The undiluted stock solution is stable for 12 months from the date of receipt when stored under these conditions. Staining solutions should be prepared and used in new polypropylene clear containers (e.g., Rubbermaid® recycling #5 plastics). The diluted stain, in pH 7.5-8.0 buffer, is stable for at least 24 hours at ambient temperature when protected from light. Staining solutions prepared in water are less stable than those prepared in buffer. We recommend dividing the stock solution of dye into smaller aliquots that will thaw faster (e.g. 50 μl/vial).

Handling and Disposal

Because SYBR® Green I Stain binds to nucleic acids, it should be handled with care and disposed of properly. Refer to the Material Safety Data Sheet (MSDS) for recommended safety precautions. Stain solutions should be disposed by passing through activated charcoal followed by incineration of the charcoal. For adsorption on activated charcoal, consult Sambrook, *et al., "Molecular Cloning: A Laboratory Manual",* E.9. Solutions can also be passed through Schleicher & Schuell®'s S&S® Extractor Ethidium Bromide Waste Reduction System, followed by the incineration of the filter. We strongly recommend using double gloves in addition to lab coat and safety glasses when handling the DMSO stock solution.

General Guidelines for Staining with SYBR® Green I Stain

- **Before opening, each vial should be allowed to warm to room temperature and then briefly centrifuged in a microcentrifuge to deposit the DMSO/stain solution at the bottom of the vial.**

- New clear polypropylene containers (e.g., Rubbermaid® recycling #5 plastics) should be obtained for use with SYBR® Green I Stain (new polypropylene containers appear to bind less SYBR® Green I Stain than other plastics). When stored in polypropylene containers, the diluted stain can be used for up to 24 hours and will stain several gels (2 to 3) with little decrease in sensitivity. The containers should be rinsed with distilled water (do not use detergents) after each use and dedicated to **SYBR® Green I Stain use only.**

- SYBR® Green I Stain binds to glass and some non-polypropylene plastics which may result in a decrease or elimination of signal from your sample.

- A working SYBR® Green I Staining Solution should be prepared fresh at a 1:10,000 dilution of stock dye in a pH 7.5 to 8.0 buffer (e.g., TAE, TBE, or TE).

- Agarose gels should be cast no thicker than 4 mm. As gel thickness increases, diffusion of the dye into the gel is decreased lowering the detection of DNA.
- For recording your results on film, we recommend using the SYBR® Green Gel Stain Photographic Filter (Catalog No. 50530). This filter is of deep yellow cellophane having an overall size of 3" x 3" (75 mm x 75 mm). If this filter does not fit your camera, a Wratten® #15 or a Tiffen® #15 photographic filter of the appropriate diameter (purchased from the camera system manufacturer or a local photography shop) will work. The red/orange filter used to photograph ethidium bromide stained gels should not be used with gels stained with SYBR® Green I Stain.

Protocol for Post-staining Gels

Follow the steps below to stain DNA after electrophoresis

1. Remove the concentrated stock solution of SYBR® Green Stain from the freezer and allow the solution to thaw at room temperature.
2. Spin the solution in a microcentrifuge to collect the dye at the bottom of the tube.
3. Dilute the 10,000X concentrate to a 1X working solution (1 µl per 10 ml), in a pH 7.5-8.5 buffer, in a clear plastic polypropylene container. Prepare enough staining solution to just cover the top of the gel.
4. Remove the gel from the electrophoresis chamber.
5. Place the gel in staining solution.
6. Gently agitate the gel at room temperature.
7. Stain the gel for 15–30 minutes. The optimal staining time depends on the thickness of the gel, concentration of the agarose, and the fragment size to be detected. Longer staining times are required as gel thickness and agarose concentration increase.
8. Remove the gel from the staining solution and view with a 300 nm UV transilluminator, CCD camera or Clare Chemical's Dark Reader® transilluminator. SYBR® Green Stained Gels do not require destaining. The dyes fluorescence yield is much greater when bound to DNA than when in solution.

Staining vertical gels with SYBR® Green Stains

Incorporating SYBR® Green Stain into the gel or prestaining the DNA for use in a vertical format is not recommended. The dye binds to glass or plastic plates and DNA may show little to no signal. Gels should be post-stained as described in the previous section.

Follow this procedure when staining vertical gels with SYBR® Green Stain

1. Remove the concentrated stock solution of SYBR® Green Stain from the freezer and allow the solution to thaw at room temperature.
2. Spin the solution in a microcentrifuge to collect the dye at the bottom of the tube.
3. Dilute the 10,000X concentrate to a 1X working solution, in a pH 7.5-8.5 buffer, in a clear plastic polypropylene container. Prepare enough staining solution to just cover the top of the gel.
4. Remove the gel from the electrophoresis chamber.
5. Open the cassette and leave the gel in place on one plate.
6. Place the plate, gel side up in a staining container.
7. Gently pour the stain over the surface of the gel.
8. Stain the gel for 5-15 minutes.
9. View with a 300 nm UV transilluminator, CCD camera or Clare Chemical's Dark Reader® transilluminator. SYBR® Green Stained Gels do not require destaining. The dye's fluorescence yield is much greater when bound to DNA than when in solution.

Protocol for Adding Dye to Loading Buffer

SYBR® Green I Stain can be added directly to the loading buffer at a final concentration of 1:1000. First prepare a 1:100 dilution of SYBR® Green I Stain in high-quality anhydrous DMSO. The 1:100 dilution can be stored in the freezer and reused. Add 1 µl of this dilution to 9 µl-10 µl of your sample before loading.

Photography

Gels stained with SYBR® Green I Stain exhibit negligible background fluorescence allowing long film exposures when detecting small amounts of DNA. When used with 300 nm transillumination and Polaroid® Type 57 Film, a 0.5-1 second exposure using an f-stop of 4.5 is adequate.
In the photographs, the DNA will appear as bright bands against a gray gel. For Polaroid® Type 55 Film, a 15-45 second exposure using an f-stop of 4.5 is adequate. When used with 254 nm epi-illumination (especially with a hand-held lamp), exposures of the order of 1-1.5 minutes may be required for maximal sensitivity. With 254 nm epi-illumination, the DNA will appear as bright bands against a black background. The SYBR® Green Stain Photographic Filter is recommended for all photography.

Application Notes

- SYBR® Green I Stain can be removed from double-stranded DNA by ethanol precipitation. Isopropanol precipitation is somewhat less effective at removing the dye; butanol extraction, chloroform extraction and phenol extraction do not remove the dye efficiently.
- Gels previously stained with ethidium bromide can subsequently be stained with SYBR® Green I Stain following the standard protocol for post-staining. There will be some decrease in sensitivity when compared to a gel stained only with SYBR® Green I Stain.
- Do not dilute the SYBR® Green I Stain stock solution in glass containers because SYBR® Green I Stain will bind to glass. Dilute the stock solution in a polypropylene container.
- The inclusion of SYBR® Green I Stain in cesium chloride density gradient plasmid preparations is not recommended. The effect of the dye on the buoyant density of DNA is unknown.
- SYBR® Green I Stain does not appear to interfere with enzymatic reactions.
- We recommend the addition of 0.1% to 0.3% SDS in the prehybridization and hybridization solutions when performing Southern blots on gels stained with SYBR® Green I Stain.

- SYBR® Green I Stain bound to double-stranded DNA fluoresces green under UV transillumination. Gels that contain DNA with single-stranded regions may fluoresce orange rather than green.

- We do not recommend photographing gels with a 254 nm transilluminator. An outline of the UV light source can appear in photographs. A filter that will allow a 525 nm transmission and exclude other wavelengths (e.g., those in the infrared) is required.

- SYBR® Green I Stain is not compatible with GelBond® Film or GelBond® PAG Film.

Decontamination

Staining solutions should be disposed of by passing through activated charcoal followed by incineration of the charcoal. For adsorption on activated charcoal, consult Sambrook, *et al*., pp. 6.16 – 6.19, (1989). Solutions can also be passed through Schleicher & Schuell®'s S&S® Extractor Ethidium Bromide Waste Reduction System, followed by the incineration of the filter.

References

Schneeberger, C., et al. 1995. *PCR Methods & App. 4:234-238*

Ordering Information

Catalog No.	Description	Size
50512	SYBR® Green I Nucleic Acid Gel Stain	2 x 500 µl
50513	SYBR® Green I Nucleic Acid Gel Stain	10 x 50 µl
50530	SYBR® Green Gel Stain Photographic Filer 3" square (Wratten® #15)	each

Related Products

Agarose
MDE® Gel Solution
AccuGENE® Electrophoresis Buffers

For More information contact Technical Service at (800) 521-0390 or visit our website at www.Lonza.com.

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