Revised: 03–April–2001

# SYBR® Green II RNA Gel Stain

**Quick Facts**

***Storage upon receipt:***
- –20°C
- Desiccate
- Protect from light

***Ex/Em:*** 254, 497/520 nm, bound to nucleic acid

## Introduction

Molecular Probes' SYBR® Green II RNA gel stain is one of the most sensitive dyes known for detecting RNA in electrophoretic gels. As little as 100 pg RNA or single-stranded DNA per band can be detected in a SYBR Green II–stained agarose or polyacrylamide gel using 254 nm epi-illumination Polaroid® 667, black-and-white print film and a SYBR Green gel stain photographic filter (S-7569).[1,2] Even with 300 nm transillumination, as little as 500 pg RNA per band can be detected. SYBR Green II RNA gel stain is significantly more sensitive than ethidium bromide, the most commonly used stain for detecting nucleic acids in gels (Figure 1). 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. Note that our detection limits are based on results obtained with a FotoDyne® Foto/UV® 450 ultraviolet transilluminator in combination with Polaroid 667 film. Video cameras and CCD cameras in general have a different spectral response than black-and-white print film and thus may not exhibit the same sensitivity.

On denaturing agarose/formaldehyde gels and polyacrylamide/urea gels, the sensitivity of SYBR Green II RNA 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 ng RNA per band in a SYBR Green II dye-stained agarose/formaldehyde gel or polyacrylamide/urea gel using 254 nm epi-illumination, and about 4 ng RNA per band using 300 nm transillumination.

The remarkable sensitivity of SYBR Green II RNA 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 complex is more than seven times greater than that of the RNA/ethidium bromide complex (~0.07).[3] The affinity of SYBR Green II RNA 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 RNA 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 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 RNA gel stain does not interfere with transfer of RNA to filters or subsequent hybridization in Northern blot analysis as long as 0.1%–0.3% SDS is included in prehybridization and hybridization buffers.

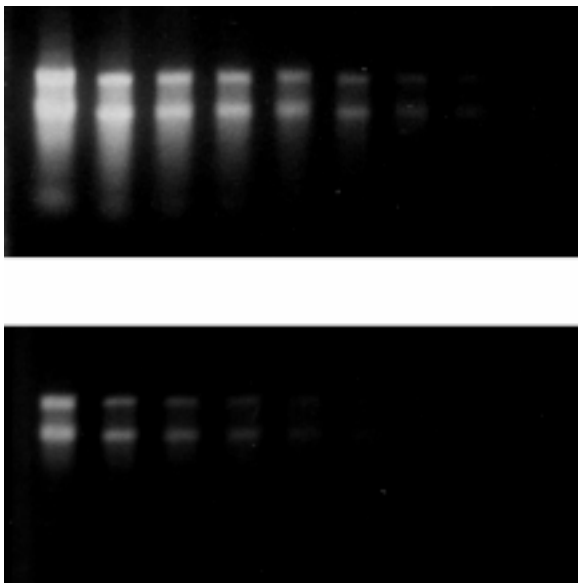


***Figure 1****. Dilution series of Escherichia coli ribosomal 16S and 23S RNA electrophoresed in 1% agarose gels. The gels contain an identical twofold dilution series of RNA. The gel shown in panel A was stained for 20 minutes with SYBR Green II RNA gel stain (using a 1:10,000 dilution of the stock reagent) and not destained. The gel shown in panel B was stained for 20 minutes with 5 µg/mL ethidium bromide, then destained for an additional 20 minutes. The SYBR Green II dye-stained gel was excited using 254 nm epi-illumination and the ethidium bromide–stained gel using 300 nm transillumination (Fotodyne® Foto UV 450 ultraviolet transilluminator). Although the SYBR Green II dye-stained gel can be excited at 300 nm, epi-illumination at 254 nm resulted in the best sensitivity in our hands. Both gels were photographed with Polaroid 667 black-and-white print film, using a SYBR Green gel stain photographic filter (SYBR Green II dye-stained gel) or an ethidium bromide gel stain photographic filter (ethidium bromide–stained gel).*

SYBR Green II RNA gel stain can be used to:

- Analyze small aliquots from RNA preparations before Northern blotting, start-site mapping or cDNA preparation
- Visualize the migration behavior of 5S rRNA species after high-resolution denaturing gradient electrophoresis (DGGE) [4]
- Stain DNA in single-strand conformation polymorphism (SSCP) [5]
- Stain DNA before amplification by PCR [6]

## Materials

### Contents

SYBR Green II stain is provided as a 10,000X concentrate in DMSO in the following sizes:

- 500 µL (S-7564), sufficient to stain ~100 minigels
- 1 µL (S-7568), sufficient to stain ~50 minigels
- 20 vials (S-7586), each containing 50 µL

We also offer SYBR Green II stain the SYBR Green Nucleic Acid Gel Stain Starter Kit (S-7580), which includes:

- 50 µL SYBR Green I stain
- 50 µL SYBR Green II stain
- SYBR Green/Gold gel stain photographic filter

### Storage

Upon receipt, store the dye frozen at -20°C, protected from light in a desiccator. When stored properly, the SYBR Green II stain stock solution in DMSO is stable for six months to one year.

### Handling and Disposal

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

**We must caution** that no data are available addressing the mutagenicity or toxicity of SYBR Green II RNA 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 RNA gel stain should be poured through activated charcoal before disposal. The charcoal must then be incinerated to destroy the dye.

### Spectral Characteristics

SYBR Green II RNA gel stain may be used with commonly available ultraviolet epi- and transilluminator excitation sources, as well as hand-held ultraviolet lamps. SYBR Green II RNA 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 dye-stained RNA is centered at 520 nm. These spectral characteristics make SYBR Green II RNA gel stain compatible with a wide variety of gel reading instruments, ranging from those with ultraviolet epi- and transillumination to argon-ion laser and mercury-arc lamp excitation gel scanners.

## Experimental Protocols

### Staining RNA Following Electrophoresis

**1.1** Perform electrophoresis on nondenaturing gels or on denaturing polyacrylamide/urea or agarose/formaldehyde gels according to standard techniques.[7] SYBR Green II RNA gel stain has not been tested with other gel matrices.

**1.2** Dilute the stock SYBR Green II RNA gel stain. For nondenaturing 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 denaturing agarose/formaldehyde gels, we recommend a 1:5000 dilution in TBE. Staining with SYBR Green II reagent is pH sensitive. For optimal sensitivity, verify that the pH of the staining solution at the temperature used for staining is between 7.5 and 8.0 (preferably pH 8.0).

**1.3** Place the gel in a staining container, such as a Petri dish or the top of a pipet-tip box. 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.

**1.4** Agitate the gel gently 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.

### Visualizing and Photographing Stained Gels

**2.1** Illuminate the stained gel using 300 nm ultraviolet transillumination, or for greater sensitivity, 254 nm epi-illumination.

**2.2** For optimal sensitivity with black and white film, SYBR Green II–stained gels should be photographed through Molecular Probes' SYBR Green gel stain photographic filter (S-7569). 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–stained gels.

**2.3** 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 epi-illumination (especially with a hand-held lamp), exposures on the order of 1–1.5 minutes may be required for maximal sensitivity.

## References

**1.** FASEB J 8, A1266 (1994); **2.** Biomedical Products 19, 68 (1994); **3.** Cytometry 7, 508 (1986); **4.** Appl Environ Microbiol 62, 1969 (1996); **5.** Diagnostic Mol Pathol 5, 260 (1996); **6.** Proc Natl Acad Sci USA 94, 10745 (1997); **7.** Sambrook, J., Fritsch, E.F. and Maniatis, T., *Molecular Cloning: A Laboratory Manual, Second Edition*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (1989).

## Product List

*Current prices may be obtained from our Web site or from our Customer Service Department.*

| Cat # | Product Name | Unit Size |
|---|---|---|
| S-7564 | SYBR® Green II RNA gel stain *10,000X concentrate in DMSO* | 500 µL |
| S-7568 | SYBR® Green II RNA gel stain *10,000X concentrate in DMSO* | 1 mL |
| S-7586 | SYBR® Green II RNA gel stain *10,000X concentrate in DMSO* *special packaging* | 20x50 µL |
| S-7580 | SYBR® Green Nucleic Acid Gel Stain Starter Kit | 1 kit |

## Contact Information

Further information on Molecular Probes' products, including product bibliographies, is available from your local distributor or directly from Molecular Probes. Customers in Europe, Africa and the Middle East should contact our office in Leiden, the Netherlands. All others should contact our Technical Assistance Department in Eugene, Oregon.

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