

Instructions for using SYBR® Green II Nucleic Acid Gel Stain

Introduction

SYBR® Green II Nucleic Acid Gel Stain is one of the most sensitive dyes known for detecting RNA in electrophoretic gels. SYBR® Green II Nucleic Acid Gel Stain can be detected as little as 100 pg of single-stranded DNA per band in agarose or polyacrylamide gels using 254 nm epi-illumination, black and white Polaroid® 667 Print Film and a SYBR® Green Gel Stain Photographic Filter (Catalog No. 50530)^(1,2). Even with 300 nm transillumination, as little as 500 pg RNA per band can be detected. Our SYBR® Green II Nucleic Acid Gel Stain is significantly more sensitive than ethidium bromide, the most commonly used stain for detecting nucleic acids in gels. With 300 nm transillumination and photography through an orange-red gelatin filter, ethidium bromide's sensitivity limit in a standard agarose minigel is about 1.5 ng single-stranded nucleic acid per band.

With denaturing agarose-formaldehyde gels and polyacrylamide-urea gels, the sensitivity of SYBR® Green II Nucleic Acid Gel Stain is somewhat reduced, though still superior to that of ethidium bromide. To achieve maximal sensitivity with ethidium bromide, agarose-formaldehyde gels must be washed for several hours prior to staining. In contrast, *without any washing or destaining steps*, we have been able to detect 1.0 ng/band RNA in a SYBR® Green II Stained agarose-formaldehyde gel or polyacrylamide-urea gel using 254 nm epi-illumination, and about 4.0 ng RNA per band using 300 nm transillumination. This remarkable sensitivity of SYBR® Green II Nucleic Acid Gel Stain for detecting RNA can be attributed to several factors, including superior fluorescence quantum yield, binding affinity, and fluorescence enhancement. Although it is not selective for RNA staining, this dye exhibits a higher quantum yield when bound to RNA (~0.54) than to double-stranded DNA (~0.36). This property is somewhat unusual among nucleic acid stains; most show far greater quantum yields and fluorescence enhancements when bound to double-stranded nucleic acids. The fluorescence quantum yield of the RNA/SYBR® Green II Stain complex is more than seven times greater than that of the RNA/ethidium bromide complex (~0.07)⁽³⁾. The affinity of SYBR® Green II Nucleic Acid Gel Stain for RNA is also higher than that of ethidium bromide, and its fluorescence enhancement upon binding RNA is well over an order of magnitude greater. Because SYBR® Green II Nucleic Acid Gel Stain has a low intrinsic fluorescence, there is no need to destain gels to remove free dye. The fluorescence of RNA/SYBR® Green II Stain complexes is not quenched in the presence of urea or formaldehyde, eliminating the need to wash these denaturants out of gels prior to staining.

In addition, staining agarose-formaldehyde gels with SYBR® Green II Nucleic Acid Gel Stain does not interfere with transfer of RNA to filters or subsequent hybridization in Northern blot analysis.

SYBR® Green II Nucleic Acid Gel Stain not only should facilitate the detection of viroid RNAs and multicopy cellular RNA species, but may also prove useful in applications, such as single-strand conformation polymorphism (SSCP) analysis⁽⁴⁾, that require extremely sensitive detection techniques. Conventional SSCP analysis requires radioactive hybridization probes. Although nonisotopic techniques for SSCP analysis have been developed⁽⁵⁻⁸⁾, they require long complex procedures, such as silver staining or chemiluminescence-mediated signal amplification. Ethidium bromide was used to develop a method for nonisotopic SSCP in precast polyacrylamide minigels⁽⁹⁾. Not only is this nonisotopic SSCP technique simple, rapid and reproducible, but it allows precise temperature control - an important parameter in SSCP analysis. SYBR® Green II Nucleic Acid Gel Stain should prove more sensitive than ethidium bromide in such applications.

Storage and Handling

Storage

SYBR® Green II Nucleic Acid Gel Stain is provided either as a 2 x 500 µl dye solution in high-quality, anhydrous DMSO (Catalog No. 50522) or as a set of 10 vials, each containing 50 µl of DMSO stock solution (Catalog No. 50523). One ml of the concentrated reagent will prepare a total of 10 liters of working stain solution, which is sufficient to stain more than 100 agarose or polyacrylamide minigels. When stored frozen at -20°C, protected from light, and desiccated this stock solution is stable for six months to one year. We recommend dividing the stock solution of dye into smaller aliquots that will thaw faster e.g. 50 µl/vial). Staining reagent diluted in buffer can be stored protected from light either at 4°C for several weeks or at room temperature for three or four days. Staining solutions prepared in water are less stable than those prepared in buffer and must be used within 24 hours to ensure maximal staining sensitivity. In addition, staining solutions prepared in buffers with pH below 7.5 or above 8.0 are less stable and show reduced staining efficacy. We recommend storing aqueous stain solutions in plastic rather than glass, as the stain may absorb to glass surfaces.

Handling and Disposal

No data is available addressing the mutagenicity or toxicity of SYBR® Green II Nucleic Acid Gel Stain. Because this reagent binds to nucleic acids, it should be treated as a potential mutagen and used with appropriate care. The DMSO stock solution should be handled with particular caution as DMSO is known to facilitate the entry of organic molecules into tissues. We strongly recommend using double gloves when handling the DMSO stock solution. As with all nucleic acid stains, solutions of SYBR® Green II Nucleic Acid Gel Stain should be poured through activated charcoal before disposal. The charcoal must then be incinerated to destroy the dye. Refer to the MSDS for safety precautions.

Spectral Characteristics

Fluorescence Spectra

SYBR® Green II Nucleic Acid Gel Stain may be used with commonly available ultraviolet epi- and transilluminator excitation sources, blue-light transilluminators such as the Clare Chemical Dark Reader® transilluminator, as well as hand held ultraviolet lamps. SYBR® Green II Nucleic Acid Gel Stain is maximally excited at 497 nm, but also has a secondary excitation peak centered near 254 nm. The fluorescence emission of SYBR® Green II stained RNA is centered at 520 nm. These spectral characteristics make SYBR® Green II Nucleic Acid Gel Stain compatible with a wide variety of gel reading instruments, ranging from those with ultraviolet epi- and transillumination to argon laser and mercury-arc lamp excitation gel scanners.

SYBR® Green Gel Stain Photographic Filter

For optimal sensitivity with black and white film, SYBR® Green II Stained gels should be photographed through Lonza's SYBR® Green Gel Stain Photographic Filter (Catalog No. 50530). A number of other yellow or green gelatin or cellophane filters (available from Kodak® through photography equipment suppliers) can also be used for photography, but most will provide slightly reduced sensitivity. The orange-red filters used to photograph ethidium bromide-stained gels should not be used with SYBR® Green II RNA Stained gels. The SYBR® Green Gel Stain Photographic Filter should be stored in a cool, dry place and handled only at its edges. If the filter needs to be cut to fit into a specialized holder, we recommend first placing it between two sheets of clean, fairly stiff paper and then cutting out the desired shape with scissors.

Application Notes

SYBR® Green II Stain can be removed from the nucleic acid 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 II 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 II Nucleic Acid Gel Stain.

Do not dilute SYBR® Green II Stain stock solution in glass containers because SYBR® Green II Nucleic Acid Gel Stain will bind to glass. Dilute the stock solution in a polypropylene container.

SYBR® Green II Nucleic Acid Gel 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 Northern or Southern blots on gels stained with SYBR® Green II Nucleic Acid Gel Stain.

Nucleic acid bound SYBR® Green II Nucleic Acid Gel Stain fluoresces green under UV transillumination.

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 II Nucleic Acid Gel Stain is not compatible with GelBond® Flim or GelBond® PAG Film.

Experimental Protocols

Staining Following Electrophoresis

Perform electrophoresis on non-denaturing gels or on denaturing polyacrylamide-urea or agarose-formaldehyde gels according to standard techniques⁽¹⁰⁾. SYBR® Green II Nucleic Acid Gel Stain has not been tested with other gel matrices.

1. **Allow each vial to warm to room temperature and then briefly centrifuged in a microfuge to deposit the DMSO solution at the bottom of the vial.**
2. Dilute the stock SYBR® Green II Nucleic Acid Gel Stain. For non-denaturing gels and denaturing polyacrylamide-urea gels, we recommend a 1:10,000 dilution in TBE (89 mM Tris base, 89 mM Boric acid, 1 mM EDTA, pH 8). **For staining agarose-formaldehyde gels, or glyoxal-denatured RNA, use a 1:5,000 dilution in TBE.**
3. Place the gel in a staining container, such as the top of a pipet-tip box. Do not use glass or non-polypropylene plastic staining containers. Add enough staining solution to cover the gel. Protect the staining container from light by covering it with aluminum foil or placing it in the dark. There is no need to wash urea or formaldehyde out of gels prior to staining.
4. Gently agitate the gel at room temperature. The optimal staining time is typically 10-40 minutes for polyacrylamide gels and 20-40 minutes for agarose gels. The staining time may vary depending on the thickness of the gel and the percentage of agarose or polyacrylamide. No destaining is required. The staining solution may be stored in the dark (preferably refrigerated) and reused three to four times.

Protocol for Adding Dye to Loading Buffer

SYBR® Green II Stain can be added directly to the loading buffer at a final concentration of 1:1,000. First prepare a 1:100 dilution of SYBR® Green II Nucleic Acid Gel 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.

Visualizing and Photographing Stained Gels.

Illuminate the stained gel using 300 nm ultraviolet transillumination, or for greater sensitivity, 254 nm epillumination.

Photograph the gel with Polaroid® 667 Black and White Print Film using a SYBR® Green Gel Stain Photographic Filter. Stained gels have negligible background fluorescence, allowing long film exposures when detecting small amounts of RNA. For 300 nm transillumination, typically a 1-2 second exposure using an f-stop of 4.5 is adequate. For 254 nm epillumination (especially with a hand held lamp), exposures on the order of 1-1.5 minutes may be required for maximal sensitivity.

Warnings and Precautions

These products are manufactured for Lonza Rockland, Inc., by Molecular Probes, Inc. and are sold for Research Use Only. Not for use in diagnostic procedures. SYBR® Green II Nucleic Acid Gel Stain is the subject of patent applications filed by Molecular Probes, Inc., and is not available for resale or other commercial purposes without specific permission of Molecular Probes, Inc. SYBR® is a trademark of Molecular Probes, Inc.

References

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10. *Molecular Cloning: A Laboratory Manual*, Second Edition, J. Sambrook, E.F. Fritsch and T. Maniatis, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (1989).

Ordering information

Catalog No.	Description	Size
50522	SYBR® Green II RNA Gel Stain	2 x 500 µl
50523	SYBR® Green II RNA Gel Stain	10 x 50 µl
50530	SYBR® Green Photographic Filter	3" square

Related Products

Lonza Agarose Products
MDE® Gel Solution
Latitude® Precast Gels
Reliant® Gel System
AccuGENE® MOPS Buffer

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

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